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A generic model of electron transport in mitochondria

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Abstract

In this paper, a simplified, generic model of mitochondrial metabolism is explored. In particular the following question is addressed: To what extent are phenomena observed in experiments and simulations of mitochondrial metabolism generic, in the sense that they *must* occur in *all* models with this basic structure? Of particular interest are the electron transport chain and oxidative phosphorylation, and how flux through the system and the redox states of intermediates respond to physiologically important stimuli. These stimuli include changes in substrate supply ($NADH/FADH_2$), in oxygenation, and in membrane proton gradient/ATP demand. Analytical techniques are used to show that certain experimentally observed effects must occur in the generic model. These include the responses of both flux and redox states to changed substrate and oxygen concentrations. At the same time other effects, such as the responses of redox states to changes in proton gradient, are dependent on the details of the model, and are not common to every model with the same basic structure. The phenomenon of saturation in response to large inputs is also discussed.

Keywords: Mitochondria; Electron transport; Model

1. Introduction

Central to aerobic respiration in cells are the processes of electron transport and oxidative phosphorylation in mitochondria. In these processes NADH and $FADH_2$ produced by glycolysis and the TCA cycle are oxidised leading ultimately to the production of ATP. The mechanisms involved are complex, but summaries can be found in many modern biochemistry textbooks such as Garrett and Grisham (1995) or Bhagavan (2002). A comprehensive review of much of the biology of interest here can be found in Brown (1992). The author's own interest in mitochondrial metabolism sprang initially from attempts to model the regulation of cerebral blood flow (CBF) (Banaji et al., 2005). Several pathways of CBF regulation involve metabolic by-products such as lactate and adenosine which serve as sensors of metabolic state, and for this reason it was felt that a model of CBF regulation should include at least a caricature of oxidative metabolism. In addition, decreases in electron transport activity are implicated in a number of cerebral diseases such as Parkinson's disease, Huntington's disease, and Alzheimer's disease (Davey et al., 1998). The experimental study of energy transduction in cerebral mitochondria has been ongoing for several decades (Scott and Nicholls, 1980), and elucidation of some of the mechanisms involved is still in progress (Belevich et al., 2006).

There are several widely known models of electron transport and oxidative phosphorylation. These include the models of Magnus and Keizer (1997), those of Korzeniewski (1996) and Korzeniewski and Zoladz (2001), and other recent additions (e.g. Farmery and Whiteley, 2001; Beard, 2005). In some cases such models have been incorporated in more complete models of mitochondrial metabolism which include the TCA cycle (e.g. Cortassa et al., 2003). These ordinary differential equation (ODE) models have been designed with numerical data in mind, and reflecting the complexity of the processes involved, the functional forms are quite involved. At the same time, of necessity, a number of processes are omitted or lumped together. While simulating the detailed model in

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Korzeniewski and Zoladz (2001) (which will be referred to as the "Korzeniewski model") the following questions arose:

- If there is a change in the size of a parameter, but not its sign, can the model behave qualitatively differently?
- If a function in the model is replaced with another function with the same qualitative features (e.g. strictly increasing) can the model behave qualitatively differently?

These are not questions which can be answered in full generality with numerical simulations. However, simulations suggest that in some cases the structure of the interactions alone might determine qualitative model behaviour, and thus the answer to the above questions could be "no". To confirm if this is so, a generic ODE model of electron transport is constructed, which reproduces the structure of the electron transport chain (ETC) and is amenable to analysis. This basic structure, consisting of a chain of coupled redox reactions interacting with a potential, occurs in all the numerical models (e.g. Magnus and Keizer, 1997; Korzeniewski, 2001; Beard, 2005), though these models all contain additional processes such as transport processes.

Once the generic model is constructed, it is analysed. Ever since the seminal paper of Kacser and Burns (1973), metabolic control analysis (MCA) techniques have been widely used in biochemistry to study how steady state fluxes and metabolite concentrations respond to changes in input parameters (e.g. enzyme concentrations). The equivalent mathematical process is parameter sensitivity analysis. Parameter sensitivity analysis involves the construction of a matrix whose entries—essentially the control coefficients of MCA—describe how model steady state outputs such as fluxes and concentrations respond to model inputs such as enzyme concentrations and activations, substrate inflow and outflow rates, etc.

The information encoded in the sensitivity matrix is local information in that it describes the response of outputs to inputs *at particular values of the inputs and the outputs*. In a general nonlinear system (e.g. Ihekwaba et al., 2004) parameter sensitivities are highly dependent on parameter values. One question of interest is whether, in a given class of models, a given parameter sensitivity is always of a particular sign. The process of deciding this question can be seen as a *qualitative* parameter sensitivity analysis on the class of models. Once the generic model is constructed, it is shown that the model always has a unique steady state, and a qualitative sensitivity analysis is carried out on it. It is shown that the responses of steady state fluxes to three model inputs always have a particular sign. On the other hand the responses of metabolite concentrations have a particular sign in response to two inputs, but not to a third.

2. Observations and assumptions

Before constructing and analysing the generic model, it is helpful to list some effects of interest observed in experiments, and in simulations of the Korzeniewski model. This helps in the construction of the generic model, and also provides specific questions to be answered.

The first set of observations is about the rate of oxygen consumption in mitochondria, which is referred to as the *flux* through the system. This is a measurable quantity sometimes referred to as the "respiration rate". It is known that this rate is able to respond, up to a point, to:

- changes in substrate supply (e.g. upregulation of the TCA cycle),
- changes in energy demand due to more rapid ATP turnover or membrane depolarisation by pharmacological agents and
- oxygen levels.

These effects will be referred to as *supply*, *demand* and *oxygenation*, or collectively as the *inputs*. Generally, increasing any of the inputs should cause increases in flux (for example Brown, 1992; Rigoulet et al., 1998; Boschmann et al., 1996; Schonfeld et al., 1983). Consistent with the experimental data, this behaviour also occurs in simulations of the Korzeniewski model. Typical behaviour is shown in Fig. 1.

The second set of observations is about the redox states of various participants in the ETC, such as NAD/NADH, ubiquinone, the various cytochromes, etc. In general these redox states change in response to changes in the inputs, but importantly, these changes do not simply reflect changes in flux through the system. As techniques to measure the redox states of these chemicals in vivo develop (Cooper and Springett, 1997; Springett et al., 2000; McGown et al., 2003), it is potentially of clinical importance to understand what they do tell us about the state of a cell.

Experimental data on how redox states change in response to different stimuli is not easy to come by, largely because redox states in the ETC are still quite hard to measure. It is, however, assumed that increasing substrate supply causes a decrease in the level of oxidation of all members of the ETC. The response of certain ETC components to changes in oxygen levels is documented—e.g. cytochrome *c* oxidase becomes more oxidised in response to increased oxygen levels (Wilson et al., 1988). Again it is assumed that this behaviour is reproduced in other intermediates in the ETC. In response to changes in demand the picture is more cloudy. There is evidence that the oxidation level of cytochrome *c* oxidase increases in response to functional activation which would correspond to an increase in demand (Brown and Brand, 1985; Wobst et al., 2001). However, simulations of the Korzeniewski models are not unambiguous, and suggest that changes in either direction are possible in response to changes in

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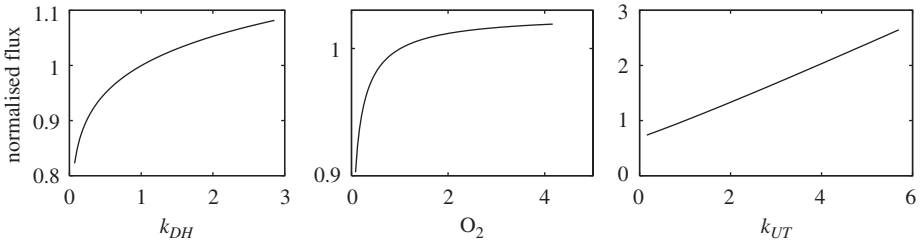

Fig. 1. Normalised flux against changes in supply, oxygenation, and demand in the Korzeniewski model for intact skeletal muscle in Korzeniewski and Zoladz (2001), available at Korzeniewski. In each case all parameters except one are fixed at values quoted at Korzeniewski. In the first case, the parameter k_{DH} representing NADH supply is varied. In the second case oxygen level is varied. In the final experiment the parameter k_{UT} representing ATP usage is varied. For ease of presentation, the parameters are normalised against some value (28000 μM min^{-1} for k_{DH}, 240 μM for O_2 and 686 μM min^{-1} for k_{UT}). Flux is taken as the activity of complex IV (v_{C4} in the model), again normalised to some typical value (300 μM min^{-1}). We see that flux increases with each input as expected.

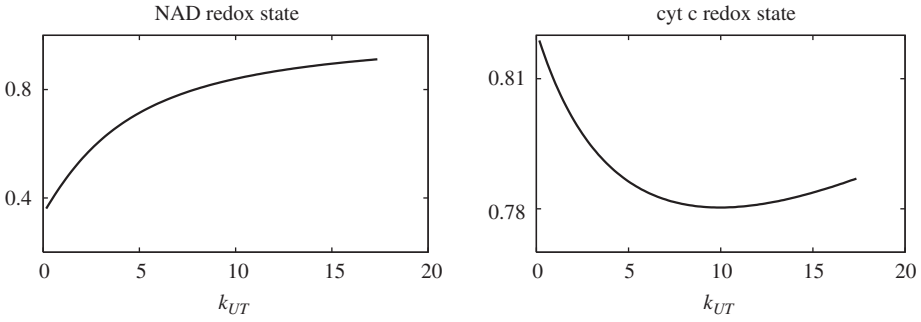

Fig. 2. The change in oxidised proportions of NAD and cytochrome c in the Korzeniewski model for intact skeletal muscle in response to changes in the demand parameter k_{UT} (normalised to 686 μM min^{-1}). Although NAD oxidation always increases with demand, cytochrome c oxidation appears to show biphasic behaviour. All other details are as for Fig. 1.

demand. The behaviour of the Korzeniewski model for intact skeletal muscle is illustrated in Fig. 2 where it can be seen that although the response of NAD redox state to changes in demand is monotonic, this is not true for the response of cytochrome c redox state.

