UNIDIRECTIONAL CALCIUM AND NUCLEOTIDE FLUXES IN SARCOPLASMIC RETICULUM

I. Interpretation of Flux Ratios for Different Reaction Schemes

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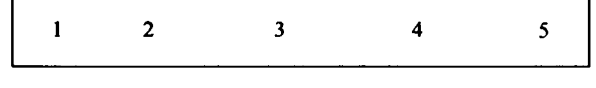
ABSTRACT The relation between unidirectional calcium and nucleotide fluxes was examined for different ATPase reaction schemes of the sarcoplasmic reticulum. The schemes considered differed in the order of sorption and desorption of calcium, ATP, and ADP. The results suggest that the theoretical relation between calcium and nucleotide fluxes depends on the reaction scheme and that experimental measurements can distinguish among them. The results obtained are generally valid and do not depend on assumptions of equilibrium or pseudoequilibrium between intermediate states of the pump.

INTRODUCTION

Although it is generally acknowledged that calcium transport in sarcoplasmic reticulum (SR) is intimately associated with activity of its Ca + Mg ATPase (Hasselbach and Makinose, 1961; Ebashi and Lipmann, 1962), there remains some uncertainty about the precise mechanism of coupling of calcium transport to ATPase activity. Two possible views have recently been reported. In one view, the driving force for transport is provided by a change in the structure of water within a narrow cleft of the calcium pump (Wiggins, 1982). The change in the structure of water would increase the activity of the calcium ion such that it could diffuse downhill towards a region of high calcium activity upon the opening of a selective channel. In an alternate view, active transport involves a pump having a different affinity for its transported ion when the binding site is accessible to different sides of the membrane (Inesi et al., 1982; Giumaraes-Motta and DeMeis, 1980; Inesi et al., 1980; Takakuwa and Kanazawa, 1982). The essential difference between these views is that selectivity is provided by a gated channel in the first view and by a binding site in the second, and that free-energy transduction is provided through water in the first and through protein conformation in the second. In the first view, the ATPase would alter the activity of a variety of ions and there is some evidence for this (Wiggins, 1980). However, the Ca + Mg ATPase from skeletal SR is known to bind calcium cooperatively and with high affinity (Inesi et al., 1980; Verjovski-Almeida and Silva, 1981). The ATPase can exist in several phosphorylated forms (Yamada and Ikemoto, 1980) that differ in their sensitivity to ADP and in ion binding. The phosphoenzyme formed first in the reaction mechanism is denoted E_1P and is sensitive to ADP in the sense that addition of ADP causes a rapid disappearance of E_1P and formation of ATP, (Takisawa and Tonomura, 1979; Shigekawa and Akowitz, 1979; Takakuwa and Kanazawa, 1979). This reaction is the nucleoside diphosphokinase reaction. The E_1P possesses a high affinity for calcium in the external medium (Takakuwa and Kanazawa, 1981). The second phosphoenzyme, E_2P, cannot form ATP upon ADP addition and can be converted to E_1P provided the calcium concentration is high (Shigekawa and Dougherty, 1978). Nakamura and Tonomura (1982) have shown that the formation of [^{32}P]ATP from ADP and [^{32}P]EP increases with calcium with an apparent affinity of 0.3 mM. Takakuwa and Kanazawa (1979) observed that the ADP-sensitive phosphoenzyme (E_1P) was slowly converted to the ADP-insensitive form (E_2P) by chelation of calcium, and ADP sensitivity was restored by addition of calcium. More recently, Takisawa and Makinose (1983) have shown a high correlation between occluded calcium and the amount of E_1P. The stoichiometry suggests that two calcium atoms are bound to each molecule of E_1P. All of the authors in the foregoing discussion are in agreement that ADP desorbs from the pump before calcium desorption on the inner surface of the membrane. This conclusion can be reached for cardiac SR as well by comparing the results of Dupont (1980) for skeletal SR with those of Sumida et al. (1978) for cardiac SR. The following minimal reaction scheme is compatible with these observations.



Scheme I

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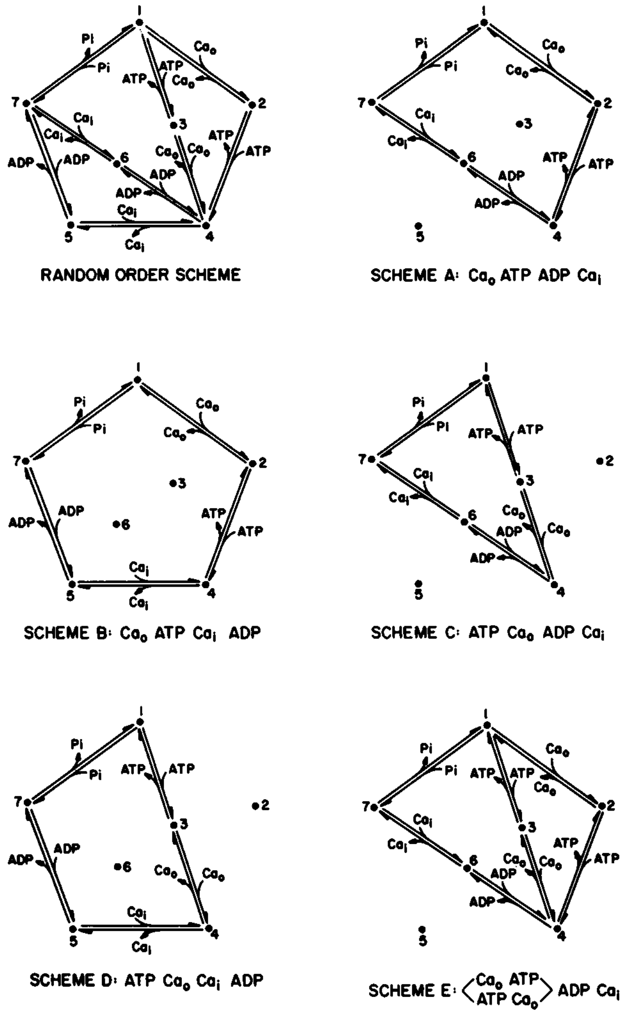
It was found that ATP-ADP exchange is 4 to 10 times faster than the rate of calcium exchange (Waas and Hasselbach, 1981; Takenaka et al., 1982), while GTP-GDP exchange approximately matched calcium exchange (Ronzani et al., 1979). Ronzani et al. (1979) therefore called into question the coupling of nucleotide diphosphokinase activity and calcium translocation. Takenaka and co-workers (1982) conclude "... that most of the ATP⇌ADP exchange is not coupled with calcium movement." We disagree with this statement because the concept of coupling is being inappropriately applied. Because nucleotide exchange takes place over a set of intermediates different from those involved in calcium exchange, it follows that nucleotide exchange will occur at a different rate. In particular, calcium exchange takes place over a set of intermediates that encompass the intermediates participating in nucleotide exchange. In this case (Scheme I) nucleotide exchange must be greater than Ca exchange × $1/n$, where n is the number of calcium atoms transported per turnover of enzyme. If steps (*2*) and (*3*) are much faster than either steps (*1*) and (*4*), or both, then nucleotide exchange would be much faster than calcium exchange.

