Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 5352–5356, August 1985
Biophysics

Variable stoichiometry in active ion transport: Theoretical analysis of physiological consequences

(transport thermodynamics/transport kinetics/ion pumps/Ca^{2+} ATPase)

EDWARD A. JOHNSON, CHARLES TANFORD, AND JACQUELINE A. REYNOLDS

Department of Physiology, Duke University Medical Center, Durham, NC 27710

Contributed by Charles Tanford, April 18, 1985

ABSTRACT **Active ion transport systems with fixed stoichiometry are subject to a thermodynamic limit on the ion concentration gradients that they can generate and maintain, and their net rates of transport must inevitably decrease as this limit is approached. The capability to vary stoichiometry might thus be physiologically advantageous: a shift to lower stoichiometry (fewer ions pumped per reaction cycle) at increasing thermodynamic load could increase the limit on the supportable concentration gradient and could accelerate the rate of transport under high-load conditions. Here we present a theoretical and numerical analysis of this possibility, using the sarcoplasmic reticulum ATP-driven Ca pump as the example. It is easy to introduce alternate pathways into the reaction cycle for this system to shift the stoichiometry (Ca^{2+}/ATP) from the normal value of 2:1 to 1:1, but it cannot be done without simultaneous generation of a pathway for uncoupled leak of Ca^{2+} across the membrane. This counteracts the advantageous effect of the change in transport stoichiometry and a physiologically useful rate acceleration cannot be obtained. This result is likely to be generally applicable to most active transport systems.**

Catalytic pathways for both primary and secondary active ion transport are often represented by single unbranched reaction cycles, with a strictly ordered sequence of steps (1–3) or by extensions of such cycles that allow for random order of addition of reactants. An inherent property of such cycles is that the transport stoichiometry is fixed, regardless of external conditions or reaction rate, and a consequence of fixed stoichiometry is that the transport reaction must have a uniquely defined equilibrium state, which governs precisely the conditions (changes in membrane potential or reactant concentrations) under which the transport reaction must decrease to zero net rate and subsequently reverse its direction (4, 5).

Constant stoichiometry has a disadvantage from a physiological point of view, which is conveniently illustrated by reference to the ATP-driven sarcoplasmic reticulum (SR) Ca pump. This pump has an established transport stoichiometry of $Ca^{2+}/ATP = 2$ when the ions are pumped against a trivial thermodynamic load (e.g., into empty or nearly empty SR vesicles). The 2:1 stoichiometry is energetically economical for part of the pump's physiological function, minimizing the amount of ATP required for bulk transport under conditions in which transport is easy—i.e., immediately after a muscle contraction when the cytoplasmic Ca^{2+} concentration is still near its peak. However, constancy in this stoichiometry dictates that the net transport rate must decrease and eventually become zero as the ion concentration gradient increases—i.e., as $[Ca^{2+}]$ goes down in the cytoplasm and increases in the SR lumen. For the final stages of Ca^{2+} removal in the muscle relaxation process, and for maintenance of low cytoplasmic Ca^{2+} when the muscle is at rest, a transport stoichiometry of $Ca^{2+}/ATP = 1$ (though thermodynamically inefficient) might then be kinetically useful. The thermodynamic limiting Ca^{2+} concentration gradient (reversal point) that the pump could support is much higher for a 1:1 stoichiometry. Creation of a supergradient may not in itself be a beneficial or even feasible goal, but rates increase when a system is further removed from equilibrium and a 1:1 pump could accordingly still operate at a vigorous rate under conditions in which a 2:1 stoichiometry pump would virtually have come to a standstill.

It is plausible that a transport system with built-in variable stoichiometry can provide a means of coping with the variation in load to which an ion pump may be subject in the course of normal operation, and this paper presents a theoretical inquiry into this possibility, continuing to focus on the SR Ca pump as a convenient example. We consider additions to the conventional fixed stoichiometry reaction cycle for this system that would lead to variable stoichiometry and then evaluate the resulting effects on physiological function. We also calculate the effects that these changes would have on laboratory experiments that are commonly used to measure transport stoichiometry, generally under conditions far from the physiological steady state.