Another phenomenon of interest is saturation. A variety of biological assumptions would imply that the extent to which an increased input can increase flux should diminish as the input becomes large. Saturation is observed in response to all the inputs in the Korzeniewski model. It is noteworthy that as an input is increased, and the system approaches maximum flux, the ETC intermediates do not in general approach complete reduction or oxidation.

Having summarised what is known from experiments, and from simulations of the Korzeniewski model, the conclusions of this paper are now foreshadowed: It will be shown that the generic model behaves as follows:

- increasing substrate supply increases flux and causes a decrease in the level of oxidation of all members of the ETC,
- increasing oxygen causes an increase in flux and an increase in the oxidation of all members of the ETC,
- increasing demand causes an increase in flux and can have a variety of effects on the redox states of members of the ETC and
- saturation occurs quite generally because of the structure of the system and independently of any detailed assumptions (e.g. Michaelis–Menten dynamics of some step.) Redox states at saturation are generally non-maximal.

3. The generic model

3.1. The basic reaction scheme

The task of constructing the model is now tackled. The theoretical starting point will be the chain of coupled redox reactions illustrated in Fig. 3. Individual members of the cycle can represent complexes or redox pairs within a complex (the Cu_A centre in cytochrome c oxidase for example).

Each chemical in the chain can exist in an oxidised state A_i and a reduced state B_i. Reactions converting oxidised to reduced states and vice versa are coupled to each other. Because the total quantity—oxidised plus reduced—of any chemical in the chain is conserved, reduced forms of the chemicals are not explicitly introduced. Instead, the concentration of oxidised A_i is referred to as a_i, and the total concentration of $A_i + B_i$ is assumed constant at $a_{i,max}$.

The three inputs outlined above are introduced as the three parameters shown in Fig. 3: a "push-side" stimulus z which increases the rate of conversion of A_1 to B_1; an "oxygen-like" stimulus w which increases the rate of oxidation of the final chemical in the chain; and a parameter ψ representing ATP demand or the proton

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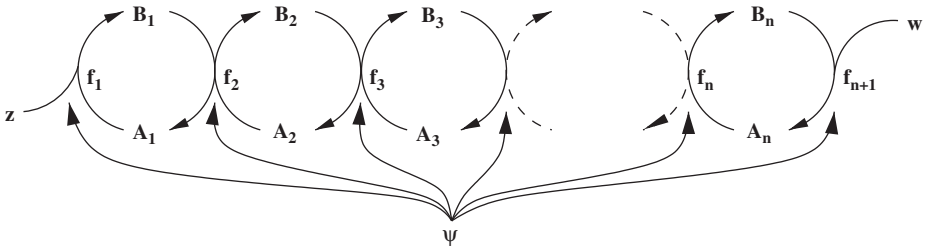

Fig. 3. A schematic representation of the reaction network of interest. The quantities A_i and B_i refer to oxidised and reduced states of the ETC intermediates. The functions f_i define the rates of reaction of the $n+1$ coupled redox reactions. z, w and ψ are parameters which reflect supply, oxygen levels, and demand/proton gradient.

gradient. This final parameter is discussed more fully in Section 7, followed by discussion in Section 8 of a more complicated model where ψ is a variable created by some of the redox reactions. This first model with ψ as a parameter can be regarded as representing an experimental situation where the experimentalist has direct control over the proton gradient and membrane potential.

w and z take values in $[0,\infty)$ while $\psi \in (-\infty,\infty)$. Although in theory ψ can take negative values since the proton density could be higher within the mitochondria than outside, in practice the regime $\psi \geqslant 0$ is of most physiological relevance. The following set of differential equations for the evolution of the a_i are obtained:

$$\dot{a}_1 = -f_1(z,a_1,\psi)+f_2(a_1,a_2,\psi), \tag{1}$$

$$\dot{a}_i = -f_i(a_{i-1},a_i,\psi)+f_{i+1}(a_i,a_{i+1},\psi), \quad i=2,\ldots,n-1, \tag{2}$$

$$\dot{a}_n = -f_n(a_{n-1},a_n,\psi)+f_{n+1}(a_n,w,\psi). \tag{3}$$

The quantities f_i represent the rates of reactions in the chain as illustrated in Fig. 3. Any steady states of the system must satisfy:

$$0 = -f_1(z,a_1,\psi)+f_2(a_1,a_2,\psi), \tag{4}$$

$$0 = -f_i(a_{i-1},a_i,\psi)+f_{i+1}(a_i,a_{i+1},\psi), \quad i=2,\ldots,n-1, \tag{5}$$

$$0 = -f_n(a_{n-1},a_n,\psi)+f_{n+1}(a_n,w,\psi). \tag{6}$$

All f_i are assumed to be once differentiable in all their arguments with continuous derivatives.

Note that the phase space of this system is the closed n-dimensional box defined by the equations:

$$0 \leqslant a_i \leqslant a_{i,max}, \quad i=1,\ldots,n. \tag{7}$$

3.2. Assumptions

In this section conditions are placed on the functions f_i which are both biologically reasonable and very general. The following notation is used for the derivatives of the functions f_i:

$$f_{ij} \equiv \frac{\partial f_i}{\partial a_j}, \quad f_{i\psi} \equiv \frac{\partial f_i}{\partial \psi}, \quad f_{1z} \equiv \frac{\partial f_1}{\partial z}, \quad f_{n+1,w} \equiv \frac{\partial f_{n+1}}{\partial w}. \tag{8}$$

It will be clear from the context whether a term refers to the value of a derivative at a particular point, or as a function of the variables.

The first set of assumptions is that at finite substrate concentrations, all reaction rates are finite. This means that f_1 is bounded on $[0,a_{1,max}]$ for any fixed z and ψ, but may tend to infinity as $z\to\infty$ for fixed a_1. For $i=2,\ldots,n$, at any fixed ψ each f_i is bounded on its domain of definition. Similarly f_{n+1} is bounded on $[0,a_{n,max}]$ for any fixed w and ψ, but may tend to infinity as $w\to\infty$.

Reasonable definitions of a "push" stimulus (such as increased activity of the TCA cycle), and an oxygen-like stimulus, give the following four equations:

$$f_1(0,\cdot,\cdot) = 0, \tag{9}$$

$$f_{1z} > 0 \quad \text{for } a_1 > 0, \tag{10}$$

$$f_{n+1}(\cdot,0,\cdot) = 0, \tag{11}$$

$$f_{n+1,w} > 0 \quad \text{for } a_n < a_{n,max}. \tag{12}$$

These equations simply tell us that without the stimuli the reactions cannot proceed, and that increasing the stimulus causes an increase in the reaction rate. Since ψ represents a potential against which at least some of the reactions must do work the following relations are obtained:

$$f_{i\psi} \leqslant 0, \quad i=1,\ldots,n+1. \tag{13}$$

If ψ does not affect the ith reaction then of course $f_{i\psi}=0$ everywhere. Moreover, if ψ does inhibit the ith reaction (i.e. $f_{i\psi}\neq 0$), then sufficiently large values of ψ make the rate of reaction arbitrarily small, i.e.

$$f_{i\psi} \neq 0 \quad \text{implies} \quad \lim_{\psi\to\infty} f_i(\cdot,\cdot,\psi) = 0. \tag{14}$$

This reflects the fact that the energy required to pump a proton against a chemical and electrical gradient becomes large as the gradient increases. The following equations imply that no reaction can proceed in the absence of any of its substrates:

$$f_i(\cdot,0,\cdot) = 0, \quad i=1,\ldots,n, \tag{15}$$

$$f_i(a_{i-1,max},\cdot,\cdot) = 0, \quad i=2,\ldots,n+1. \tag{16}$$

The final set of conditions imply that increased substrate concentration increases the rate of reaction unless one of

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the substrates is entirely absent:

$$f_{11} > 0 \quad \text{if } z > 0, \tag{17}$$

$$f_{ii} > 0 \text{ if } a_{i-1} < a_{i-1,max}, \ \ i = 2, \ldots, n, \tag{18}$$

$$f_{i+1,i} < 0 \text{ if } a_{i+1} > 0, \ \ i = 1, \ldots, n-1, \tag{19}$$

$$f_{n+1,n} < 0 \text{ if } w > 0. \tag{20}$$

These equations mean that the system is cooperative (Hirsch and Smith, 2005) in the interior of the phase space, which will prove to be of importance later. Note that functions which are linear, exponential, saturating, sigmoidal, etc. all fulfil the conditions above. An example of a typical function $f_i, i \neq 1, n+1$ is illustrated in Fig. 4.