In an ATP-driven pump, coupling is a concept that relates flow of chemical reaction to flow of ion across the membrane. The important observables are the net and unidirectional flows for the reaction and for the ion. If coupling of calcium transport can be described by a reaction scheme such as that in Scheme I, then the ratios of unidirectional fluxes will be defined by the scheme. Because our observations on cardiac SR suggest a different relation between nucleotide and calcium exchanges than in skeletal SR, we have investigated the relation between nucleotide and calcium fluxes for different reaction schemes. The results suggest that the unidirectional fluxes can be used to distinguish different reaction schemes.



RANDOM ORDER SCHEME

SCHEME A: Ca_o ATP ADP Ca_i

SCHEME B: Ca_o ATP Ca_i ADP

SCHEME C: ATP Ca_o ADP Ca_i

SCHEME D: ATP Ca_o Ca_i ADP

SCHEME E: $\left\langle\begin{matrix}\text{Ca}_o & \text{ATP}\\ \text{ATP} & \text{Ca}_o\end{matrix}\right\rangle$ ADP Ca_i

FIGURE 1 Possible simplified reaction schemes for the SR Ca-ATPase. The reaction schemes differ in their order of sorption and desorption of extravesicular calcium (Ca_o), intravesicular calcium (Ca_i), ATP, and ADP. Schemes *A–E* represent various simplifications of the random-order scheme.

Reaction Schemes

The reaction schemes we will consider are shown in Fig. 1. These schemes differ in their order of binding of ATP, Ca_o, Ca_i, and ADP. In the random-order scheme, (Fig. 1) Ca_o and ATP bind in random order and ADP and Ca_i desorb in random order. All of the other schemes correspond to the deletion of one or more branches of this random-order scheme.

In these schemes the pump is viewed as an enzyme that can exist in some finite number of different states. States of the pump may be distinguished by a particular conformation, association with a ligand or a particular chemical state, such as being phosphorylated. Ligands may also be viewed as being distributed among a variety of states, such as being bound to intermediates of the pump or being free within an enclosed or excluded aqueous compartment. Transitions between adjacent intermediates may require ligand binding or desorption or some conformational or chemical change. The mechanism of the pump in this paradigm is explained by a description of the intermediates and the rate constants, which describe transitions between intermediates. An example of a transition between two adjacent states is

$$\underset{(1)}{\text{E}} \underset{\text{Ca}_o}{\overset{\text{Ca}_o}{\rightleftharpoons}} \underset{(2)}{\text{E Ca}_o}, \tag{1}$$

which describes the binding of Ca to E when read from left to right and desorption of Ca when read in the opposite direction. The fluxes of E and Ca in Eq. 1 are

$$J_{\text{lr}} = k_{\text{lr}}\,[\text{Ca}_o]\,[\text{E}] \tag{2}$$

$$J_{\text{rl}} = k_{\text{rl}}\,[\text{E Ca}], \tag{3}$$

where J_{lr} is the flux for the sorption reaction and J_{rl} is the flux for the desorption reaction.

Calcium flux in SR can be measured only when calcium is removed from the outside compartment. In the schemes of Fig. 1, calcium flux corresponds to a flow of pump units over a set of several intermediates. Similarly, ATP-ADP exchange occurs only when the pump completes a transition over a set of intermediates that begins with sorption of ATP or ADP and ends in desorption of ADP or ATP, respectively.

Unidirectional Fluxes Over Several Intermediates

Consider only three consecutive states in a reaction mechanism as shown below

$$\xrightarrow{J_{net}} \underset{1}{\cdot} \underset{J_{21}}{\overset{J_{12}}{\rightleftharpoons}} \underset{2}{\cdot} \underset{J_{32}}{\overset{J_{23}}{\rightleftharpoons}} \underset{3}{\cdot} \xrightarrow{J_{net}} \tag{4}$$

where J_{ij} is the flux from state i to state j and $J_{ij} = \alpha_{ij}N_i$, where N_i is the population of state i and α_{ij} is the pseudo-first-order rate constant that incorporates the concentration of ligand being bound, if any, in the transition $N_i \rightharpoonup N_j$. By steady state we mean $dN_i/dt = 0$. The unidirectional flux from N_1 to N_3 is (Stein, 1976; Gregariou, 1981)

$$J_{1\rightarrow 3} = \frac{J_{12} \cdot J_{23}}{J_{21} + J_{23}}, \tag{5}$$

where the arrow denotes a flux between more than two adjacent states. In this relation, the unidirectional flux is corrected for the flux that reaches state 2 and then returns to state 1. The unidirectional flux from state 1 to state 3 is the flux from state 1 to state 2 times the fraction of this flux that reaches state 3. From the definition of J_{ij}, Eq. (5) can be rewritten as

$$J_{1\rightarrow 3} = \frac{\alpha_{12}N_1 \cdot \alpha_{23}N_2}{\alpha_{21}N_2 + \alpha_{23}N_2} \tag{6}$$

$$J_{1\rightarrow 3} = \frac{\alpha_{12} \cdot \alpha_{23}}{\alpha_{21} + \alpha_{23}} N_1. \tag{7}$$

The combined constant

$$\alpha' = \frac{\alpha_{12} \cdot \alpha_{23}}{\alpha_{21} + \alpha_{23}} \tag{8}$$

is equivalent to the net rate constant described by Cleland (1975) except it is not necessary to assume a unidirectional step in which the reverse rate constant is zero. Unidirectional flux in the reverse direction is

$$J_{3\rightarrow 1} = \frac{J_{32} \cdot J_{21}}{J_{21} + J_{23}}. \tag{9}$$

If flux occurs over a longer set of intermediates, as shown in Eq. 10, then we apply Eq. 5 twice

$$\xrightarrow{J_{net}} \underset{1}{\cdot} \underset{J_{21}}{\overset{J_{12}}{\rightleftharpoons}} \underset{2}{\cdot} \underset{J_{32}}{\overset{J_{23}}{\rightleftharpoons}} \underset{3}{\cdot} \underset{J_{43}}{\overset{J_{34}}{\rightleftharpoons}} \underset{4}{\cdot} \xrightarrow{J_{net}} . \tag{10}$$

The result is

$$J_{1\rightarrow 4} = \frac{J_{1\rightarrow 3} \cdot J_{34}}{J_{3\rightarrow 1} + J_{34}} \tag{11}$$

$$J_{4\rightarrow 1} = \frac{J_{3\rightarrow 1} \cdot J_{43}}{J_{3\rightarrow 1} + J_{34}}. \tag{12}$$

Insertion of $J_{1\rightarrow 3}$ and $J_{3\rightarrow 1}$ from Eqs. 5 and 9 gives

$$J_{1\rightarrow 4} = \frac{J_{12} \cdot J_{23} \cdot J_{34}}{J_{32} \cdot J_{21} + J_{21} \cdot J_{34} + J_{23} \cdot J_{34}} \tag{13}$$

$$J_{4\rightarrow 1} = \frac{J_{43} \cdot J_{32} \cdot J_{21}}{J_{32} \cdot J_{21} + J_{21} \cdot J_{34} + J_{23} \cdot J_{34}}. \tag{14}$$

For the simple fluxes in a linear sequence, we can stepwise apply the relation

$$J_{1\rightarrow n} = \frac{J_{1\rightarrow n-1} \cdot J_{n-1,n}}{J_{n-1\rightarrow 1} + J_{n-1,n}} \tag{15}$$

to obtain the unidirectional flux.