Ca PUMP REACTION CYCLE

Our basic reaction cycle, shown in Fig. 1, is an extension of the generally accepted minimal cycle of de Meis and coworkers (2). The central feature of this cycle is the alternation of the transport protein between two distinct conformations, E_1 and E_2. The Ca^{2+} binding sites (two per molecule) are accessible from the cytoplasm in E_1 and from the SR lumen in E_2, and the binding affinity is high in E_1 and low in E_2. E_1 has a high-affinity binding site for ATP, and ATP bound to this site creates a "high energy" phosphoenzyme intermediate ($Ca_2E_1{\sim}P$) if and only if both Ca^{2+} sites are filled. E_2 is the state from which Ca^{2+} and P_i are discharged when the pump operates in the forward direction, with a "low energy" phosphoenzyme (Ca_2E_2–P $\rightleftharpoons$ CaE_2–P $\rightleftharpoons$ E_2–P) as intermediate. The de Meis cycle *per se*, represented by the *boxed species* in Fig. 1, is ordinarily presented as perfectly ordered, even with respect to the order of addition of reactants. It is known, however, that Ca^{2+} and ATP can bind to E_1 in random order and we assume that P_i and Ca^{2+} from the SR lumen side can likewise bind in random order to E_2. (In the forward operation of the pump, Ca^{2+} will normally dissociate first from Ca_2E_2–P because conversion of E_2–P to $E_2{\cdot}P_i$ is much slower than ion dissociation from a weak binding site.) This randomness in binding order has been incorporated into Fig. 1, leading to several new intermediates and to extensive branching of the reaction pathway. However, randomness in

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: SR, sarcoplasmic reticulum.

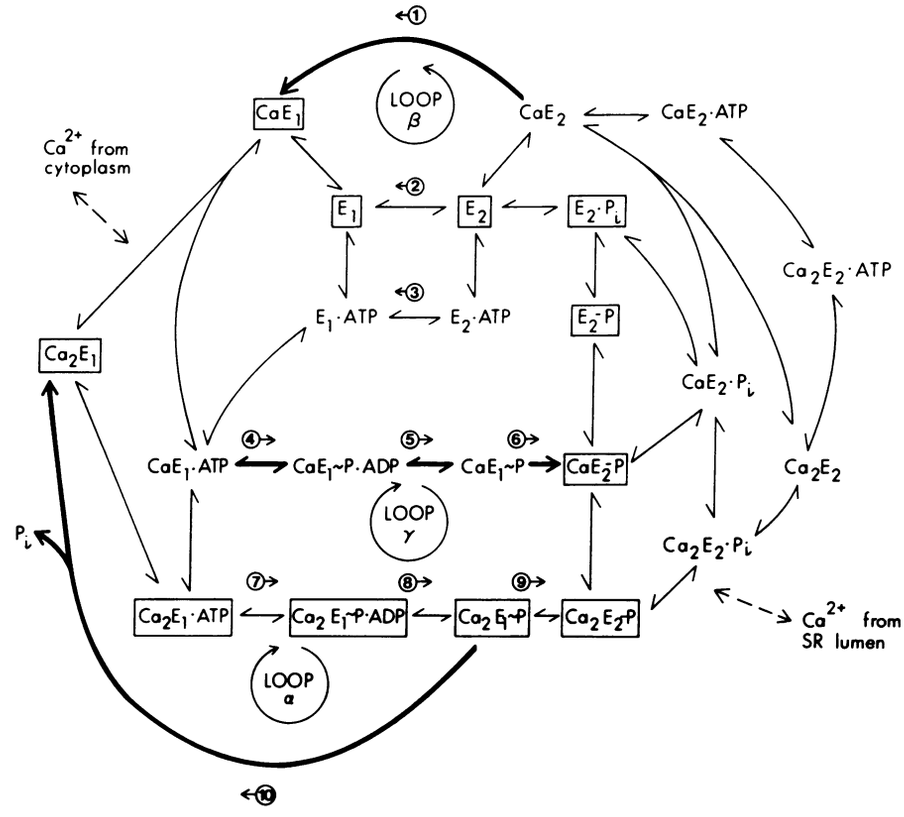

FIG. 1. Reaction cycle for the SR Ca pump. Boxed species represent the ordered "minimal" reaction cycle of de Meis and co-workers (2). Additional intermediates joined to the boxed species by narrow lines allow for randomness in the order of binding of substrates but have no effect on stoichiometry. *Heavy lines* (steps 1, 4, 5, 6, and 10) represent new intermediates and/or transitions that lead to partial uncoupling and thereby affect transport stoichiometry. Arrows on numbered steps indicate the direction of reaction for equilibrium and rate constants cited in the text. The diagram does not explicitly show where ATP, ADP, and P_i enter or leave the cycle (except in step 10); this should be obvious from the compositions of intermediates. The diagram also does not explicitly incorporate essential roles played by Mg^{2+} and K^+. Parameters used for calculations are intended to apply to experimental conditions in which these ions are present at concentrations satisfying all requirements.