4. Normal behaviour of the system

Frequently it will be convenient to rewrite system (1)–(3) in simpler vector notation as

$$\dot{\mathbf{a}} = \mathbf{F}(\mathbf{a}, z, w, \psi), \tag{21}$$

where

$$\mathbf{a} = [a_1, a_2, \ldots, a_n]^T,$$

and

$$\mathbf{F} = [-f_1 + f_2, -f_2 + f_3, \ldots, -f_n + f_{n+1}]^T.$$

In this section the existence of a unique equilibrium for the system will be shown—i.e. the existence of a unique function $\mathbf{a}(z, w, \psi)$ satisfying

$$0 = \mathbf{F}(\mathbf{a}(z, w, \psi), z, w, \psi). \tag{22}$$

Subsequently it will be shown that this equilibrium is globally stable. Local stability amounts to the condition that the Jacobian $\mathbf{J_F}$ has negative eigenvalues at the equilibrium which is shown in Appendix A. Global stability can be proved by a variety of methods, and here a general result on cooperative, tridiagonal systems will be used.

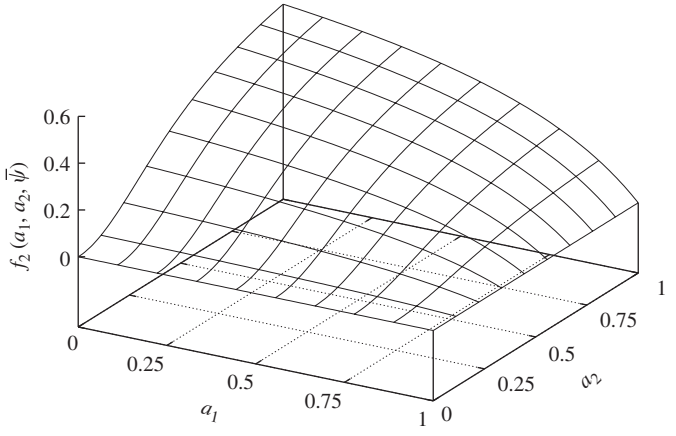

Fig. 4. A typical f_i for fixed $\psi = \bar{\psi}$, decreasing in its first argument, increasing in its second, and zero along the lines $a_{i-1} = a_{i-1,max}$ and $a_i = 0$. In the example plotted, $i = 2$ and $a_{1,max} = a_{2,max} = 1$.

In subsequent sections the response of the unique equilibrium to the three parameters z, w and ψ is examined. This amounts to exploring the three equations:

$$\mathbf{J_F}\frac{d\mathbf{a}}{d\mu} + \frac{d\mathbf{F}}{d\mu} = 0, \quad \mu = z, w, \psi. \tag{23}$$

The structure of $\mathbf{J_F}$ and of $d\mathbf{F}/d\mu$ in each case will allow conclusions about $d\mathbf{a}/d\mu$.

4.1. Existence of a unique equilibrium

The following statement of the intermediate value theorem (IVT) will be used repeatedly:

> A real function f which is strictly decreasing on a closed interval $[a, b]$ and such that $f(a)f(b) \leqslant 0$, has a unique zero in $[a, b]$. If $f(a)f(b) < 0$, then the unique zero is in (a, b).

z and w are fixed at non-zero values, and ψ is also fixed. Explicit reference to any of the parameters is omitted below. Thus for the time-being, by an abuse of notation, $f_i(a_{i-1}, a_i)$ will mean the value of this function at some fixed ψ, while $f_1(a_1)$ will mean the value of f_1 at some fixed z and ψ and $f_{n+1}(a_n)$ will mean the value of f_{n+1} at some fixed w and ψ.

With fixed values of the parameters, the aim is to prove the existence of a unique equilibrium of the form $(a_1, a_2, \ldots, a_n)$ which solves Eqs. (4)–(6). Fix $a_2 = \bar{a}_2$. Then by assumption $f_1(0) = 0$, $f_1(a_{1,max}) > 0$, $f_2(0, \bar{a}_2) \geqslant 0$, $f_2(a_{1,max}, \bar{a}_2) = 0$. The situation is illustrated in Fig. 5.

The IVT can be applied to the strictly decreasing function $g(a_1) \equiv f_2(a_1, \bar{a}_2) - f_1(a_1)$ to get

$$g(0) = f_2(0, \bar{a}_2) - f_1(0) \geqslant 0, \tag{24}$$

$$g(a_{1,max}) = f_2(a_{1,max}, \bar{a}_2) - f_1(a_{1,max}) < 0. \tag{25}$$

Thus for any $\bar{a}_2$, there exists a unique value $a_1(\bar{a}_2)$ such that

$$-f_1(a_1(\bar{a}_2)) + f_2(a_1(\bar{a}_2), \bar{a}_2) = 0. \tag{26}$$

The above argument holds for every $\bar{a}_2 \in [0, a_{2,max}]$. Note that the value of a_1 which solves this equation is strictly less than $a_{1,max}$ for any $\bar{a}_2$.

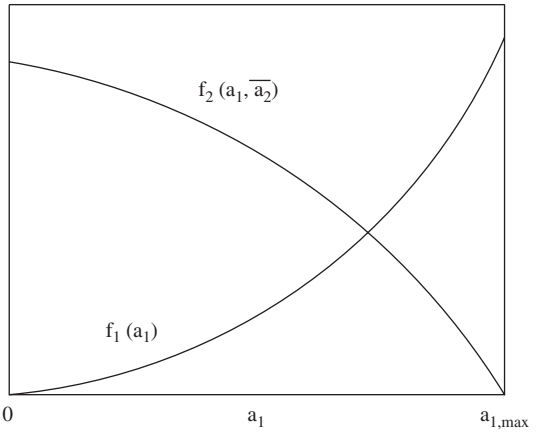

Fig. 5. There is always a unique solution to the equation $f_1(a_1) = f_2(a_1, \bar{a}_2)$ for any $\bar{a}_2 \geqslant 0$.

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To continue the argument, a_2 is now allowed to vary and it is confirmed that the function $a_1(a_2)$ is strictly increasing on $[0, a_{2,max}]$. Differentiating the relation $0 = -f_1(a_1(a_2)) + f_2(a_1(a_2), a_2)$ with respect to a_2 gives

$$0 = -f_{11}\frac{da_1}{da_2} + f_{21}\frac{da_1}{da_2} + f_{22}, \tag{27}$$

which solves to give

$$\frac{da_1}{da_2} = \frac{f_{22}}{(f_{11} - f_{21})} > 0. \tag{28}$$

From Eqs. (18) and (19), $f_{11} - f_{21} > 0$ and the inequality follows because both numerator and denominator are strictly positive ($f_{11} > 0$ because $z > 0$, while $f_{22} > 0$ because a_1 is strictly less than $a_{1,max}$).

Next, behaviour of the function $\tilde{f}_2(a_2) \equiv f_2(a_1(a_2), a_2)$ is investigated. $\tilde{f}_2$ is the function f_2 restricted to the surface $a_1(a_2)$. Note that

$$\frac{d\tilde{f}_2}{da_2} = f_{21}\frac{da_1}{da_2} + f_{22}. \tag{29}$$

Comparing this equation with Eq. (27) gives immediately that $d\tilde{f}_2/da_2 > 0$. Thus $\tilde{f}_2$ is an increasing function of a_2.

So far it has been proved that there exists a surface $a_1(a_2)$ which solves Eq. (4), that a_1 increases with a_2 on this surface, and that f_2 evaluated on this surface is an increasing function of a_2. The argument for the remaining equations is now iterative. For the equation:

$$0 = -f_2(a_1, a_2) + f_3(a_2, a_3), \tag{30}$$

f_2 is replaced by $\tilde{f}_2$ and identical arguments to those used above provide a function $a_2(a_3)$ which solves Eq. (30).

This process continues. Finally,

$$0 = -\tilde{f}_n(a_n) + f_{n+1}(a_n), \tag{31}$$

which has a unique solution a_n immediately by the IVT because of the monotonicity of the two functions. Thus for fixed non-zero w and z it is possible to find values $(a_1, a_2, \ldots, a_n)$ which solve Eqs. (4)–(6). The special case $z = 0$ is trivial. One can simply check by back-substitution that the vector $\mathbf{a} = (a_{1,max}, a_{2,max}, \ldots, a_{n,max})$ satisfies the equation. Uniqueness follows by noting that $f_{n+1}(a_n) = 0$ is solved uniquely by $a_n = a_{n,max}$, $f_n(a_{n-1}, a_{n,max}) = 0$ is solved uniquely (for fixed non-zero w) by $a_{n-1} = a_{n-1,max}$, etc. Similarly for $w = 0$, the vector $\mathbf{a} = (0, 0, \ldots, 0)$ is the unique solution. For both z and w zero there is no unique solution. This doubly degenerate situation corresponding to no substrate supply and no oxygen (and of course no flux) is ignored.

Having proved the existence of a unique equilibrium for generic parameter values, it is now shown that this equilibrium is globally stable. Existing theory shows that if a system is cooperative and tridiagonal on some positively invariant, bounded region of phase space, then any trajectory entering this region must converge to some member of the equilibrium set (Smillie, 1984), and in fact it is possible to find explicitly a Lyapunov function for such systems (Fiedler and Gedeon, 1999). The system presented here fulfils these requirements on the interior of the phase space. On the boundary the system is not cooperative, because some derivatives are zero. It is shown in Appendix B that this is not a serious problem, because for non-zero w and z no trajectory can lie entirely on the boundary. Thus all trajectories must enter the interior of the phase space, and thence converge to the unique equilibrium.

5. Response of the system to changes in supply

In the arguments above, the parameters z, w and ψ have been fixed. Now they are allowed to vary and the question of how the unique equilibrium migrates through phase space is tackled. The first input to examine is the supply z. It will be shown that all intermediates become more reduced in response to increasing supply, and that the flux through the system increases. Limiting behaviour in response to large z is explored. Later it will be shown that any analysis of the response to z carries over immediately to w.