The fluxes given by Eqs. 13 and 14 correspond to fluxes of the pump. That is, $J_{1\rightarrow 4}$ gives the rate at which pump units at state 1 complete a trip to state 4. If we incorporate a ligand binding at the transition $N_1 \rightharpoonup N_2$ and desorption of ligand at transition $N_3 \rightharpoonup N_4$, as shown below

$$\longrightarrow \underset{1}{\cdot} \underset{L_o}{\overset{L_o}{\rightleftharpoons}} \underset{2}{\cdot} \rightleftharpoons \underset{3}{\cdot} \underset{L_i}{\overset{L_i}{\rightleftharpoons}} \underset{4}{\cdot} \longrightarrow \tag{16}$$

then $J_{1\rightarrow 4}$ is also the unidirectional flux of L_o to L_i. Flux of ligands is thereby related to the flux of pump units through their available states.

Ratio of Unidirectional Fluxes

The ratio of unidirectional fluxes from Eqs. 13 and 14 is

$$\frac{J_{1\rightarrow 4}}{J_{4\rightarrow 1}} = \frac{J_{12} \cdot J_{23} \cdot J_{34}}{J_{43} \cdot J_{32} \cdot J_{21}} \tag{17}$$

substituting in for $J_{ij} = \alpha_{ij} N_i$, we have

$$\frac{J_{1\rightarrow 4}}{J_{4\rightarrow 1}} = \frac{\alpha_{12} \cdot \alpha_{23} \cdot \alpha_{34}}{\alpha_{43} \cdot \alpha_{32} \cdot \alpha_{21}} \frac{N_1}{N_4}. \tag{18}$$

Only the population of states at the ends of the exchange sequence appear in the equation. If N_1 and N_4 are in pseudoequilibrium, then the ratio of unidirectional fluxes will be constant. The unidirectional fluxes are related to J_{net} by

$$J_{1\rightarrow 4} - J_{4\rightarrow 1} = J_{net} \tag{19}$$

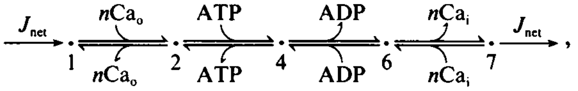
so that

$$\frac{J_{1\rightarrow 4}}{J_{4\rightarrow 1}} = \frac{J_{net} + J_{4\rightarrow 1}}{J_{4\rightarrow 1}} > 1.0 \tag{20}$$

provided J_{net} is greater than zero.

Evaluation of Binding Sequences

Single cycle Scheme A in Fig. 1 invokes the sorption and desorption sequence shown in Eq. 21



$$\xrightarrow{J_{net}} \underset{1}{\cdot} \underset{nCa_o}{\overset{nCa_o}{\rightleftharpoons}} \underset{2}{\cdot} \underset{ATP}{\overset{ATP}{\rightleftharpoons}} \underset{4}{\cdot} \underset{ADP}{\overset{ADP}{\rightleftharpoons}} \underset{6}{\cdot} \underset{nCa_i}{\overset{nCa_i}{\rightleftharpoons}} \underset{7}{\cdot} \xrightarrow{J_{net}}, \quad (21)$$

Scheme A

where n calcium atoms are viewed as binding simultaneously in a single step. This is not true of skeletal SR (Inesi et al., 1980; Verjovski-Almeida and Silva, 1981). The consequence of this assumption will be discussed later. The unidirectional calcium influx (Jf), unidirectional calcium efflux (Jr), unidirectional forward nucleotide flux (JF), and unidirectional reverse nucleotide flux (JR) are given as

$$Jf = nJ_{1\rightarrow 7} \qquad (22)$$

$$Jr = nJ_{7\rightarrow 1} \qquad (23)$$

$$JF = J_{2\rightarrow 6} \qquad (24)$$

$$JR = J_{6\rightarrow 2}, \qquad (25)$$

where $J_{1\rightarrow 7}$, $J_{7\rightarrow 1}$, $J_{2\rightarrow 6}$, and $J_{6\rightarrow 2}$ are the fluxes of pump units between the states noted. Stepwise application of Eq. 15 gives

$$Jf = \frac{nJ_{12} \cdot J_{24} \cdot J_{46} \cdot J_{67}}{J_{24} \cdot J_{46} \cdot J_{67} + J_{64} \cdot J_{42} \cdot J_{21} + J_{42} \cdot J_{21} \cdot J_{67} + J_{46} \cdot J_{67} \cdot J_{21}} \qquad (26)$$

$$Jr = \frac{nJ_{76} \cdot J_{64} \cdot J_{42} \cdot J_{21}}{J_{24} \cdot J_{46} \cdot J_{67} + J_{64} \cdot J_{42} \cdot J_{21} + J_{42} \cdot J_{21} \cdot J_{67} + J_{46} \cdot J_{67} \cdot J_{21}} \qquad (27)$$

$$JF = \frac{J_{24} \cdot J_{46}}{J_{46} + J_{42}} \qquad (28)$$

$$JR = \frac{J_{64} \cdot J_{42}}{J_{46} + J_{42}}. \qquad (29)$$

These may also be written as

$$Jf = \frac{nJ_{12} \cdot J_{2\rightarrow 6} \cdot J_{67}}{J_{6\rightarrow 2} \cdot J_{21} + J_{21} \cdot J_{67} + J_{2\rightarrow 6} \cdot J_{67}} \qquad (30)$$

$$JF = J_{2\rightarrow 6} \qquad (31)$$

$$Jr = \frac{nJ_{76} \cdot J_{6\rightarrow 2} \cdot J_{21}}{J_{6\rightarrow 2} \cdot J_{21} + J_{21} \cdot J_{67} + J_{2\rightarrow 6} \cdot J_{67}} \qquad (32)$$

$$JR = J_{6\rightarrow 2} \qquad (33)$$

so the apparent coupling ratios are

$$\frac{Jf}{JF} = \frac{nJ_{12} \cdot J_{67}}{J_{6\rightarrow 2} \cdot J_{21} + J_{21} \cdot J_{67} + J_{2\rightarrow 6} \cdot J_{67}} \qquad (34)$$

$$\frac{Jr}{JR} = \frac{nJ_{76} \cdot J_{21}}{J_{6\rightarrow 2} \cdot J_{21} + J_{21} \cdot J_{67} + J_{2\rightarrow 6} \cdot J_{67}}. \qquad (35)$$

