Phosphorylation of the Sodium−Potassium Adenosinetriphosphatase Proceeds through a Rate-Limiting Conformational Change Followed by Rapid Phosphoryl Transfer†

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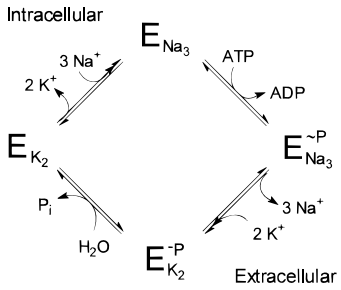
ABSTRACT: The sodium−potassium adenosinetriphosphatase of sheep kidney, preincubated with sodium and magnesium ($E{\cdot}Na_3$), reacts with 0.01−2.00 mM ATP to form covalent phosphoenzyme (E-P). The first order rate constant for phosphorylation increases hyperbolically with ATP concentration with a maximum value of $(4.6 \pm 0.9) \times 10^2$ s^{-1} and $K_{0.5} = 75 \pm 25$ μM (pH 7.4, 25 °C, 120 mM NaCl, and 3 mM $MgCl_2$). If the phosphoryl-transfer step were rate-limiting, the approach to equilibrium to give 50% E-P in the presence of ADP would follow $k_{obsd} = k_f + k_r = 9.2 \times 10^2$ s^{-1}. However, the formation of phosphoenzyme from $E{\cdot}Na_3$ with 1.0 mM ATP plus 2.0 mM ADP proceeds to 50% completion with $k_{obsd} = (4.2 \pm 0.8) \times 10^2$ s^{-1}. This result shows that phosphoryl transfer from bound ATP to the enzyme is not the rate-limiting step for phosphoenzyme formation from $E{\cdot}Na_3$. The result is consistent with a rate-limiting conformational change of the $E{\cdot}Na_3{\cdot}$ATP intermediate that is followed by rapid phosphoryl transfer, with $k_{cat} \geq 3000$ s^{-1}.

The sodium−potassium adenosinetriphosphatase is a membrane-bound enzyme that is found in nearly all animal cell membranes and uses energy from the hydrolysis of ATP to transport three Na^+ ions out of the cell and two K^+ ions into the cell with each turnover. The structure and function of the enzyme have been reviewed by Cantley (1981), Glynn (1985), Post (1989), Skou and Essman (1992), and Robinson and Pratap (1993). This electrogenic reaction cycle generates an outward movement of positive charge that is used to establish a resting membrane potential or action potentials of excitable cells, and the electrochemical potential gradient that is established for Na^+ ions is coupled to the transport of sugars, amino acids, and other compounds into the cell. The sodium−potassium ATPase is also essential for the function of mammalian kidneys, the maintenance of osmotic stability in animal cells, and the high concentration of intracellular K^+ that is required for the activity of many enzymes. Finally, the heat that is generated by the sodium pump is thought to account for over 20% of the basal metabolic rate of adult mammals (Glynn, 1985).

The chemical reaction of ATP hydrolysis is coupled to the physical reaction of sodium and potassium transport by alternating changes of the specificities for catalysis of the chemical and vectorial reactions, as shown in Scheme 1 (Abeles *et al*., 1992). Binding of three Na^+ ions to the intracellular sites activates the enzyme for phosphorylation of a β-carboxyl group of aspartate at the active site by ATP. Phosphorylation changes the vectorial specificity and allows the Na^+ ions to dissociate into the extracellular medium. Binding of two K^+ ions from the extracellular solution changes the chemical specificity for catalysis and allows the phosphoenzyme to react with water. Dephosphorylation changes the vectorial specificity and allows the two K^+ ions to dissociate into the cytoplasm of the cell (Skou, 1965; Post, 1965; Post *et al*., 1969, 1972; Post & Sen, 1973). These alternating changes of the specificities for catalysis of the chemical and vectorial reactions require that neither the chemical reaction of ATP hydrolysis nor the vectorial reaction of sodium and potassium transport takes place unless the other reaction also occurs (Jencks, 1980, 1982, 1983, 1989).

Scheme 1



We report here kinetic measurements using the rapid mix−quench technique which demonstrate that a conformational change of the $E{\cdot}Na_3{\cdot}$ATP complex, formed from the sodium−potassium ATPase of sheep kidney in the presence of ATP and Na^+, is rate-limiting for phosphoenzyme formation. The fact that a kinetically significant conformational change occurs following the binding of ATP to $E{\cdot}Na_3$ shows that enzyme with Na^+ bound to the interior, high-affinity sites is not yet in a form capable of reacting with ATP. This means that changes in the vectorial specificity for the binding/dissociation of Na^+ and K^+ on the two sides of the membrane and in the chemical specificity for reaction with ATP/ADP do not occur simultaneously, as implied in the classical "E_1-E_2" model for active transport (Albers, 1967; Post *et al*., 1969, 1972), but occur in separate steps along the reaction pathway.