5.1. Response of redox states to changes in z

It is proved that as z increases the steady state value of each of the a_i decreases, i.e. $da_i/dz < 0$. Fixing w and ψ and differentiating $0 = \mathbf{F}(\mathbf{a}, z, w, \psi)$ with respect to z gives

$$0 = \mathbf{J_F}\frac{d\mathbf{a}}{dz} + \frac{\partial \mathbf{F}}{\partial z}. \tag{32}$$

In this relation, $\mathbf{J_F}$ is the Jacobian of the system, $\partial\mathbf{F}/\partial z = (-f_{1z}, 0, \ldots, 0)^T$ describes how the various reactions depend on z, and the quantity to be determined is $d\mathbf{a}/dz$ which describes how the redox states change with supply.

It is easy to calculate $\mathbf{J_F}$

$$\mathbf{J_F} = \begin{bmatrix} -f_{11} + f_{21} & f_{22} & 0 & \cdots & 0 & 0 \\ -f_{21} & -f_{22} + f_{32} & f_{33} & \cdots & 0 & 0 \\ 0 & -f_{32} & -f_{33} + f_{43} & \cdots & 0 & 0 \\ \vdots & \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & 0 & 0 & \cdots & -f_{n-1,n-1} + f_{n,n-1} & f_{nn} \\ 0 & 0 & 0 & \cdots & -f_{n,n-1} & -f_{nn} + f_{n+1,n} \end{bmatrix}.$$

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Writing $x_i \equiv \mathrm{d}a_i/\mathrm{d}z$ and $g_i \equiv (\mathbf{J_F}(\mathrm{d}\mathbf{a}/\mathrm{d}z))_i$ Eq. (32) is examined componentwise and it is shown that all x_i must be negative. First it is shown inductively that all x_i have the same sign. That x_n and x_{n-1} must both have the same sign (or both be zero) follows from the final component in Eq. (32):

$$g_n = 0 = -f_{n,n-1}x_{n-1} + (f_{n+1,n} - f_{nn})x_n. \tag{33}$$

Noting that $-f_{n,n-1} > 0$ and $f_{n+1,n} - f_{nn} < 0$ gives the result. The inductive step involves showing that if x_j has the same sign as x_n, then so does x_{j-1}. Summing components j to n gives

$$\sum_{i=j}^{n} g_i = -f_{j,j-1}x_{j-1} - f_{jj}x_j + f_{n+1,n}x_n = 0,$$
$$j = 2, \ldots, n-1. \tag{34}$$

Noting that $-f_{j,j-1} > 0$, $-f_{jj} < 0$ and $f_{n+1,n} < 0$ implies immediately that x_{j-1} must have the same sign as x_j and x_n. Thus all x_i must have the same sign.

The possibility that all $x_i = 0$ is quickly dismissed because this contradicts $g_1 = f_{1z} > 0$. All that remains to be shown is that all x_i are negative. Summing all the equations gives

$$\sum_{i=1}^{n} g_i = -f_{11}x_1 + f_{n+1,n}x_n = f_{1z}. \tag{35}$$

Since $f_{11} > 0, f_{n+1,n} < 0$ and $f_{1z} > 0$, this implies that at least one of x_1 and x_n is negative. Since all x_i have the same sign, they must all be strictly negative.

It is time to pause to examine the implications of this argument. The conclusion is that as the push z is increased, the steady state concentration of each of the oxidised chemicals in the chain decreases. This result is independent of any nonlinearities in the functions f_i as long as they are strictly monotonic.

5.2. Response of the flux to changes in z

Since the quantity of interest here is z, the other parameters (w and ψ) are assumed fixed and omitted as arguments of functions. It is trivial to confirm that flux increases with increasing z. At steady state, flux through the system for any z is

$$R(z) \equiv f_1(z, a_1(z)) = f_2(a_1(z), a_2(z)) = \cdots = f_{n+1}(a_n(z)). \tag{36}$$

Differentiating $R(z) = f_{n+1}(a_n(z))$ gives

$$\frac{\mathrm{d}R}{\mathrm{d}z} = \frac{\partial f_{n+1}}{\partial a_n}\frac{\mathrm{d}a_n}{\mathrm{d}z} > 0. \tag{37}$$

The inequality follows since both terms in the product are negative. So, as expected, flux increases with increasing substrate supply.

5.3. Limiting behaviour for large z

Limiting behaviour of both flux and redox states as z becomes very large are explored. It will be seen that rate-saturation arises independently of the functional forms for the reaction rates (for example all reactions could follow mass-action dynamics or Michaelis–Menten dynamics and saturation would arise in either case).

First, at fixed w and ψ, the functions $f_2, \ldots, f_{n+1}$ are bounded functions of their arguments. Let

$$R_t = \min\{\max(f_2), \max(f_3), \ldots, \max(f_{n+1})\}, \tag{38}$$

R_t provides an upper bound on the steady state flux. Thus $R(z) \leqslant R_t$ is an increasing bounded function, and hence reaches a limiting value R_∞ as $z \to \infty$. Further, $\lim_{z\to\infty}(\mathrm{d}a_n/\mathrm{d}z) = 0$, since $\mathrm{d}a_n/\mathrm{d}z$ is strictly negative, but $a_n \geqslant 0$. Eq. (37) now implies that $\lim_{z\to\infty}(\mathrm{d}R/\mathrm{d}z) = 0$. Thus the assumptions imply saturating behaviour of R for large z.

Since R tends smoothly to some maximum value, and the a_i decrease, one might at first expect that as z increases concentrations of each of the a_i tend to zero, i.e. all chemicals become maximally reduced. In fact:

- with the possible exception of a_1, the concentration of each a_i must tend to some non-zero limiting value as z increases and
- if f_1 is unbounded in z (for any fixed a_1) then a_1 approaches zero as z increases.

The first observation implies that increasing the push cannot cause arbitrarily high reduction levels in members of the chain, (and equivalently, as will be shown later, increasing oxygenation cannot cause arbitrarily high oxidation levels). In fact, if ψ and w are fixed, the chain has a limiting state of maximum flux and maximum reduction in response to increased push. Since the existence of this limiting state is independent of the details of the model, it always makes sense to ask whether the normal "operating point" of the system is near or far from this limiting state.

The assumption in the second observation is satisfied in the Korzeniewski model, where the push parameter (termed k_{DH} in those models) reflects the rate of reduction of NAD occurring in processes external to the ETC such as the TCA cycle, glycolysis, etc. In an in vitro context z might refer to the concentration of an enzyme such as Glucose-6-P-dehydrogenase responsible for the conversion of NAD to NADH (in the presence of a large supply of Glucose-6-P) (Rigoulet et al., 1998).

To verify the first statement, it suffices to note that $R_\infty > 0$ and $f_i(\cdot, 0) = 0 \neq R_\infty$ for $i = 2, \ldots, n$. As the limiting flux is non-zero it is intuitively clear that a_i cannot tend to zero for $i \geqslant 2$, since each quantity is the substrate for some reaction in the chain whose other substrates are bounded. To understand the second statement, note that although $f_1(z, a_1)$ itself is not necessarily bounded for any fixed a_1, clearly $f_1(z, a_1(z)) < R_t$ along the solution curve

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since the other f_i are bounded. Moreover,

$$\frac{\mathrm{d}f_1(z,a_1(z))}{\mathrm{d}z}=\frac{\mathrm{d}R}{\mathrm{d}z}>0 \tag{39}$$

from Eq. (37). It has been noted that each a_i is a bounded decreasing function of z so they must all tend to unique limits $a_i(\infty)$ as $z\to\infty$. In fact if f_1 becomes unbounded for any finite a_1, this implies that $a_1(\infty)=0$. This gives the system of equations:

$$f_2(a_1(\infty),a_2(\infty))=R_\infty, \tag{40}$$

$$f_i(a_{i-1}(\infty),a_i(\infty))=R_\infty,\quad i=3,\ldots,n, \tag{41}$$

$$f_{n+1}(a_n(\infty))=R_\infty. \tag{42}$$

With $a_1(\infty)$ known, these n independent equations uniquely determine $a_2(\infty),\ldots,a_n(\infty)$ and R_∞.

It must be stressed that although the response to increased z is saturation, this does not mean that the sensitivity to changes in supply always decreases for increased supply. This is only the case in the limit.

6. Response of the system to changes in oxygen

Having analysed the effect of changes in substrate supply the next parameter to examine is w, the oxygen level. This case is very simple to handle because there is a duality between the effects of z and w on the system. To see this duality, let $b_i=a_{i,max}-a_i$ be the concentration of the reduced form of A_i. Define:

$$\tilde{f}_1(b_1,z,\psi)=f_1(z,a_{1,max}-b_1,\psi), \tag{43}$$

$$\tilde{f}_i(b_i,b_{i-1},\psi)=f_i(a_{i-1,max}-b_{i-1},a_{i,max}-b_i,\psi),\quad i=2,\ldots,n \tag{44}$$

$$\tilde{f}_{n+1}(w,b_n,\psi)=f_{n+1}(a_{n,max}-b_n,w,\psi). \tag{45}$$

Note that the arguments in the functions have been swapped. Since $\dot{b}_i=-\dot{a}_i$ this gives the following system of ODEs for the b_i:

$$\dot{b}_1=-\tilde{f}_2(b_2,b_1,\psi)+\tilde{f}_1(b_1,z,\psi), \tag{46}$$

$$\dot{b}_i=-\tilde{f}_{i+1}(b_{i+1},b_i,\psi)+\tilde{f}_i(b_i,b_{i-1},\psi),\quad i=2,\ldots,n-1 \tag{47}$$

$$\dot{b}_n=-\tilde{f}_{n+1}(w,b_n,\psi)+\tilde{f}_n(b_n,b_{n-1},\psi). \tag{48}$$

Renaming z as w, w as z, b_i as a_{n+1-i} and $\tilde{f}_i$ as f_{n+2-i} gives back the system in Eqs. (1)–(3) satisfying conditions (9)–(20). If the reader wants a pictorial idea of what has been done, this is best achieved by imagining Fig. 3 rotated through 180°. This makes it easy to see that swapping w and z, reversing the order of the subscripts, and swapping oxidised and reduced forms of each chemical gives us back a formally identical system[2].