Substituting in $J_{21} = J_{12} - J_{net}$ and $J_{2\rightarrow 6} = J_{6\rightarrow 2} + J_{net}$, we derive

$$\frac{Jf}{JF} = n\frac{J_{12} \cdot J_{67}}{J_{6\rightarrow 2}(J_{21} + J_{67}) + J_{12} \cdot J_{67}} \qquad (36)$$

since $J_{6\rightarrow 2}\,(J_{21} + J_{67}) > 0$, because by definition all J_{ij} and $J_{i\rightarrow j} \geq 0$, it follows that

$$\frac{Jf}{JF} < n. \qquad (37)$$

Similarly,

$$\frac{Jr}{JR} = n\frac{J_{21} \cdot J_{76}}{J_{2\,6}(J_{12} + J_{76}) + J_{21} \cdot J_{76}} < n. \qquad (38)$$

The ratio of these coupling ratios, from Eqs. 34 and 35, is

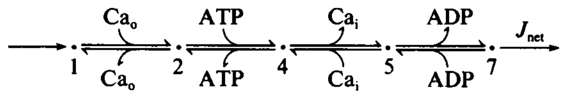
$$\frac{(Jf/JF)}{(Jr/JR)} = \frac{J_{12} \cdot J_{67}}{J_{21} \cdot J_{76}}, \qquad (39)$$

which can be rewritten as

$$\frac{(Jf/JF)}{(Jr/JR)} = \frac{J_{12}}{J_{12} - J_{net}}\frac{J_{67}}{J_{67} - J_{net}}. \qquad (40)$$

Since $J_{net} > 0$ at steady state calcium uptake and ATPase rate, reaction Scheme A predicts a ratio of unidirectional flux ratios that is greater than one.

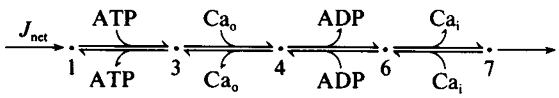
A similar procedure may be used to evaluate the consequences of reaction Schemes *B–C*, Fig. 1. The linear representation of these schemes and their predictions are given below



$$\longrightarrow \underset{1}{\cdot} \underset{Ca_o}{\overset{Ca_o}{\rightleftharpoons}} \underset{2}{\cdot} \underset{ATP}{\overset{ATP}{\rightleftharpoons}} \underset{4}{\cdot} \underset{Ca_i}{\overset{Ca_i}{\rightleftharpoons}} \underset{5}{\cdot} \underset{ADP}{\overset{ADP}{\rightleftharpoons}} \underset{7}{\cdot} \xrightarrow{J_{net}} \quad (41)$$

Scheme B

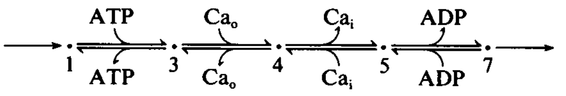
$$\frac{(Jf/JF)}{(Jr/JR)} = \frac{J_{12}}{J_{12} - J_{net}}\frac{J_{57} - J_{net}}{J_{57}} \qquad (42)$$



$$\xrightarrow{J_{net}} \underset{1}{\cdot} \underset{ATP}{\overset{ATP}{\rightleftharpoons}} \underset{3}{\cdot} \underset{Ca_o}{\overset{Ca_o}{\rightleftharpoons}} \underset{4}{\cdot} \underset{ADP}{\overset{ADP}{\rightleftharpoons}} \underset{6}{\cdot} \underset{Ca_i}{\overset{Ca_i}{\rightleftharpoons}} \underset{7}{\cdot} \longrightarrow \quad (43)$$

Scheme C

$$\frac{(Jf/JF)}{(Jr/JR)} = \frac{J_{13} - J_{net}}{J_{13}}\frac{J_{67}}{J_{67} - J_{net}} \qquad (44)$$



$$\longrightarrow \underset{1}{\cdot} \underset{ATP}{\overset{ATP}{\rightleftharpoons}} \underset{3}{\cdot} \underset{Ca_o}{\overset{Ca_o}{\rightleftharpoons}} \underset{4}{\cdot} \underset{Ca_i}{\overset{Ca_i}{\rightleftharpoons}} \underset{5}{\cdot} \underset{ADP}{\overset{ADP}{\rightleftharpoons}} \underset{7}{\cdot} \longrightarrow \quad (45)$$

Scheme D

$$\frac{(Jf/JF)}{(Jr/JR)} = \frac{J_{13} - J_{net}}{J_{13}}\frac{J_{57} - J_{net}}{J_{57}}. \qquad (46)$$

Because the pump is at steady state and $J_{net} > 0$, there is a definite prediction for $(Jf/JF)/(Jr/JR)$ for single cycle Schemes A and D. Scheme A requires that $(Jf/JF)/(Jr/JR) > 1.0$, while Scheme D requires $(Jf/JF)/(Jr/\mathrm{JR}) < 1.0$. The observed ratios can be used to rule out one of these schemes. For reaction Schemes B and C $(Jf/JF)/(Jr/JR)$ can be greater or less than 1.

State Populations

For this discussion, we will limit consideration to a single cycle containing five states, as shown for example by Scheme *A* in Fig. 1. At steady state the population of each state is constant, so we write

$$\frac{\mathrm{d}N_1}{\mathrm{d}t} = 0 = -\alpha_{12}N_1 - \alpha_{17}N_1 + \alpha_{21}N_2 + \alpha_{71}N_7 \tag{47a}$$

$$\frac{\mathrm{d}N_2}{\mathrm{d}t} = 0 = -\alpha_{21}N_2 - \alpha_{23}N_2 + \alpha_{12}N_1 + \alpha_{32}N_3 \tag{47b}$$

$$\frac{\mathrm{d}N_4}{\mathrm{d}t} = 0 = -\alpha_{42}N_4 - \alpha_{46}N_4 + \alpha_{24}N_2 + \alpha_{64}N_4 \tag{47c}$$

$$\frac{\mathrm{d}N_6}{\mathrm{d}t} = 0 = -\alpha_{64}N_6 - \alpha_{67}N_6 + \alpha_{46}N_4 + \alpha_{76}N_7 \tag{47d}$$

$$\frac{\mathrm{d}N_7}{\mathrm{d}t} = 0 = -\alpha_{76}N_7 - \alpha_{71}N_7 + \alpha_{17}N_1 + \alpha_{67}N_6. \tag{47e}$$