binding order alone does not have an effect on transport stoichiometry. It is easily verified from Fig. 1 that the new loops created by the connecting lines of regular thickness do not permit completion of any reaction cycle that would lead to a stoichiometry other than the 2:1 stoichiometry of the original de Meis cycle. The same statement applies to another element of randomness that we have incorporated into the cycle—namely, the likelihood that ATP can bind to E_2 before its conversion to E_1. This, too, has no effect on transport stoichiometry but, as we have shown in an earlier paper (6), can affect the kinetic response to changes in ATP concentration.

In all, the scheme of Fig. 1 (before introduction of pathways leading to variable stoichiometry) has 20 reaction intermediates and requires solution of 20 simultaneous differential equations for kinetic analysis. It is actually the same scheme that was used in previous papers (6, 7) for discussion of the kinetics of activation of the SR Ca pump by ATP and cytoplasmic Ca^{2+}. Previously used rate and equilibrium constants, listed in the fourth column of Table 1 in ref. 7, have been retained for the present analysis. These parameters quantitatively account for equilibrium Ca^{2+} binding to the pump protein at pH 7 and for kinetic activation of the pump reaction in leaky SR vesicles by ATP and cytoplasmic Ca^{2+}, deviating from experimental data only in that the degree of cooperativity of kinetic activation by Ca^{2+} is somewhat lower than most (but not all) investigators have reported.

Fig. 1 also shows, by means of *heavy* lines, three types of alterations in the pathway that affect the coupling between ATP hydrolysis and Ca^{2+} transport and thereby affect the transport stoichiometry. One of these (loop α), created by reaction step 10, permits the uncoupled hydrolysis of ATP. The second (loop β), created by reaction step 1, permits the uncoupled leak of Ca^{2+} from high concentration in the SR into the cytoplasm, doing so by removing the restriction that the $E_2 \rightleftharpoons E_1$ transition requires unoccupied Ca^{2+} binding sites. In the actual analytical treatment of loop β we have allowed for the likelihood that ATP may bind to E_2 before this transition occurs (6) by permitting transformation of CaE_2·ATP into CaE_1·ATP as well as CaE_2 into CaE_1. This additional step is omitted from Fig. 1 to avoid undue cluttering.

The third way we have used to obtain variable stoichiometry is intuitively the most attractive. It is created by permitting phosphorylation of E_1 and subsequent ion translocation to occur when only one of the E_1 binding sites for Ca^{2+} is occupied, as indicated by reactions 4–6 in Fig. 1. The upper part of the reaction scheme, plus steps 4–6, now leads to a complete cycle with a 1:1 stoichiometry, and it is intuitively obvious that this cycle will tend to become more important than the competing cycle via steps 7–9 at low cytoplasmic Ca^{2+} concentrations, where the steady-state level of CaE_1·ATP will exceed the steady-state level of Ca_2E_1·ATP. Fig. 1 shows, however, that introduction of steps 4–6 has the unwelcome side effect of introducing loop γ into the reaction scheme, the overall reaction for which is net transfer of a Ca^{2+} ion from one side of the membrane to the other. Under conditions of high load, the thermodynamically favored direction of this loop must be clockwise, corresponding to a pathway for leakage of Ca^{2+} from the SR lumen into

the cytoplasm. As we shall see, this loop prevents realization of the potential benefits of the shift in stoichiometry.

Fig. 1 shows no direct kinetic link between $CaE_1{\sim}P$ and $Ca_2E_1{\sim}P$. The latter form of the enzyme is slow in releasing Ca^{2+} to the cytoplasmic medium and is often called an "occluded" state (8, 9), and we have assumed that binding of Ca^{2+} to the hypothetical intermediate $CaE_1{\sim}P$ to form $Ca_2E_1{\sim}P$ is likewise slow (with or without attached ADP) in relation to the overall rate.