[2]This implies that there is a symmetry in the *class* of systems being studied in the sense that performing the above transformations on any member of the class gives us another member of the class. The transformation described can be seen as a particular action of the group $\mathbb{Z}_2$ on the system, since performing the transformation twice returns the original system.

Thus all the results from the analysis of the effects of supply carry over directly to the case of oxygenation. The following conclusions can immediately be listed:

(1) Increasing w increases the flux.
(2) Increasing w causes an increase in the oxidised states a_i for all i and reducing w causes a decrease in the oxidised states (i.e. hypoxia has the same qualitative effect as increased supply on redox states, but of course reduces the flux).
(3) As w is increased, the respiration rate approaches a limit, and with the possible exception of a_n, the values of a_i tend to values less than $a_{i,max}$.
(4) If f_{n+1} is unbounded as w increases for any fixed $a_n\neq a_{n,max}$, then $a_n\to a_{n,max}$ as w is increased.

The penultimate observation is interesting. It is generally accepted that in most tissues, including the brain (Rolett et al., 2000), oxygen exerts little control on the respiration rate until it drops significantly below normal. That the limiting redox states in response to large supply of oxygen are in general sub-maximal is consistent with data on cytochrome c in Wilson et al. (1988).

7. Response of the system to changes in ψ

The final parameter to examine is ψ. It will be seen that ψ is a very different parameter from z and w, not least because it can affect a number of reactions in the chain. As a result it controls chain activity in a more complex way than the other parameters. In this section it is assumed that ψ inhibits some non-empty subset of the reactions, i.e. $\partial f_i/\partial\psi<0$ for at least one i.

The parameter ψ can be identified with the proton gradient. This gradient inhibits the extrusion of protons from the matrix by two means: firstly, acting as a chemical gradient; secondly, by giving rise to the membrane potential. The second effect is in fact the dominant effect in normal circumstances. The two effects of the proton gradient are treated as a single effect, assuming that they always work in the same direction. This is consistent with the Korzeniewski model where the membrane potential and proton gradient are tightly coupled. For the time-being the fact that in addition to inhibiting the reactions, ψ is produced by the reactions will be ignored. The incorporation of the production of ψ (a negative feedback loop) causes complications discussed later.

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7.1. Response of the redox states to changes in ψ

z and w are fixed at non-zero values, and omitted as arguments to the functions. As for z and w, the first question to ask is what effect a change in ψ will have on the steady state concentrations of the various a_i. The response begins with the familiar equation:

$$0 = \mathbf{J_F}\frac{\mathrm{d}\mathbf{a}}{\mathrm{d}\psi} + \frac{\partial \mathbf{F}}{\partial \psi}. \tag{49}$$

This time let $x_i \equiv \mathrm{d}a_i/\mathrm{d}\psi$ and $g_i = (\mathbf{J_F}(\mathrm{d}\mathbf{a}/\mathrm{d}\psi))_i$.

Now $\partial\mathbf{F}/\partial\psi = (-f_{1\psi} + f_{2\psi}, -f_{2\psi} + f_{3\psi}, \ldots, -f_{m\psi} + f_{n+1,\psi})^T$, and unlike in the cases of z and w, it does not have sufficient structure to allow any conclusions. Clearly, given any n real numbers $q_1, \ldots, q_n$ one can choose values of $f_{i\psi} \leqslant 0$ such that $f_{i+1,\psi} - f_{i\psi} = q_i$. This implies that any vector $\mathbf{x}$ is a solution to $0 = \mathbf{J_F}\mathbf{x} + (\partial\mathbf{F}/\partial\psi)$ for some choice of $f_{i\psi}$. This observation means that the behaviour of redox states in response to changes in ψ depends on the details of a model and cannot be predicted for a generic model.

This is not the end of the story, though. There is a special case, of possible physiological significance described below, where the signs of some of the x_i are determined. This is when some initial or final set of $f_{i\psi}$ are ordered with respect to the rest in the following sense:

$$|f_{i\psi}| \leqslant |f_{j\psi}| \quad \text{for all } j > i, \;\; i = 1, \ldots, i_{max}, \tag{50}$$

or

$$|f_{i\psi}| \leqslant |f_{j\psi}| \quad \text{for all } j < i, \;\; i = i_{min}, \ldots, n+1. \tag{51}$$

In the first case it can be shown that $x_i \leqslant 0$ for $i = 1, \ldots, i_{max}$, and analogously in the second that $x_i \geqslant 0$ for $i = i_{min}, \ldots, n$. Moreover unless all $f_{i\psi}$ are equal, and hence all x_i are zero, the results are strict—i.e. $x_i < 0$ in the first case, and $x_i > 0$ in the second case. Outline proofs of these statements are presented in Appendix C.

In the case of the ETC there is at least one situation where the above result is relevant. If there are redox reactions at one of the ends of the ETC which are *not* involved with pumping protons, and hence which are not inhibited by ψ, then the effect of changes in ψ on the redox state of these pairs becomes predictable. As an example, assume that proton gradient does not affect the initial reduction of NAD (which is actually external to the ETC). Then one can predict that an increased proton gradient will lead to an increase in NADH. Intuitively, the proton gradient inhibits reactions further down the chain leading to the build-up of NADH. This is borne out by data is Brandes and Bers (1999) where an increase in workload (i.e. a decrease in ψ) causes a rapid decrease in NADH levels. There, however, after the initial decrease, NADH levels recover due probably to the activation of pyruvate dehydrogenase and/or the TCA cycle, which are external to the model. The prediction is also confirmed by simulation results for the Korzeniewski model presented in Fig. 2.

7.2. Response of the flux to changes in ψ

Define $R(\psi) \equiv f_1(a_1(\psi), \psi) = f_2(a_1(\psi), a_2(\psi), \psi) = \cdots = f_{n+1}(a_n(\psi), \psi)$. Despite limited information on how redox states change with ψ, it can be shown that the steady state flux $R(\psi)$ is a strictly decreasing function of ψ, and $\lim_{\psi\to\infty} R(\psi) = 0$. That $\lim_{\psi\to\infty} R(\psi) = 0$ follows immediately from the fact that the a_i are bounded and the assumption that $\lim_{\psi\to\infty} f_i(\cdot,\cdot,\psi) = 0$ (Section 3.2). Intuitively it is obvious that $R(\psi)$ decreases with ψ since each reaction is either inhibited or unaffected by ψ, but the calculation involves considerably more work than the equivalent calculation for z or w.

Note that at steady state

$$R'(\psi) \equiv \frac{\mathrm{d}R(\psi)}{\mathrm{d}\psi} = f_{11}x_1 + f_{1\psi} \tag{52}$$

$$= f_{21}x_1 + f_{22}x_2 + f_{2\psi} \tag{53}$$

$$\vdots \tag{54}$$

$$= f_{n,n-1}x_{n-1} + f_{nn}x_n + f_{n\psi} \tag{55}$$

$$= f_{n+1,n}x_n + f_{n+1,\psi}. \tag{56}$$

In an abuse of notation f_{ij} now refers to the value of f_{ij} evaluated at the steady state, and x_i to the value of $\partial a_i/\partial\psi$ evaluated at the steady state. Solving for $R'(\psi)$ involves showing that one can write

$$R'(\psi) = f_{11}x_1 + f_{1\psi} \tag{57}$$

$$= \tilde{f}_{22}x_2 + \tilde{f}_{2\psi} \tag{58}$$

$$\vdots$$

$$= \tilde{f}_{nn}x_n + \tilde{f}_{n\psi} \tag{59}$$

$$= f_{n+1,n}x_n + f_{n+1,\psi}, \tag{60}$$

where $\tilde{f}_{ii} > 0$ and $\tilde{f}_{i\psi} \leqslant 0$, and the conclusion will follow by eliminating x_n from the final two equations.

The construction is as follows. x_1 is eliminated from

$$f_{11}x_1 + f_{1\psi} = f_{21}x_1 + f_{22}x_2 + f_{2\psi} = R'(\psi), \tag{61}$$

to get

$$R'(\psi) = \frac{f_{21}}{f_{11} - f_{21}}(f_{2\psi} - f_{1\psi} + f_{22}x_2) + f_{22}x_2 + f_{2\psi}. \tag{62}$$

Defining

$$\delta_1 = -\frac{f_{21}}{f_{11} - f_{21}}, \tag{63}$$

it is immediate that $0 < \delta_1 < 1$, so that

$$R'(\psi) = f_{22}(1-\delta_1)x_2 + f_{2\psi}(1-\delta_1) + \delta_1 f_{1\psi}. \tag{64}$$

Define $\tilde{f}_{22} = (1-\delta_1)f_{22}$ and $\tilde{f}_{2\psi} = (1-\delta_1)f_{2\psi} + \delta_1 f_{1\psi}$. Clearly $\tilde{f}_{22} > 0$. If $f_{1\psi} = 0$ and $f_{2\psi} = 0$ then $\tilde{f}_{2\psi} = 0$ and otherwise $\tilde{f}_{2\psi} < 0$. The construction for $\tilde{f}_{33}, \tilde{f}_{3\psi}$ etc. is formally identical. If for some m, $f_{m\psi} < 0$, then $\tilde{f}_{n\psi} < 0$ for all $n \geqslant m$.