Four of these five relations are independent. With the conservation relation

$$N_{\mathrm{TOT}} = N_{\mathrm{i}} \tag{48}$$

we may eliminate one equation and solve for the N_{i}'s as a function of the complete set of α_{ij}'s. The solution may be obtained by linear algebra (Cornish-Bowden, 1976) or by Hill's diagram method (Hill, 1977). For reaction Scheme A from Eqs. 26–29 we have

$$\frac{Jf}{Jr} = \frac{J_{12} \cdot J_{24} \cdot J_{46} \cdot J_{67}}{J_{21} \cdot J_{42} \cdot J_{64} \cdot J_{76}} \tag{49}$$

$$\frac{JF}{JR} = \frac{J_{24} \cdot J_{46}}{J_{42} \cdot J_{64}}. \tag{50}$$

Inserting $J_{\mathrm{ij}} = \alpha_{\mathrm{ij}}N_{\mathrm{i}}$, we obtain

$$\frac{Jf}{Jr} = \frac{\alpha_{12}N_1 \cdot \alpha_{24}N_2 \cdot \alpha_{46}N_4 \cdot \alpha_{67}N_6}{\alpha_{21}N_2 \cdot \alpha_{42}N_4 \cdot \alpha_{64}N_6 \cdot \alpha_{76}N_7} = \frac{\alpha_{12} \cdot \alpha_{24} \cdot \alpha_{46} \cdot \alpha_{67}}{\alpha_{21} \cdot \alpha_{42} \cdot \alpha_{64} \cdot \alpha_{76}} \frac{N_1}{N_7} \tag{51}$$

$$\frac{JF}{JR} = \frac{\alpha_{24}N_2 \cdot \alpha_{46}N_4}{\alpha_{42}N_4 \cdot \alpha_{64}N_6} = \frac{\alpha_{24} \cdot \alpha_{46}}{\alpha_{42} \cdot \alpha_{64}} \frac{N_2}{N_6}. \tag{52}$$

The actual populations N_1, N_7, N_2, and N_6 will depend on the complete set of α_{ij}'s, which can then be inserted into Eqs. 51 and 52. The α_{ij}'s, as mentioned earlier, are pseudo-first-order rate constants that incorporate the concentration of ligand if one is bound in the transition $N_{\mathrm{i}} \rightarrow N_{\mathrm{j}}$. For Scheme A, for example, we have

$$J_{12} = \alpha_{12}N_1 = \beta_{12}[\mathrm{CAO}]N_1, \tag{53}$$

where β_{12} is a true second-order rate constant. From the relation $\alpha_{12} = \beta_{12}[\mathrm{CAO}]$, clearly the population of any state N_{i} is influenced by the concentration of all ligands. When ratios of state populations are obtained, however, certain ligand concentrations drop out of the expression.

For Schemes A–D we have obtained expressions for Jf/Jr and JF/JR. For these expressions we have assumed that $\alpha_{71} = \beta_{71}[\mathrm{Pi}] = 0$ since in our experimental observations the Pi concentration has been kept quite low and ample evidence suggests β_{71} is small. For Scheme A the result is

$$\frac{Jf}{Jr} = 1 + \frac{\beta_{71}}{\beta_{76}} \frac{\left\{\frac{\beta_{24}\beta_{46}\beta_{67}}{\beta_{42}\beta_{21}\beta_{64}}[\mathrm{ATP}] + [\mathrm{ADP}] + \frac{\beta_{67}(\beta_{46} + \beta_{42})}{\beta_{64}\beta_{42}}\right\}}{[\mathrm{ADP}]\,[\mathrm{CAI}]} \tag{54}$$

$$\frac{JF}{JR} = 1 + \frac{\frac{\beta_{71}}{\beta_{76}} \frac{\beta_{67}(\beta_{46} + \beta_{42})}{\beta_{64}\beta_{42}}}{[\mathrm{ADP}]\left([\mathrm{CAI}] + \frac{\beta_{71}}{\beta_{76}}\right)}, \tag{55}$$

which can be simplified to

$$\frac{Jf}{Jr} = 1 + \frac{k_1(k_2[\mathrm{ATP}] + [\mathrm{ADP}] + k_3)}{[\mathrm{ADP}]\,[\mathrm{CAI}]} \tag{56}$$

$$\frac{JF}{JR} = 1 + \frac{k_1k_3}{[\mathrm{ADP}]\,([\mathrm{CAI}] + k_1)}. \tag{57}$$

In an analogous way, the following expressions for Schemes B–D can be derived

Scheme B

$$Jf/Jr = 1 + \frac{k_4(k_5[\mathrm{ATP}] + k_6)}{[\mathrm{CAI}](k_1 + [\mathrm{ADP}])} \tag{58}$$

$$JF/\mathrm{JR} = 1 + \frac{k_4(k_6 + [\mathrm{CAI}])}{[\mathrm{CAI}]\,[\mathrm{ADP}]} \tag{59}$$

Scheme C

$$Jf/Jr = 1 + \frac{k_1(k_7 + [\mathrm{ADP}])}{[\mathrm{CAI}]\,[\mathrm{ADP}]} \tag{60}$$

$$JF/JR = 1 + \frac{k_1(k_8[\mathrm{CAO}] + k_7)}{[\mathrm{ADP}](k_1 + [\mathrm{CAI}])} \tag{61}$$

Scheme D

$$Jf/Jr = 1 + \frac{k_4k_9}{[\mathrm{CAI}](k_1 + [\mathrm{ADP}])} \tag{62}$$

$$JF/JR = 1 + \frac{k_4(K_{10}[\mathrm{CAO}] + [\mathrm{CAI}] + k_9)}{[\mathrm{ADP}]\,[\mathrm{CAI}]} \tag{63}$$

where

$$k_1 = \frac{\beta_{71}}{\beta_{76}} \tag{64}$$

$$k_2 = \frac{\beta_{24}\beta_{46}\beta_{67}}{\beta_{42}\beta_{21}\beta_{64}} \tag{65}$$

$$k_3 = \frac{\beta_{67}(\beta_{46} + \beta_{42})}{\beta_{46}\beta_{42}} \tag{66}$$

$$k_4 = \frac{\beta_{71}}{\beta_{75}} \tag{67}$$

$$k_5 = \frac{\beta_{57}\beta_{24}\beta_{45}}{\beta_{54}\beta_{21}\beta_{42}} \tag{68}$$

$$k_6 = \frac{\beta_{57}(\beta_{45} + \beta_{42})}{\beta_{54}\beta_{42}} \tag{69}$$

$$k_7 = \frac{\beta_{67}(\beta_{46} + \beta_{43})}{\beta_{64}\beta_{43}} \tag{70}$$

$$k_8 = \frac{\beta_{67}\beta_{34}\beta_{46}}{\beta_{31}\beta_{43}\beta_{64}} \tag{71}$$

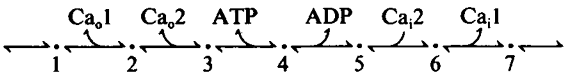
$$k_9 = \frac{\beta_{57}(\beta_{45} + \beta_{43})}{\beta_{54}\beta_{43}} \tag{72}$$

$$k_{10} = \frac{\beta_{34}\beta_{45}\beta_{57}}{\beta_{54}\beta_{43}\beta_{31}}. \tag{73}$$

Nonsimultaneous Calcium Binding

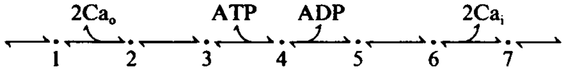
As mentioned earlier, the single cycle Schemes A–D all envision that calcium binding at the outside surface and calcium desorption at the inner surface occurs in a single step in which n calcium atoms are involved, where n is usually considered to be equal to the stoichiometry of the pump. However, outside calcium binds to the pump in a cooperative way, indicating sequential binding (Inesi et al., 1980; Verjorski-Almeida and Silva, 1981). This presents no difficulties for the conclusion drawn about the ratio of unidirectional flux ratios (Eqs. 40, 42, 44, and 46) for the single cycle Schemes A–D provided that nucleotide does not bind between two calcium-binding steps or nucleotide does not desorb between two calcium desorption steps.