THERMODYNAMIC CONSTRAINTS

For all but the new (heavy line) transitions of Fig. 1 we have employed the same cycle parameters used previously. When two states in this previously used cycle are linked by a new connection, then the equilibrium constant for the overall reaction along the new pathway is fixed by the equilibrium constants between the same points along the old pathway. This principle, previously applied by us to the loop in Fig. 1 containing steps 2 and 3 (6), unambiguously determines the equilibrium constants for reactions 1 and 10 in Fig. 1 and for the product of equilibrium constants in the sequence of reactions 4–6. Because these transformations alter reaction stoichiometry by total or partial uncoupling between ATP hydrolysis and ion transport, the resulting equilibria greatly favor one direction of reaction over the other, as indicated by the arrows on the heavy lines in Fig. 1. We obtain specifically the following values (subscripts referring to the numbered steps in Fig. 1): $K_1 = 500$ and K_{eq} for the corresponding step with bound ATP = 6000; $K_{10} = 120$ M (note that P_i is released in this step and that the numerical value of K_{10} is equal to the P_i concentration required to reverse the step); and $K_4K_5K_6 = 0.36$ M. The high value for the latter product (compared to $K_7K_8K_9 = 7.2 \times 10^{-6}$ M) may be taken as the physiologically desired result, making translocation of the single Ca^{2+} ion in this sequence inherently more favorable than enforced translocation of two Ca^{2+} ions along steps 7–9.

The fixed value for $K_4K_5K_6$ still permits freedom to assign any desired values to two of the three individual equilibrium constants. We have assumed that neither the ADP dissociation constant nor the phosphoenzyme formation constant depends on whether one or two Ca^{2+} ions are bound—i.e., we have set $K_4 = K_7$ and $K_5 = K_8$, which leads to $K_6/K_9 = 5 \times 10^4$. This is a reasonable figure since we have the same amount of free energy available from the conversion of E~P to E–P in both reactions but are using it to move only one Ca^{2+} ion uphill in step 6, whereas two are moved in step 9. (We have also made calculations allowing for a difference between K_4 and K_7, with no significant effect on the results.)

RESULTS

To determine the effects of each variable stoichiometry pathway in Fig. 1, we started with the reference cycle in which all of the reactions marked by heavy lines were switched off and then calculated the change in kinetic behavior resulting from a gradual increase in the rate of each alternate pathway in turn.

To obtain kinetic behavior relevant to physiological function, we need to focus on the rate of Ca^{2+} flux from a maximal cytoplasmic Ca^{2+} concentration approaching 1×10^{-5} M (10) down to 1×10^{-7} M, possibly 5×10^{-8} M (11). The SR lumen Ca^{2+} concentration rises as the cytoplasmic concentration decreases. Since much of the accumulated Ca^{2+} is bound to Ca^{2+} binding proteins, the binding affinities for these proteins help determine free $[Ca^{2+}]$ in the SR as a function of total uptake, and this dictates that $[Ca^{2+}]$ in the SR will probably be between 1 and 3 mM during most of the uptake process. We have used a concentration of 2 mM for the calculations presented here. We have assumed ATP, ADP, and P_i concentrations to remain constant during the uptake process at 5 mM, 0.02 mM, and 5 mM, respectively.

We have also simulated a laboratory experiment in which Ca^{2+} is pumped against zero load or nearly zero load by use of SR vesicles with a low Ca^{2+} content and very low levels of ADP and P_i in the extravesicular solution. This experiment, with varying extravesicular Ca^{2+} concentration, has been carried out by Makinose and The (12). They found that the transport stoichiometry remained constant at $Ca^{2+}/ATP = 2.0$ at all extravesicular Ca^{2+} concentrations down to nearly 10^{-7} M, dropping off only below that point. Intravesicular $[Ca^{2+}]$ was at 5 μM in these experiments, far below the required concentration for significant association with the Ca^{2+} binding sites in the E_2 state.

Loop α. A complete cycle around loop α (downhill direction) corresponds to the uncoupled hydrolysis of one molecule of ATP. As k_{10} is increased relative to k_9, the rate of useless hydrolysis of ATP increases relative to the productive coupled reaction, and the transport stoichiometry (Ca^{2+}/ATP) moves smoothly from 2 to 0. It is difficult to imagine how this manner of producing variable stoichiometry can be physiologically helpful, and our calculations confirm this.