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Finally, Eqs. (59) and (60) imply that

$$x_n = \frac{f_{n+1,\psi} - \tilde{f}_{n\psi}}{\tilde{f}_{nn} - f_{n+1,n}}, \tag{65}$$

so that

$$\begin{aligned} R'(\psi) &= f_{n+1,n}x_n + f_{n+1,\psi} \\ &= \frac{f_{n+1,n}}{\tilde{f}_{nn} - f_{n+1,n}}(f_{n+1,\psi} - \tilde{f}_{n\psi}) + f_{n+1,\psi}. \end{aligned} \tag{66}$$

Since

$$\delta_n \equiv -\frac{f_{n+1,n}}{\tilde{f}_{nn} - f_{n+1,n}} \quad \text{satisfies} \quad 0 < \delta_n < 1. \tag{67}$$

We have

$$R'(\psi) = (1 - \delta_n)f_{n+1,\psi} + \delta_{n-1}\tilde{f}_{m\psi}. \tag{68}$$

In the special case that all of the $f_{m\psi} = 0$ for $m < n+1$, then $\tilde{f}_{m\psi} = 0$, but in this case for a non-trivial system, $f_{n+1,\psi} < 0$. Thus either $f_{n+1,\psi} < 0$ or $\tilde{f}_{m\psi} < 0$, and the above equation implies that $R'(\psi) < 0$. Thus as long as ψ affects a single reaction, increasing ψ will always decrease the flux, whatever the effect on individual redox states in the chain.

8. The full model: incorporating the production of ψ

This essentially concludes the analysis of the model defined by differential Eqs. (1)–(3), and conditions (9)–(20). What remains is to say something about the more complicated situation where ψ is itself created by the reactions. Incorporation of the production of ψ into the model introduces a negative feedback loop into the system. Intuitively this ought not to have too dramatic an effect on much of the behaviour described so far, but the question is what can be said mathematically.

The generic version of such an augmented system looks like:

$$\dot{a}_1 = -f_1(z, a_1, \psi) + f_2(a_1, a_2, \psi), \tag{69}$$

$$\dot{a}_i = -f_i(a_{i-1}, a_i, \psi) + f_{i+1}(a_i, a_{i+1}, \psi), \quad i = 2, \ldots, n-1, \tag{70}$$

$$\dot{a}_n = -f_n(a_{n-1}, a_n, \psi) + f_{n+1}(a_n, w, \psi), \tag{71}$$

$$\dot{\psi} = P(\psi, f_1, f_2, \ldots, f_n) - L(u, \psi). \tag{72}$$

The final equation tells us that ψ may be produced by a given reaction and decays/is used up at a rate dependent on its value. P is the rate of production of ψ and depends on the various reaction rates. L is the rate of "decay" of ψ, either as leak or via the production of ATP. u is a parameter reflecting any effect which can cause an increase in the rate of decay of ψ, and is discussed below. Of course one expects $P(\psi, 0, 0, \ldots, 0) = 0$, since if all the reactions are halted, then ψ is not being produced. Elementary assumptions would suggest that $P(\psi, f_1, f_2, \ldots, f_{n+1})$ takes the form $Z(\psi)(p_1 f_1 + p_2 f_2 + \cdots + p_{n+1} f_{n+1})$. One can think of each constant $p_i \geqslant 0$ as the stoichiometry describing how many protons are pumped into the mitochondrial intermembrane space by reaction i. The function $Z(\psi)$ allows the possibility of "redox slip" (Brand et al., 1994), i.e. a changing number of protons pumped by a given reaction with changing ψ. Thus $Z(\psi)$ is either a constant or a positive but decreasing function of ψ. Although redox slip does not appear to be very important in normal circumstances (Brand et al., 1994; Canton et al., 1995), its inclusion does not affect the analysis carried out below, as the replacement of $Z(\psi)$ by a constant makes no difference to the signs of any of the quantities calculated below.

On the other hand the decay L is assumed to be strictly increasing in ψ and in u. The additional parameter u is allowed to take arbitrarily large positive values and controls the use of energy in the system. It could define the phosphorylation potential—essentially the ratio $[ADP][P_i]/[ATP]$ or alternatively it could define any quantity which increases the flow of protons down their gradient through the membrane—for example the concentration of an uncoupler (Scott and Nicholls, 1980; Joyce et al., 2003). $L(u, 0) = 0$ for all u, but $L(0, \psi) > 0$ for $\psi > 0$, reflecting the fact that even when there is no energy use, ψ decays due to passive leak effects.

8.1. Existence of a unique steady state

It is already known from the analysis of the situation without feedback that for any fixed values of z, w and ψ the equations for the dynamics of a_i have a solution, and that treating this solution as a function of ψ gives $f_1(z, a_1(\psi), \psi) = f_2(a_1(\psi), a_2(\psi), \psi) = \cdots = f_{n+1}(a_n(\psi), w, \psi) \equiv R(\psi)$. Moreover, $R(\psi)$ is a strictly decreasing function of ψ which approaches 0 as $\psi \to \infty$. It is helpful now and for arguments later to define $Q \equiv \sum_{i=1}^{n+1} p_i$. For non-trivial feedback there is at least one $p_i \neq 0$, and so $Q > 0$. From now on this is assumed, as the case with all $p_i = 0$ is simply the case without feedback which has already been analysed.

For fixed $u = \overline{u}$, a steady state in the full model now exists as long as there is a ψ which satisfies:

$$\tilde{P}(\psi) \equiv Z(\psi)R(\psi)Q = L(\overline{u}, \psi). \tag{73}$$

Looking first at the left hand side of this equation, the fact that $R(\psi)$ is strictly decreasing and $Z(\psi)$ is decreasing or constant gives

$$\frac{d\tilde{P}}{d\psi} = \left(\frac{dZ}{d\psi}R(\psi) + \frac{dR}{d\psi}Z(\psi)\right)Q < 0. \tag{74}$$

Eq. (73) also gives that $\tilde{P}(0) \neq 0$ (since $R(0) \neq 0$), and $\lim_{\psi\to\infty} \tilde{P}(\psi) = 0$ (since $\lim_{\psi\to\infty} R(\psi) = 0$—see Section 7.2).

On the right hand side of Eq. (73), $L(\overline{u}, \psi)$ is strictly increasing in ψ, with $L(\overline{u}, 0) = 0$. These facts combine to provide a familiar situation where the IVT can be used.

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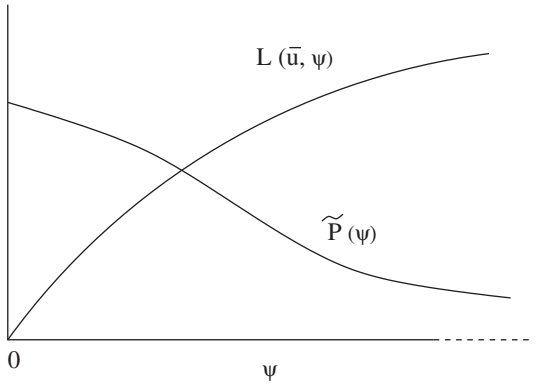

Fig. 6. There is always a unique solution to the equation $\tilde{P}(\psi) = L(\overline{u}, \psi)$ for any fixed $\overline{u}$.

Although the interval is no longer bounded, the fact that $\lim_{\psi\to\infty}\tilde{P}(\psi) = 0$ means that for some large enough $\overline{\psi}$, $\tilde{P}(\overline{\psi}) - L(\overline{u}, \overline{\psi}) < 0$—see Fig. 6. Because $\tilde{P}(\overline{\psi}) - L(\overline{u}, \psi)$ is monotonic the steady state defined by this equation is unique. Thus in the model with feedback the unique steady state survives.

8.2. Variation of the steady state with u

Of interest in this section is how the position of the steady state varies with changes in the value of the usage parameter u. At any value of u there is a value $\psi(u)$ which solves $\tilde{P}(\psi(u)) = L(u, \psi(u))$. Differentiating this equation with respect to u gives

$$\frac{\mathrm{d}\psi}{\mathrm{d}u}\left(\frac{\mathrm{d}Z}{\mathrm{d}\psi}R(\psi) + \frac{\mathrm{d}R}{\mathrm{d}\psi}Z(\psi)\right)Q = \frac{\partial L}{\partial u} + \frac{\partial L}{\partial \psi}\frac{\mathrm{d}\psi}{\mathrm{d}u}, \tag{75}$$

from which

$$\frac{\mathrm{d}\psi}{\mathrm{d}u} = \frac{\frac{\partial L}{\partial u}}{\left(\frac{\mathrm{d}Z}{\mathrm{d}\psi}R(\psi) + \frac{\mathrm{d}R}{\mathrm{d}\psi}Z(\psi)\right)Q - \frac{\partial L}{\partial \psi}}. \tag{76}$$

The top of this fraction is positive and the bottom is negative, so ψ decreases as u increases, as one might expect. Thus the rate $R(\psi(u))$ increases with u since $\mathrm{d}R/\mathrm{d}u = (\mathrm{d}R/\mathrm{d}\psi)(\mathrm{d}\psi/\mathrm{d}u)$ and both terms in the product are negative. Depending on the experimental situation to be modelled $\partial L/\partial u$ might describe the sensitivity of Complex V to changes in the phosphorylation potential, or alternatively how the leak current changes with the addition of uncouplers. $\partial L/\partial\psi$ contains information about how the leak current and the activity of Complex V change with changes in the proton gradient.