To see this, consider the three schemes shown below



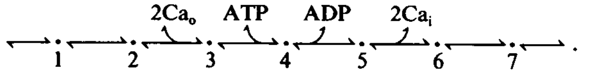
(74)

Scheme 1



(75)

Scheme 2



(76)

Scheme 3

For Scheme 2 we derive

$$\frac{Jf/JF}{Jr/JR} = \frac{J_{12}J_{23}J_{56}J_{67}}{J_{21}J_{32}J_{65}J_{76}}. \tag{77}$$

Since $J_{12} = J_{\text{net}} + J_{21}$, $J_{23} = J_{\text{net}} + J_{32}$, $J_{56} = J_{\text{net}} + J_{65}$ and $J_{67} = J_{\text{net}} + J_{76}$, it follows that this ratio of unidirectional flux ratios must be greater than one. Clearly, this also holds for Scheme 3

$$\frac{Jf/JF}{Jr/JR} = \frac{J_{23} \cdot J_{56}}{J_{32}\, J_{65}} > 1.0. \tag{78}$$

For Scheme 1, the total unidirectional fluxes of calcium are

$$Jf_t = Jf_1 + Jf_2 \tag{79}$$

and

$$Jr_t = Jr_1 + Jr_2, \tag{80}$$

where Jf_1 and Jr_1 are the calcium fluxes involving binding site 1 and Jf_2 and Jr_2 are those for site 2. The calcium fluxes are related by

$$Jf_1 = \frac{J_{12} \cdot J_{67} \cdot Jf_2}{J_{21} \cdot Jr_2 + J_{67}J_{21} + J_{67}Jf_2} \tag{81}$$

$$Jr_1 = \frac{J_{76}J_{21}Jr_2}{J_{21}Jr_2 + J_{21}J_{67}Jf_2}. \tag{82}$$

Substituting these into Eqs. 79 and 80 and rearranging, we obtain

$$\frac{Jf_t}{Jr_t} = \frac{Jf_2}{Jr_2}\frac{(J_{12}J_{67} + J_{21}J_{67} + Jf_2J_{67} + J_{21}Jr_2)}{(J_{21}J_{67} + J_{21}J_{76} + Jf_2J_{67} + J_{21}Jr_2)}. \tag{83}$$

Since $J_{12} = J_{\text{net}} + J_{21}$ and $J_{67} = J_{\text{net}} + J_{76}$, we have

$$\frac{Jf_t}{Jr_t} = \frac{Jf_2}{Jr_2}\left(1 + \frac{J_{\text{net}}(J_{67} + J_{21})}{J_{21}J_{67} + J_{21}J_{76} + Jf_2J_{67} + J_{21}Jr_2}\right). \tag{84}$$

So

$$\frac{Jf_t}{Jr_t} > \frac{Jf_2}{Jr_2}. \tag{85}$$

Since stoichiometry cancels in these ratios, Jf_2/Jr_2 is the same as Jf/Jr for Scheme 3

$$\frac{Jf_2}{Jr_2} = \frac{J_{23}J_{34}J_{45}J_{56}}{J_{32}J_{43}J_{54}J_{65}} > \frac{JF}{JR} = \frac{J_{34}J_{45}}{J_{43}J_{54}} \tag{86}$$

and therefore, from Eqs. 85 and 86

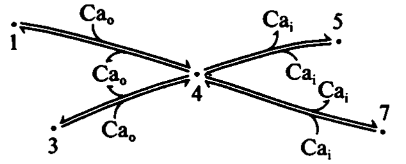
$$\frac{Jf_t/Jr_t}{JF/JR} = \frac{Jf_t/JF}{Jr_t/JR} > 1.0. \tag{87}$$

Nonsimultaneous calcium binding does not change the conclusions reached about single cycles A–D. Such binding, however, will alter the concentration dependence on [CAO] given in Eqs 56–63.

Unidirectional Flux Over Branches

Reaction Schemes A–D are not necessarily mutually exclusive. If the calcium pump can bind calcium and ATP in random order and desorb calcium and ADP in random order, then the overall processes would be more complicated. The scheme depicting random sorption desorption is shown in Fig. 1.

In this case, calcium flux occurs when the pump completes transitions from state 1 through states 2 and 4 to end at state 5; from state 1 through states 2, 4, and 6 to end at state 7; from state 3 through 4 to end at 5; and from state 3 through states 4 and 6 to end at state 7. The flux of calcium is shown in Scheme II.



Scheme II

We reduce the flux of pump from states 1 through 2 to state 4 to that given by application of Eq. 15, and similarly reduce the flux from the state 4 through state 6 to state 7 to a single unidirectional flux

$$J_{124} = \frac{J_{12} \cdot J_{24}}{J_{24} + J_{21}} \tag{88a}$$

$$J_{421} = \frac{J_{42} \cdot J_{21}}{J_{24} + J_{21}} \tag{88b}$$

$$J_{467} = \frac{J_{46} \cdot J_{67}}{J_{67} + J_{64}} \tag{88c}$$

$$J_{764} = \frac{J_{76} \cdot J_{64}}{J_{67} + J_{64}}. \tag{88d}$$

Next we reduce the combined fluxes in branches 124 and 34 to a single set of equivalent fluxes, and similarly reduce branches 467 and 45 to two equivalent unidirectional fluxes

$$\longrightarrow \underset{1}{\cdot} \underset{J_{4\to1}}{\overset{J_{1\to4}}{\rightleftharpoons}} \underset{4}{\cdot} \underset{J_{7\to4}}{\overset{J_{4\to7}}{\rightleftharpoons}} \underset{7}{\cdot} \longrightarrow \tag{89}$$

where

$$J_{1\to4} = J_{124} + J_{34} \tag{90a}$$

$$J_{4\to1} = J_{421} + J_{43} \tag{90b}$$

$$J_{4\to7} = J_{45} + J_{467} \tag{90c}$$

$$J_{7\to4} = J_{54} + J_{764}. \tag{90d}$$

The overall unidirectional calcium flux is given by application of Eq. 15 as

$$Jf = \frac{J_{1\to4} \cdot J_{4\to7}}{J_{4\to7} + J_{4\to1}} \tag{91a}$$

$$Jr = \frac{J_{1\to4} \cdot J_{4\to1}}{J_{4\to7} + J_{4\to1}}. \tag{91b}$$

Application of these methods gives the following expressions for unidirectional calcium and nucleotide fluxes