In a laboratory experiment in which Ca^{2+} is pumped against zero load or nearly zero load, reactions 7–9 become essentially irreversible. The overall stoichiometry is then determined by the relative probability, at the branching point ($Ca_2E_1{\sim}P$), of proceeding along steps 9 and 10—i.e.,

$$Ca^{2+}/ATP = \frac{2\,k_9}{k_9 + k_{10}}. \qquad [1]$$

This result is *independent* of the extravesicular Ca^{2+} concentration and therefore cannot account for the variation in transport stoichiometry observed by Makinose and The (12). Under physiological conditions, Eq. **1** no longer applies because the probability of returning to state $Ca_2E_1{\sim}P$ by reversal of step 9 increases with $[Ca^{2+}]_{SR}$, thus offering the protein additional opportunity for branching to step 10. Some calculated results are shown in Fig. 2. They are independent of $[Ca^{2+}]_{cyto}$ to below 10^{-7} M. The rate of Ca^{2+} uptake by the SR is unaffected by the changing stoichiometry; it just requires more ATP to do the same job.

Loop β. Turning on step 1 (Fig. 1) and the similar transition with bound ATP decreases overall transport stoichiometry by providing an uncoupled Ca^{2+} leak from the SR to the cytoplasm after the primary translocation (step 9) has oc-

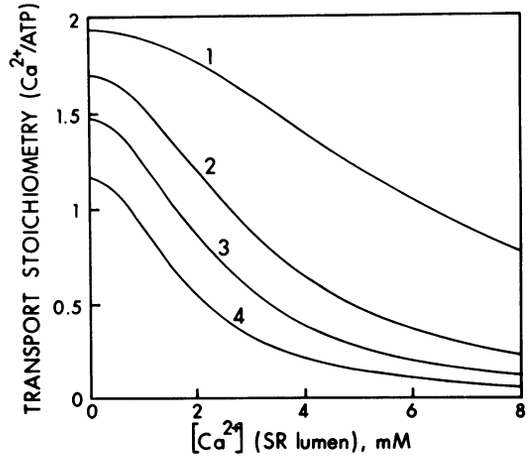

FIG. 2. Effect of loop α (uncoupled ATP hydrolysis) on transport stoichiometry. The rate constants for step 10 of Fig. 1 are 1, 5, 10, and 20 sec^{-1}, respectively, for curves 1–4. The rate constant for the directly competing reaction (k_9) is 28 sec^{-1}. Limiting stoichiometries at $[Ca^{2+}]_{SR} = 0$ obey Eq. **1**.

curred with an unaltered stoichiometry of $Ca^{2+}/ATP = 2$. To obtain a significant effect it is necessary to have a relatively high $[Ca^{2+}]$ in the SR (otherwise CaE_2 and CaE_2·ATP will not be present at significant levels); also k_1 and the corresponding rate constant for CaE_2·ATP must be comparable in magnitude to k_2 and k_3. The requirement for high $[Ca^{2+}]$ in the SR means that introduction of step 1 cannot affect laboratory experiments at low load. At high load, under physiological conditions, a decrease in transport stoichiometry is obtained. Our calculations show that a concomitant net acceleration of Ca^{2+} uptake into the SR is possible, which arises from the fact that the $E_2 \rightarrow E_1$ transition is the rate-limiting step in our model and increasing its rate via loop β raises the steady-state level of CaE_1. The flux enhancement is small and essentially independent of $[Ca^{2+}]_{cyto}$ so that no selective kinetic advantage can result.

Loop γ. As noted previously, the best hope for producing a physiologically beneficial reduction in transport stoichiometry is via steps 4–6 (Fig. 1). To "turn on" this sequence we either can set $k_4 = k_7$ and vary k_6 or can allow k_6 to be very large (because the equilibrium constant K_6 is very large) and vary k_4. We have used the latter procedure for the results reported here but have also made calculations based on variation in k_6 with similar results. The important factor is the overall rate through steps 4–6 compared to the overall rate through steps 7–9.