Analysis of the model without feedback (Section 7.1) has already defined what can and cannot be said about how steady state values of the a_i change with ψ. All of this analysis carries over immediately. Thus in general although changes in u cause predictable changes in ψ, they cause unpredictable changes in the values of a_i.

In sum:

(1) As u increases, ψ decreases, but one cannot in general predict the effect on the redox states of ETC intermediates.
(2) In the special situation where some initial/final set of the $f_{i\psi}$ are ordered with respect to the rest, the effect of u on these terminal redox reactions can be predicted.
(3) Increased u, by decreasing ψ, will always increase the flux through the ETC.

The parameter u is thus a reasonable representation of the effect, say, of increased ADP/ATP ratio during functional activation. Of course, the generic model does not account for other effects which a changed level of ADP might have external to the chain such as the activation of glycolysis or the TCA cycle.

8.3. Variation of the steady state with z and w

The next task is to ask whether the conclusions reached about the effect of changes in z and w continue to hold in the full model. As before, the vector form of the ODE system is most convenient:

$$\dot{\mathbf{a}} = \mathbf{F}(\mathbf{a}, z, w, u), \tag{77}$$

where $\mathbf{a} = (a_1, a_2, \ldots, a_n, \psi)^T$, and $\mathbf{F} = (-f_1 + f_2, \ldots, -f_n + f_{n+1}, P(\psi, f_1, f_2, \ldots, f_{n+1}) - L(u, \psi))^T$.

Differentiating $0 = \mathbf{F}(\mathbf{a}, z, w, u)$ with respect to z at the solution gives the linear system

$$0 = \mathbf{J}_\mathbf{F}\frac{\mathrm{d}\mathbf{a}}{\mathrm{d}z} + \frac{\partial \mathbf{F}}{\partial z}. \tag{78}$$

As before $g_i = (\mathbf{J}_\mathbf{F}(\mathrm{d}\mathbf{a}/\mathrm{d}z))_i$, $x_i \equiv \mathrm{d}a_i/\mathrm{d}z$, with the added quantity $x_\psi \equiv \mathrm{d}\psi/\mathrm{d}z$. Now

$$\partial\mathbf{F}/\partial z = (-f_{1z}, 0, \ldots, 0, p_1 Z(\psi) f_{1z})^T.$$

It can now be shown that just as for the system without feedback the steady state flux increases with z and the steady state values of each a_i decrease with z. Further, the steady state value of ψ increases with z.

Fixing w and u, at steady state $f_1(z, a_1(z), \psi(z)) = \cdots = f_{n+1}(a_n(z), w, \psi(z)) = R(z)$ and $QZ(\psi(z))R(z) - L(\psi(z), u) = 0$. Differentiating this equation with respect to z gives

$$Q\left(Z(\psi(z))\frac{\mathrm{d}R}{\mathrm{d}z} + R(z)\frac{\mathrm{d}Z}{\mathrm{d}\psi}x_\psi\right) - \frac{\partial L}{\partial \psi}x_\psi = 0, \tag{79}$$

i.e.

$$QZ(\psi(z))\frac{\mathrm{d}R}{\mathrm{d}z} + \left(QR(z)\frac{\mathrm{d}Z}{\mathrm{d}\psi} - \frac{\partial L}{\partial \psi}\right)x_\psi = 0. \tag{80}$$

Examining the signs of the known quantities in this equation gives that x_ψ must have the same sign as $\mathrm{d}R/\mathrm{d}z$, and must be non-zero since $Q \neq 0$.

Next it can be shown that x_n must have the opposite sign to x_ψ. This is done by differentiating the relation

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$R(z) = f_{n+1}(a_n(z), w, \psi(z))$ to get

$$\frac{dR}{dz} = f_{n+1,n}x_n + f_{n+1,\psi}x_\psi. \tag{81}$$

Since $f_{n+1,n}$ and $f_{n+1,\psi}$ are negative and dR/dz has the same sign as x_ψ, this implies that x_n must have the opposite sign to x_ψ. Since none of the other quantities are zero $x_n \neq 0$.

The proof proceeds by assuming that $x_n > 0$ and showing that this implies a contradiction. Differentiating $R = f_n(a_{n-1}, a_n, \psi)$ gives

$$\frac{dR}{dz} = f_{n,n-1}x_{n-1} + f_{nn}x_n + f_{n,\psi}x_\psi. \tag{82}$$

The last two terms on the right hand side are both positive, implying that $x_{n-1} > 0$. Inductively, differentiating the equations $R = f_i(a_{i-1}, a_i, \psi)$ for $i = n$ down to 2, gives that all the x_i must be positive. But $R = f_1(z, a_1, \psi)$ gives:

$$\frac{dR}{dz} = f_{1z} + f_{11}x_1 + f_{1\psi}x_\psi. \tag{83}$$

From above $x_n > 0$ implies that $x_\psi < 0$ and $dR/dz < 0$. So each expression on the right-hand side is positive, while the left hand side is negative. This contradiction means that in fact $x_n < 0$, while $x_\psi > 0$ (and hence $dR/dz > 0$). An identical inductive argument now gives that $x_i < 0$ for $i = 1, \ldots, n-1$. Thus the analysis from the basic model holds true:

(1) Increasing the push z decreases the oxidation level of each element of the chain.
(2) Increasing the push z increases the flux through the system.
(3) Increasing the push z increases proton gradient.

Because of the symmetry between the effects of z and w, one can immediately add that:

(1) Increasing the oxygenation w increases the oxidation level of each element of the chain.
(2) Increasing the oxygenation w increases the flux through the system.
(3) Increasing the oxygenation w increases the proton gradient.

It is reassuring to note that the introduction of dynamics for ψ does not invalidate these basic results.

8.4. Stability of the steady state in the system with feedback

The astute reader will have noticed that there has been an omission in the analysis of the system with feedback. While, in the system without feedback it was shown that the unique steady state must be globally stable, no such argument is made for the unique steady state in the augmented system. In fact without additional assumptions it cannot be shown that the steady state must always be locally stable.

The Jacobian, $\mathbf{J_F}$ is now:

$$\begin{bmatrix} -f_{11}+f_{21} & f_{22} & \cdots & 0 & -f_{1\psi}+f_{2\psi} \\ -f_{21} & -f_{22}+f_{32} & \cdots & 0 & -f_{2\psi}+f_{3\psi} \\ \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & 0 & \cdots & -f_{nn}+f_{n+1,n} & -f_{n\psi}+f_{n+1,\psi} \\ Z(\psi)(p_1f_{11}+p_2f_{21}) & Z(\psi)(p_2f_{22}+p_3f_{32}) & \cdots & Z(\psi)(p_nf_{nn}+p_{n+1}f_{n+1,n}) & -L_\psi + Z(\psi)\sum_{i=1}^{n+1}(p_if_{i\psi}) \\ & & & & +Z_\psi\sum_{i=1}^{n+1}(p_if_i) \end{bmatrix}.$$

Here $L_\psi \equiv \partial L/\partial\psi$ and $Z_\psi \equiv \partial Z/\partial\psi$. The Jacobian is the original tridiagonal Jacobian augmented with a final row and column. In the case where all the p_i are small, this must have eigenvalues with negative real parts, since eigenvalues depend continuously on the entries in the matrix (which for all $p_i = 0$ are known to be negative). It is also possible to show that the above matrix can have no *real positive or zero eigenvalues*. However, this does not rule out the possibility of imaginary eigenvalues—in other words of the system undergoing a Hopf bifurcation.

In the special case where the system is two dimensional, i.e. there is only a single redox pair, it is possible to prove that the above Jacobian must have eigenvalues with negative real parts, and indeed that the unique steady state is globally stable. Unfortunately, this analysis does not carry over simply to higher dimensions.

Rather than focussing on the Jacobian, another possible angle of attack is to use results on monotone control systems (Angeli and Sontag, 2003) to study the behaviour of this system. In order to do so, one would need to treat the original system without feedback (Eqs. (1)–(3)) as a system with "input" ψ and "output" $\sum_{i=1}^{n+1} p_i f_i$. However, an immediate problem arises: although the system is cooperative at fixed values of the parameters, it is monotone neither in its inputs nor in its outputs. No reco-ordinatisations have yet been found which will make the system monotone in its inputs and outputs.

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9. Discussion and conclusions

In this paper a generic model of electron transport and oxidative phosphorylation has been explored. To discover patterns in the behaviour of numerical models such as those in Korzeniewski and Zoladz (2001) and Magnus and Keizer (1997) generally requires numerous simulations. The aim has been not to provide an alternative to these models, but rather to discover what patterns if any we might expect *a priori* arising solely from certain structures in such models.

The main conclusion is that much of the classical behaviour of the ETC arises directly out of the basic structure of the interactions in the chain. It is possible to predict qualitative changes in flux rates and redox states in response to key inputs such as changes in supply, demand and oxygenation. In particular it has been shown that flux increases in response to increased supply, demand or oxygenation, and that chain intermediates generically become more reduced in response both to increased supply and to hypoxia. These results can be interpreted as the signs of entries in a sensitivity matrix as shown in Fig. 7.

We have also seen interesting limiting behaviour in response to large supply or oxygenation. Rate saturation arises from the structure of the reaction scheme, rather than the detailed dynamics or assumptions about the enzymatic nature of any of the reactions. Moreover in the limit of large supply or oxygenation, ETC intermediates do not become completely reduced or oxidised, with the possible exception of an initial or final element in the chain. The fact that one can reach conclusions about saturation shows that at least sometimes it is possible to reach semi-quantitative conclusions from a qualitative model.