$$Jf = \frac{\left(\frac{J_{12} \cdot J_{24}}{J_{24} + J_{21}} + J_{34}\right)\left(J_{45} + \frac{J_{46} \cdot J_{67}}{J_{67} + J_{64}}\right)}{J_{45} + \frac{J_{46} \cdot J_{67}}{J_{67} + J_{64}} + J_{43} + \frac{J_{42} \cdot J_{21}}{J_{24} + J_{21}}} \tag{92a}$$

$$Jr = \frac{\left(J_{54} + \frac{J_{76} \cdot J_{64}}{J_{67} + J_{64}}\right)\left(J_{43} + \frac{J_{42} \cdot J_{21}}{J_{21} + J_{24}}\right)}{J_{43} + \frac{J_{42} \cdot J_{21}}{J_{21} + J_{24}} + J_{45} + \frac{J_{46} \cdot J_{67}}{J_{67} + J_{64}}} \tag{92b}$$

$$JF = \frac{\left(\frac{J_{13} \cdot J_{34}}{J_{34} + J_{31}} + J_{24}\right)\left(J_{46} + \frac{J_{45} \cdot J_{57}}{J_{57} + J_{54}}\right)}{J_{46} + \frac{J_{45} \cdot J_{57}}{J_{57} + J_{54}} + J_{42} + \frac{J_{43} \cdot J_{31}}{J_{31} + J_{34}}} \tag{92c}$$

$$JR = \frac{\left(J_{64} + \frac{J_{75} \cdot J_{54}}{J_{54} + J_{57}}\right)\left(J_{42} + \frac{J_{43} \cdot J_{31}}{J_{31} + J_{34}}\right)}{J_{42} + \frac{J_{43} \cdot J_{31}}{J_{31} + J_{34}} + J_{46} + \frac{J_{45} \cdot J_{57}}{J_{57} + J_{54}}}. \tag{92d}$$

These expressions contain the expression for their respective unidirectional flux for Schemes A–D when the appropriate fluxes of the random-order scheme (Fig. 1) are set equal to zero. In principle, the unidirectional flux ratios can be obtained as before and, after solving for the state populations in terms of the complete set of rate constants, expressions can be derived for the unidirectional flux ratios in terms of the first-order rate constants and the ligand concentrations. However, for the random-order scheme, there are 48 partial diagrams as defined by Hill (1977) required to solve for the N_i's, and therefore the process becomes quite cumbersome. Since, as described in the introduction, there is good evidence that ADP desorbs before calcium, a simplified version of this scheme has been investigated (Scheme *E* in Fig. 1). In this case ATP and Ca$_o$ may bind randomly, but ADP desorbs before calcium desorbs to the inner surface. This scheme is much easier to solve. The result is

$$\frac{Jf}{Jr} = 1 + \frac{\beta_{71}\beta_{67} + \beta_{71}\beta_{64}[\mathrm{ADP}]}{\beta_{64}[\mathrm{ADP}]\beta_{76}[\mathrm{CAI}]} + \frac{\beta_{46}\beta_{67}\beta_{71}(\beta_{21} + \beta_{24}[\mathrm{ATP}])}{\beta_{76}\beta_{64}[\mathrm{ADP}][\mathrm{CAI}](\beta_{42}\beta_{21} + \beta_{21}\beta_{43} + \beta_{43}\beta_{24}[\mathrm{ATP}])} \tag{93}$$

$$\frac{JF}{JR} = 1 + \frac{\beta_{71}\beta_{67}}{\beta_{64}[\mathrm{ADP}](\beta_{71} + \beta_{76}[\mathrm{CAI}])} + \frac{\beta_{46}\beta_{67}\beta_{71}(\beta_{31} + \beta_{34}[\mathrm{CAO}])}{\beta_{64}[\mathrm{ADP}](\beta_{71} + \beta_{76}[\mathrm{CAI}])(\beta_{42}\beta_{31} + \beta_{42}\beta_{34}[\mathrm{CAO}] + \beta_{43}\beta_{31})} \tag{94}$$

$$\frac{Jf/JF}{Jr/JR} = \frac{\beta_{71} + \beta_{76}[\mathrm{CAI}]}{\beta_{76}[\mathrm{CAI}]} \cdot \left\{ \frac{\beta_{71}\beta_{67} + \beta_{64}[\mathrm{ADP}](\beta_{71} + \beta_{76}[\mathrm{CAI}]) + \dfrac{\beta_{46}\beta_{67}\beta_{71}(\beta_{21} + \beta_{24}[\mathrm{ATP}])}{\beta_{21}\beta_{42} + \beta_{21}\beta_{43} + \beta_{43}\beta_{24}[\mathrm{ATP}]}}{\beta_{71}\beta_{67} + \beta_{64}[\mathrm{ADP}](\beta_{71} + \beta_{76}[\mathrm{CAI}]) + \dfrac{\beta_{46}\beta_{67}\beta_{71}(\beta_{31} + \beta_{34}[\mathrm{CAO}])}{\beta_{31}\beta_{43} + \beta_{31}\beta_{42} + \beta_{42}\beta_{34}[\mathrm{CAO}]}} \right\}. \tag{95}$$

These expressions can be used to derive expressions for Jf/Jr, JF/JR, and $(Jf/JF)/(Jr/JR)$ for single cycles A and C by letting the appropriate rate constants be zero. For single cycle A, we have $\beta_{13} = \beta_{31} = \beta_{34} = \beta_{43} = 0$ and

Scheme A

$$\frac{Jf/JF}{Jr/JR} = \frac{\beta_{71} + \beta_{76}[\mathrm{CAI}]}{\beta_{76}[\mathrm{CAI}]} \cdot \left\{ \frac{\beta_{71}\beta_{67} + \beta_{64}[\mathrm{ADP}](\beta_{71} + \beta_{76}[\mathrm{CAI}]) + \dfrac{\beta_{46}\beta_{67}\beta_{71}(\beta_{21} + \beta_{24}[\mathrm{ATP}])}{\beta_{42}\beta_{21}}}{\beta_{71}\beta_{67} + \beta_{64}[\mathrm{ADP}](\beta_{71} + \beta_{76}[\mathrm{CAI}]) + \dfrac{\beta_{46}\beta_{67}\beta_{71}}{\beta_{42}}} \right\}. \tag{96}$$

For Scheme C, $\beta_{12} = \beta_{21} = \beta_{24} = \beta_{42} = 0$ and

Scheme C

$$\frac{Jf/JF}{Jr/JR} = \frac{(\beta_{71} + \beta_{76}[\mathrm{CAI}])}{(\beta_{76}[\mathrm{CAI}])} \cdot \left\{ \frac{\beta_{71}\beta_{67} + \beta_{64}[\mathrm{ADP}](\beta_{71} + \beta_{76}[\mathrm{CAI}]) + \dfrac{\beta_{46}\beta_{67}\beta_{71}}{\beta_{43}}}{(\beta_{71}\beta_{67} + \beta_{64}[\mathrm{ADP}](\beta_{71} + \beta_{76}[\mathrm{CAI}]) + \dfrac{\beta_{46}\beta_{67}\beta_{71}}{\beta_{43}} \dfrac{(\beta_{31} + \beta_{34}[\mathrm{CAO}])}{\beta_{31}}} \right\}. \tag{97}$$

Again, for Scheme A the ratio of unidirectional flux ratios is expected to exceed one, whereas for Scheme C the ratio of flux ratios may be greater than or less than one depending on the actual rate constants. If a random binding of ATP and $\mathrm{Ca_o}$ occurs, the ratio of unidirectional flux ratios will be given by Eq. 95.