The calculated results in Fig. 3 show that an increase in the probability of steps 4–6 produces the hoped for downward shift in the activation of the pump reaction by cytoplasmic Ca^{2+}, but only if we define "pump reaction" in terms of ATP hydrolysis. The effect on Ca^{2+} transport is in the opposite

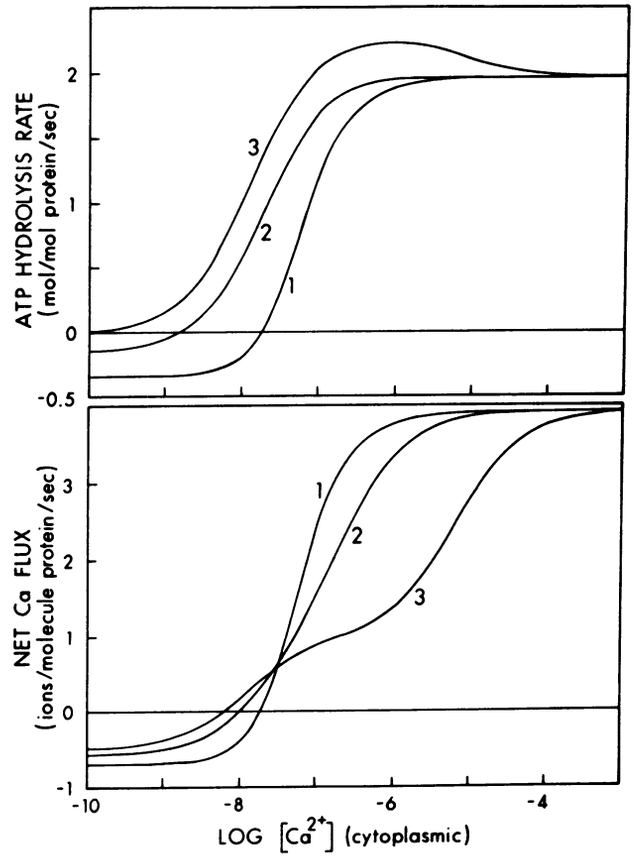

FIG. 3. Effect of transport of a single Ca^{2+} ion through steps 4–6 of Fig. 1. Curve 1 is the reference curve (pathway turned off); k_4 = 25 sec^{-1} for curve 2 and 1000 sec^{-1} for curve 3. The smallest rate constant along steps 7–9 is k_9 = 28 sec^{-1}.

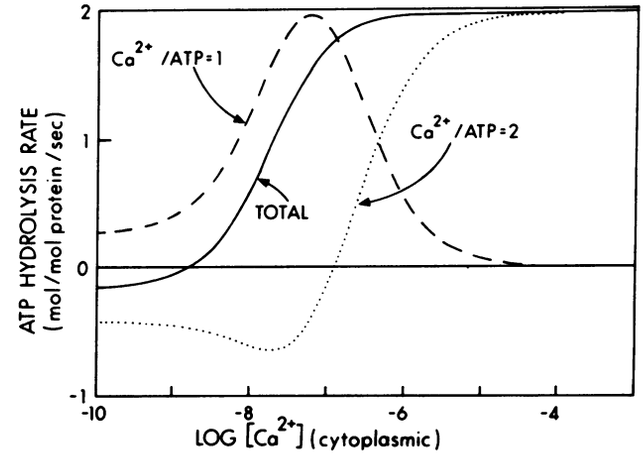

FIG. 4. Dissection of curve 2 of Fig. 3 to show separate components of ATP hydrolysis through steps 4–6 ($Ca^{2+}/ATP = 1$) and steps 7–9 ($Ca^{2+}/ATP = 2$). At low $[Ca^{2+}]_{cyto}$ the rate through steps 7–9 becomes negative (ATP is synthesized); completion of loop γ through steps 4–6 corresponds to a net Ca^{2+} leak from the SR into the cytoplasm.

direction—i.e., a substantial decrease in the rate of Ca^{2+} uptake over the physiologically significant concentration range. Fig. 4 shows the underlying cause for this result. Diversion of CaE_1·ATP in the direction of step 4 leads to depletion of the steady-state levels of CaE_1·ATP and CaE_2·ATP. This promotes reaction along steps 7–9 in the reverse direction (ATP synthesis) and continuous cycling around loop γ, which is equivalent to leakage of Ca^{2+} from the SR lumen into the cytoplasm. A small increase in Ca^{2+} uptake rate is produced near $[Ca^{2+}]_{cyto} = 10^{-8}$ M, close to the reversal point for 2:1 stoichiometry, but this is not physiologically useful since $[Ca^{2+}]_{cyto}$ does not normally fall much below 10^{-7} M even in the resting state of a muscle cell (11).