One further result is that demand, acting via changes in ψ, is a more complicated stimulus than supply or oxygenation. Increases in demand generically increase flux, but may give rise to unpredictable changes in redox states of the intermediates in the chain. In order to predict the precise changes further information is needed on the relative reaction rates of the different redox reactions, and the comparative extent to which they are influenced by the proton gradient.

	flux	redox state
substrate supply	+	-
oxygen supply	+	+
energy demand	+	±

Fig. 7. Sensitivity matrix showing the response of steady state flux through the ETC and redox states of model intermediates to increases in substrate supply, oxygen supply and energy demand.

Understanding the scope of the model is crucial to being able to interpret results presented in this paper. All processes external to the ETC and oxidative phosphorylation system are external to the model. Thus for example, the quantity u cannot be seen as a generalised measure of increase in demand in vivo. Increased workload, for example in muscle tissue during exercise, generally gives rise to diffuse changes, such as increased calcium concentration, which are able to activate a number of enzymes external to the ETC. To understand the true effects of an increase in demand on electron transport in vivo, one would need to allow for the possibility that at least two parameters—u and z in the full model—change simultaneously, with possibly ambiguous effects on redox states.

Bearing this in mind, it is possible to infer other *negative* results from the arguments in this paper. Consider a situation of ischaemia—i.e. a decrease in blood flow. This would cause a decrease in supply and in oxygenation. Both of these would cause a decrease in flux, but would have opposite effects on the oxidation states of ETC intermediates. Thus the qualitative effect of ischaemia on redox states in the ETC is not predictable from the generic model and would require a more detailed model.

Finally, it has been shown that many of the same conclusions hold both for a simple model in which the proton gradient features as a controllable parameter and in the more complex case where proton gradient is a variable itself created by the various reactions of the chain and tending to decay. Most importantly, there is a unique steady state in both cases. However, the full conditions which will ensure that the unique steady state must be stable in the model with feedback have not been defined. This is a task for future work. There are numerous other potential extensions of this work, including:

(1) Exploring the effects of activators and inhibitors of ETC proteins with a view to finding out whether their effects on redox states have fixed sign: this would complete the qualitative sensitivity analysis of the generic model.
(2) Adding auxiliary processes such as some of the feedback processes which occur outside the ETC to find out if they can introduce instabilities into the system.
(3) Constructing and analysing a similar generic model of the TCA cycle.

The final two extensions would involve analysing models with a more complex structure including branches and loops. The results in Section 8 on the model with feedback show that although it may not be possible to prove the existence of a globally stable steady state in such models, if such a steady state is assumed to exist then it may still be possible to carry out a sensitivity analysis and arrive at definite conclusions.

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Appendix A. Calculating eigenvalues of the Jacobian in the simple model

In this appendix it is shown that the eigenvalues of $\mathbf{J_F}$ for the system without feedback have negative real parts—everywhere, not just at the fixed point. First note that $\mathbf{J_F}$ is a tridiagonal sign-symmetric matrix and hence has real eigenvalues (Parter and Youngs, 1962). There are a number of equivalent ways of showing that all the eigenvalues must in fact be negative. One simple method is to show directly that the determinant of $\mathbf{J_F}-\lambda\mathbf{I}$ cannot be zero for any real non-negative λ.

This is done by triangularising the matrix $\mathbf{J_F}-\lambda\mathbf{I}$. Let $R(i)$ refer to the ith row of $\mathbf{J_F}-\lambda\mathbf{I}$ and define

$$\delta_1=\frac{-f_{21}}{\lambda-f_{21}+f_{11}}, \tag{A.1}$$

$$\delta_i=\frac{-f_{i+1,i}}{-f_{i+1,i}+(1-\delta_{i-1})f_{ii}+\lambda}, \quad i=2,\ldots,n-1. \tag{A.2}$$

For $\lambda\geqslant 0$, δ_1 satisfies $0<\delta_1<1$, and it follows inductively that $0<\delta_i<1$ for all i.

$\mathbf{J_F}-\lambda\mathbf{I}$ can be converted to upper triangular form by sequentially performing the elementary row operations:

$$R(i)\rightarrow R(i)+\delta_{i-1}R(i-1), \quad i=2,\ldots,n. \tag{A.3}$$

This triangularisation gives the matrix

$$\begin{bmatrix} -f_{11}+f_{21}-\lambda & f_{22} & \cdots & 0 \\ 0 & (-1+\delta_1)f_{22}+f_{32}-\lambda & \cdots & 0 \\ \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & \cdots & (-1+\delta_{n-1})f_{nn}+f_{n+1,n}-\lambda \end{bmatrix}.$$

The determinant of this matrix is simply the product of the diagonal elements. It is immediate that for $\lambda\geqslant 0$ all of the diagonal elements are strictly negative. Thus the determinant is non-zero. Since elementary row operations preserve the determinant, this proves that the determinant of $\mathbf{J_F}-\lambda\mathbf{I}$ cannot be zero for real non-negative λ, and hence that all eigenvalues of $\mathbf{J_F}$ are strictly negative.

The above argument of course also implies that $|\mathbf{J_F}|\neq 0$ and hence that $\mathbf{J_F}$ is invertible.

Appendix B. Behaviour on the boundary of the phase space

In this appendix it is shown that no complete trajectory of system (1)–(3) with non-zero z and w can lie on the boundary of the phase space. A point on the boundary must satisfy at least one of the $2n$ equations:

$$a_i=0, \quad i=1,\ldots,n, \tag{B.1}$$

$$a_i=a_{i,max}, \quad i=1,\ldots,n. \tag{B.2}$$

Treating the surface defined by the first n equations first, note that for $w\neq 0$, $\dot{a}_n>0$ on $a_n=0$. Thus any trajectory starting on the surface $a_n=0$ immediately leaves it. One can check that if $a_k\neq 0$, then $\dot{a}_{k-1}>0$ on $a_{k-1}=0$ for all $k=2,\ldots,n$.

Similarly for the surface defined by the final n equations, note that for $z\neq 0, \dot{a}_1<0$ on $a_1=a_{1,max}$. Thus any trajectory starting on the surface $a_1=a_{1,max}$ immediately leaves it. One can check that if $a_k\neq a_{k,max}$, then $\dot{a}_{k+1}<0$ on $a_{k+1}=a_{k+1,max}$ for all $k=1,\ldots,n-1$.

Thus for non-zero w and z any trajectory starting on the boundary of phase space immediately leaves it and enters the interior of the phase space.

Appendix C. Special cases where changes in demand have a predictable effect on redox states

In this appendix the proof of results stated in Section 7.1 is sketched. The first case where

$$|f_{i\psi}|\leqslant|f_{j\psi}| \quad \text{for all } j>i, \ \ i=1,\ldots,i_{max} \tag{C.1}$$

is treated, omitting several details since the arguments are similar to those in Section 5.2. In outline:

(1) $x_1\leqslant 0$ (because $x_1>0\Rightarrow x_n>0\Rightarrow x_1<0$—a contradiction)
(2) $x_i\leqslant 0\Rightarrow x_{i+1}\leqslant 0$ for $i=1,\ldots,i_{max}-1$.

First, $x_1>0$ implies a contradiction. Assume $x_1>0$. Adding all the components in Eq. (49) together gives:

$$\sum_{i=1}^{n} g_i=-f_{11}x_1+f_{n+1,n}x_n=f_{1\psi}-f_{n+1,\psi}\geqslant 0. \tag{C.2}$$

Since $f_{11}>0$ and $f_{n+1,n}<0$, this implies that at least one of x_1 or x_n is less than or equal to 0. Since $x_1>0$, then $x_n<0$.

The argument can be continued by noting that

$$\sum_{i=1}^{k-1} g_i=-f_{11}x_1+f_{k,k-1}x_{k-1}+f_{k,k}x_k=f_{1\psi}-f_{k\psi}\geqslant 0. \tag{C.3}$$

If $x_1>0$ and $x_k<0$, then $x_{k-1}<0$. Continuing, this leads to the conclusion that $x_1<0$ which contradicts the premise

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that $x_1 > 0$. Thus $x_1 \leqslant 0$. In fact, in the above argument if there is just one $|f_{j\psi}|$ greater than $|f_{1\psi}|$, then this rules out the possibility that $x_1 = 0$ and the result becomes strict, that is $x_1 < 0$.

If $2 \leqslant i_{max}$, an identical procedure is carried out, except now summing from the second equation onwards

$$\sum_{i=2}^{n} g_i = -f_{21}x_1 + f_{32}x_2 + f_{n+1,n}x_n = f_{2\psi} - f_{n+1,\psi} \geqslant 0. \quad \text{(C.4)}$$

It has already been shown that $x_1 \leqslant 0$ with $x_1 = 0$ if and only if all the x_i are zero and all the $f_{i\psi}$ are equal. Ignoring this possibility it is assumed that $x_1 < 0$. Then the above inequality becomes

$$f_{32}x_2 + f_{n+1,n}x_n > 0. \quad \text{(C.5)}$$

Clearly either $x_2 < 0$ or $x_n < 0$. Now a very similar chain of argument to that above leads to the conclusion that $x_2 < 0$ barring the trivial possibility that all $f_{i\psi}$ are equal and all x_i are zero. The argument continues in this way showing that in the non-trivial case, $x_i < 0$ for all $i \leqslant i_{max}$.

The second case is nearly identical. The only difference is that now the indices to be summed over are reversed. The conclusion in this case is that $x_i \geqslant 0$ for all $i \geqslant i_{min}$, with the inequality being strict unless all the $f_{i\psi}$ are equal and hence all the x_i are zero.

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