The term random order can be taken to mean simply that both sequences of binding are allowed or that the binding of one is independent of the other. In a truly random sequence, the binding of one ligand does not influence the other. The formal statement of this is

$$\beta_{12} = \beta_{34} \tag{98a}$$

$$\beta_{21} = \beta_{43} \tag{98b}$$

$$\beta_{13} = \beta_{24} \tag{98c}$$

$$\beta_{31} = \beta_{42}. \tag{98d}$$

If these constraints are placed on Scheme E, then we have

$$\frac{Jf/JF}{Jr/JR} = \frac{\beta_{71} + \beta_{76}[\mathrm{CAI}]}{\beta_{76}[\mathrm{CAI}]} \cdot \left\{ \frac{\beta_{71}\beta_{67} + \beta_{64}[\mathrm{ADP}](\beta_{71} + \beta_{76}[\mathrm{CAI}]) + \left(\dfrac{\beta_{46}\beta_{67}\beta_{71}}{\beta_{21}}\right)\left(\dfrac{\beta_{21} + \beta_{24}[\mathrm{ATP}]}{\beta_{42} + \beta_{21} + \beta_{24}[\mathrm{ATP}]}\right)}{\beta_{71}\beta_{67} + \beta_{64}[\mathrm{ADP}](\beta_{71} + \beta_{76}[\mathrm{CAI}]) + \left(\dfrac{\beta_{46}\beta_{67}\beta_{71}}{\beta_{42}}\right)\left(\dfrac{\beta_{42} + \beta_{12}[\mathrm{CAO}]}{\beta_{21} + \beta_{42} + \beta_{12}[\mathrm{CAO}]}\right)} \right\}. \tag{99}$$

The final ratio of ratios depends on the relative affinities of the pump for ATP and $\mathrm{Ca_o}$.

In the truly random scheme the pump travels over each branch equally often, $J_{124} = J_{134}$. However, the calcium or nucleotide fluxes are not equal in the two branches. It is for this reason that the complicated equations (93–95) are derived. To see this, consider that in Scheme E $\beta_{13} = \beta_{31} = 0$. That is, ATP cannot bind to the enzyme unless calcium binds first, but the calcium bound after ATP binds can exchange with extravesicular calcium. This is similar to the suggestion of Takakuwa and Kanazawa (1982) that phosphoenzyme can exchange calcium at the outside surface of the vesicle. However, when $\beta_{13} = \beta_{31} = 0$ the exchange occurs before ADP desorbs rather than afterwards as proposed by Takakuwa and Kanazawa (1981). When ATP binding is limited in this way, we have

$$\frac{Jf/JF}{Jr/JR} = \frac{\beta_{71} + \beta_{76}[\mathrm{CAI}]}{\beta_{76}[\mathrm{CAI}]} \cdot \left\{ \frac{\beta_{71}\beta_{67} + \beta_{64}[\mathrm{ADP}](\beta_{71} + \beta_{76}[\mathrm{CAI}]) + \dfrac{\beta_{46}\beta_{67}\beta_{71}(\beta_{21} + \beta_{24}[\mathrm{ATP}])}{\beta_{21}\beta_{42} + \beta_{21}\beta_{43} + \beta_{43}\beta_{24}[\mathrm{ATP}]}}{\beta_{71}\beta_{67} + \beta_{64}[\mathrm{ADP}](\beta_{71} + \beta_{76}[\mathrm{CAI}]) + \dfrac{\beta_{46}\beta_{67}\beta_{71}}{\beta_{42}}} \right\}. \tag{100}$$

If β_{42} is much less than β_{43}, and β_{71} is negligible compared with $\beta_{76}[\mathrm{CAI}]$, then the ratio of ratios will be less than one; if β_{42} is much greater than β_{43}, then the ratio of ratios will exceed one. Thus, even if both branches of the scheme are traveled unequally, the ratio of unidirectional flux ratios still depends heavily on the rate constants for ATP and $\mathrm{Ca_o}$ desorption, β_{42} and β_{43}, respectively. As a further illustration, it is known that ATP and $\mathrm{Ca_o}$ bind with high affinity, meaning that $\beta_{42} \ll \beta_{24}$, $\beta_{21} \ll \beta_{12}$, $\beta_{31} \ll \beta_{13}$, and $\beta_{43} \ll \beta_{34}$. Terms that combine two of these dissociation constants will be particularly small. When these simplifications are applied to Eq. 95 we have

$$\frac{Jf/JF}{Jr/JR} = \frac{\beta_{71} + \beta_{76}[\mathrm{CAI}]}{\beta_{76}[\mathrm{CAI}]} \cdot \left\{ \frac{\beta_{71}\beta_{67} + \beta_{64}[\mathrm{ADP}](\beta_{71} + \beta_{76}[\mathrm{CAI}]) + \dfrac{\beta_{46}\beta_{67}\beta_{71}}{\beta_{43}}}{\beta_{71}\beta_{67} + \beta_{64}[\mathrm{ADP}](\beta_{71} + \beta_{76}[\mathrm{CAI}]) + \dfrac{\beta_{46}\beta_{67}\beta_{71}}{\beta_{42}}} \right\}. \tag{101}$$

Considering only the single cycle schemes, if the ratio $(Jf/JF)/(Jr/JR)$ is less than one, the results obtained (Eqs. 40, 42, 44, and 46) indicate that Scheme A is eliminated. If we add the information that ADP desorbs before Ca_i, as discussed in the introduction, then Scheme C is the only single cycle scheme compatible with the experimental observations. Scheme C requires a binding sequence ordered as ATP · Ca_o · ADP · Ca_i. If we also consider branched schemes, Scheme E is consistent with the data provided β_{43} is considerably larger than β_{42}, where β_{43} is the rate constant for Ca_o dissociation from the Ca_o-ATP-E complex and β_{42} is the rate constant for ATP dissociation from this same complex. Thus, if the ratio $(Jf/JF)/(Jr/JR)$ is less than one, and given the information that ADP desorbs before Ca_i, only two conclusions seem possible: either the sequence is ordered as ATP · Ca_o · ADP · Ca_i or the desorption of Ca_o is much faster than the desorption of ATP from the Ca_o-ATP-E complex.

CONCLUSION

The results obtained here suggest that the measured unidirectional calcium and nucleotide fluxes can be used to distinguish among a variety of reaction schemes. This process is particularly easy if the schemes are not branched. Branching of the reaction scheme introduces considerable complexity, which limits the usefulness of the flux analysis. Nevertheless, the results suggest that a careful analysis of the concentration dependence of the unidirectional flux ratios can also distinguish between some types of branches schemes.

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