It should be noted that reversal of steps 7–9 cannot occur at low $[Ca^{2+}]_{SR}$. In the case of a laboratory experiment measuring uptake into nearly empty SR vesicles, the result of turning on steps 4–6 is therefore a smooth change in transport stoichiometry from 2:1 to 1:1, as shown by Fig. 5. The curves in Fig. 5 have the same shape as the curve obtained in this kind of experiment by Makinose and The (12). However, only curve 1 (k_4 = 10 sec^{-1}) is compatible with the observed midpoint at or below 10^{-7} M.

Reversal of the SR Pump. It is established that the 2:1 transport stoichiometry is maintained when the SR pump works in reverse, synthesizing ATP from ADP and P_i by use of a high Ca^{2+} concentration gradient as the thermodynamic

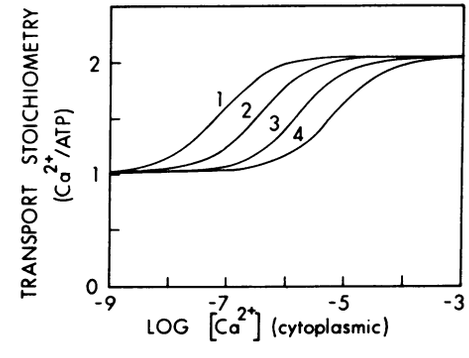

FIG. 5. Effect of steps 4–6 on transport stoichiometry in a laboratory experiment with $[Ca^{2+}]_{SR} < 50$ μM. Curves 1–4 correspond to k_4 = 10, 50, 250, and 1000 sec^{-1}, respectively. The rate-limiting step through steps 7–9 is k_9 = 28 sec^{-1}.

driving force (13). The alternative pathways provided in Fig. 1 are predicted to have no effect on stoichiometry in this experiment even when they do affect the stoichiometry for Ca^{2+} uptake and ATP hydrolysis: step 6 in loop γ is effectively irreversible for thermodynamic reasons; step 10 cannot compete with step 8 (reverse direction) at the high [ADP] used in ATP synthesis experiments; and step 1, in the absence of ATP, is very slow in comparison with phosphorylation of E_2 and subsequent translocation via step 9 (reverse direction) at high $[Ca^{2+}]_{SR}$. Failure to observe variable stoichiometry in a reversal experiment cannot therefore be used to reject the possibility of variable stoichiometry under physiological conditions.

Failure to observe variable stoichiometry in ATP-driven Ca^{2+} uptake under low-load laboratory conditions, however, is a useful rejection criterion or at least limits the magnitude of rate constants for the alternative pathways, as illustrated by the calculation in Fig. 5 relative to the experiment of Makinose and The. On the other hand, Makinose and The's result cannot be used as evidence in favor of variable pump stoichiometry. It does not necessarily mean that the stoichiometry of the pump reaction per se changes below 10^{-7} M Ca^{2+}, because the net rate of the pump reaction becomes very small under these conditions, which could mean that ion translocations not mediated by the pump (e.g., leakage of Ca^{2+} through membrane imperfections) then begin to affect the experimental measurements.

DISCUSSION

One conclusion from our analysis is that imperfect coupling in the SR Ca pump reaction cycle can be tolerated to some extent without affecting measurements of stoichiometry under normal experimental conditions (pH $\approx$7, *ca.* 0.1 M KCl, etc.). In particular, the $E_1 \rightleftharpoons E_2$ transition for unphosphorylated protein need not be strictly limited at the structural level to occurring without bound Ca^{2+}: k_1 and k_2 (Fig. 1) can have almost comparable values without serious effect. Similarly (Fig. 2), uncoupled ATP hydrolysis via step 10 can have k_{10} close to 1 sec^{-1} without measurable effect. If such deviations from perfect coupling in fact exist, they might become exaggerated under unusual experimental conditions (e.g., low or high pH) and then lead to changes in transport stoichiometry that have been reported (14). It is not immediately obvious, however, why amplification of variable stoichiometry pathways should be promoted by use of pseudosubstrates instead of ATP, as one report indicates (15). One recent report of variable stoichiometry (16) involves pumping into vesicles of very low free $[Ca^{2+}]$—i.e., it may be comparable to the experiment of Makinose and The cited in the text. It should be noted that *apparent* variable stoichiometry may be deduced from unidirectional isotope flux measurements even when the transport stoichiometry of the overall reaction is fixed (17).

A more important conclusion is that alternate pathways in Fig. 1, regardless of rate magnitude, producing easily detected variations in stoichiometry, would not result in thermodynamic or kinetic benefit for the physiological function of the SR Ca pump.

Our analysis does not of course address itself to a quite different way of producing variable stoichiometry, which would be to have entirely distinct 2:1 and 1:1 reaction cycles for the Ca pump and a separate regulatory device (sensitive to $[Ca^{2+}]_{cyto}$) for shifting from one to the other. This possibility would require high-affinity regulatory Ca^{2+} binding sites in addition to the transport sites, and for the SR Ca pump there is no experimental evidence to suggest existence of such sites.

It is not possible to say that our results must apply quite generally to transport systems of all kinds. However, one important part of our analysis is completely general, dictated by rigorous rules for cycle kinetics and for addition of loop reactions within a larger cycle (18). Introduction of a single new connection into an otherwise unbranched reaction cycle inevitably leads to three possible reaction cycles, not two. If one cycle leads to the transport of n ions per cycle and a second cycle leads to an otherwise similar reaction with a smaller number $(n - m)$ of transported ions, then there will *inevitably* be a third cycle by which (as in loop γ of Fig. 1) m ions can move from high to low concentration across the membrane. This new cycle must be energetically dissipatory and must always tend to negate any advantage that might be gained from a shift of the coupled reaction from the n cycle to the $(n - m)$ cycle, but one cannot rigorously eliminate the possibility that the negation may in some cases be <100%. It should also be mentioned that the thermodynamic principle involved here is independent of whether or not a transmembrane potential is part of the load opposing active transport. Our choice of the SR Ca pump (zero membrane potential) as the illustrative system was dictated solely by our familiarity with the system.

Most of this work was done at the Max Planck Institut für medizinische Forschung, Abteilung Physiologie, in Heidelberg, Federal Republic of Germany, and we thank Professor W. Hasselbach for providing facilities for us. Support for the work was derived from grants by the National Science Foundation, the National Institutes of Health, the Kemshall Fund, the Max Planck Institut (E.A.J.), and the Alexander von Humboldt Stiftung (C.T.).

1. Jencks, W. P. (1980) *Adv. Enzymol.* **51,** 75–106.
2. de Meis, L. & Vianna, A. L. (1979) *Annu. Rev. Biochem.* **48,** 275–292.
3. Sanders, L., Hansen, U.-P., Gradmann, D. & Slayman, C. L. (1984) *J. Membr. Biol.* **77,** 123–152.
4. Chapman, J. B. (1973) *J. Gen. Physiol.* **62,** 643–646.
5. Tanford, C. (1981) *J. Gen. Physiol.* **77,** 223–229.
6. Reynolds, J. A., Johnson, E. A. & Tanford, C. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 3658–3661.
7. Tanford, C., Reynolds, J. A. & Johnson, E. A. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 4688–4692.
8. Dupont, Y. (1980) *Eur. J. Biochem.* **109,** 231–238.
9. Takisawa, H. & Makinose, M. (1983) *J. Biol. Chem.* **258,** 2986–2992.
10. Allen, D. G. & Blinks, J. R. (1978) *Nature (London)* **273,** 509–513.
11. Cobbold, P. H. & Bourne, P. K. (1983) *Nature (London)* **312,** 444–446.
12. Makinose, M. & The, R. (1965) *Biochem. Z.* **343,** 383–393.
13. Makinose, M. & Hasselbach, W. (1971) *FEBS Lett.* **12,** 271–272.
14. Berman, M. (1982) *Biochim. Biophys. Acta* **694,** 95–121.
15. Rossi, B., Leon, F. A., Gache, C. & Lazdunski, M. (1979) *J. Biol. Chem.* **254,** 2302–2307.
16. Gafni, A. & Boyer, P. D. (1984) *Proc. Natl. Acad. Sci. USA* **82,** 98–101.
17. Chapman, J. B. (1982) *J. Theor. Biol.* **95,** 665–678.
18. Hill, T. L. (1977) *Free Energy Transduction in Biology* (Academic, New York).