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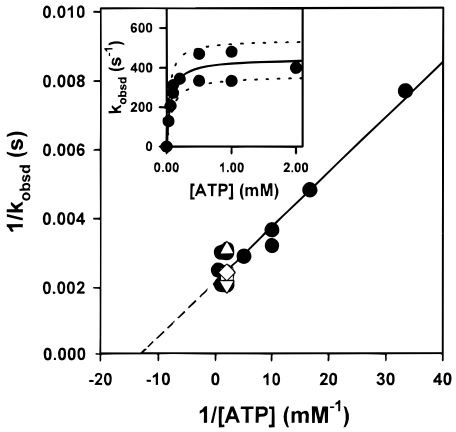


FIGURE 1: Double reciprocal plots of the pseudo-first order rate constants for phosphorylation of the ATPase at different concentrations of [γ-^{32}P]ATP at 25 °C and pH 7.4. Final conditions were 30 mM Tris buffer, 0.29 mg/mL ATPase, 120 (●, ▽, ◇), 150 (□), or 200 (△) mM NaCl, and 3 (●, □, △), 5 (▽), or 10 (◇) mM $MgCl_2$. Syringe A contained 0.58 mg/mL ATPase, syringe B contained [γ-^{32}P]ATP at twice the final concentrations, and syringe C contained 1.5 M HCl. All other components were present in syringes A and B at the final concentrations (see Methods). The solid line is drawn for a maximum rate constant of 460 s^{-1} and an equilibrium constant of 75 μM for ATP. The inset shows the same data plotted as a hyperbolic relationship. The dashed lines of the inset represent the minimum and maximum values predicted by the relative error in the values of k_f and $K_{0.5}$ for ATP.

MATERIALS AND METHODS

Materials. Reagents were generally of the highest purity available and were used without further purification. Na_2ATP and Na_2ADP were obtained from Boehringer Mannheim, and [γ-^{32}P]ATP (>99% pure) was from New England Nuclear.

Sodium−potassium adenosinetriphosphatase was prepared from sheep kidneys by a slight modification of the Jørgensen procedure (Jørgensen, 1974a). Sheep kidneys were obtained within 30 min of exsanguination and were either frozen quickly on dry ice or sliced immediately and allowed to soak overnight in an ice cold solution of 0.25 M sucrose, 20 mM histidine-HCl, 1.0 mM ethylenediaminetetraacetic acid (EDTA), 100 mM NaCl, and 30 mM Tris buffer (pH 7.5). After purification according to the method of Jørgensen [using 0.4 mg/mL sodium dodecyl sulfate (SDS) and 1.6 mg/mL protein in the SDS incubation step], the preparations catalyzed the hydrolysis of ATP with a rate of 4−9 μmol (mg of total protein)$^{-1}$ min^{-1} and appeared as only α and β chains by SDS−polyacrylamide gel electrophoresis (PAGE). The activity (≤25% of maximal activity) of the purified ATPase enzyme was inhibited 98−99% by 1.0 mM ouabain.

Methods. Sodium−potassium adenosinetriphosphatase activity was measured spectrophotometrically by coupling the production of ADP to the oxidation of NADH with pyruvate kinase (PK) and lactate dehydrogenase (LDH, Rossi *et al*., 1979). Standard conditions were 30 mM Tris buffer at pH 7.4, 110 mM NaCl, 15 mM KCl, 3 mM $MgCl_2$, 1 mM EDTA, 2.0 mM ATP, 1.5 mM PEP, 0.15 mM NADH, 50 μg/mL PK, and 50 μg/mL LDH at 37 °C. The protein concentration was estimated by the procedure of Lowry *et al*. (1951) with bovine serum albumin as a standard.

The formation of phosphoenzyme was followed by rapid mix−quench techniques (Froehlich *et al*., 1976b; Stahl & Jencks, 1984; Johnson, 1986) using a KinTek rapid-mixing apparatus. The temperature-equilibrated contents of ~15 μL sample loops filled from syringes A and B are driven into a mixing chamber, and after the contents flow through a length of narrow-bore Teflon tubing for t_1 = 2 ms to 50 s, the reaction is quenched in a second mixing chamber by 1.5 N hydrochloric acid from syringe C. The instrument tube lengths were calibrated with solutions of [γ-^{32}P]ATP, and the reaction times were confirmed from measurements of the rate constant for hydrolysis of benzylidene malononitrile in 1 N NaOH at 20 °C.

Sodium−potassium adenosinetriphosphatase was prepared for phosphorylation by mixing with buffer containing 120 mM NaCl, 3 mM $MgCl_2$, and 30 mM Tris buffer at pH 7.4 and 24 °C. The enzyme was found to be stable in this medium at room temperature over the 1 h periods in which it was used. For each reaction, 14.7 μL of the enzyme solution was loaded into sample loop A of the mixing apparatus and the reaction was started within 10 s. Bovine serum albumin (1.0 mg) was added as carrier to each quenched reaction mixture prior to precipitation by trichloroacetic acid to give a final concentration of 12%. The amount of [^{32}P]E-P in the quenched reaction mixtures was determined essentially as described by Verjovski-Almeida *et al*. (1978). E_{tot} was taken as the amount of phosphoenzyme formed after a long (>5 s) reaction. For the zero-time points, the order of addition of quenching acid and [γ-^{32}P]ATP was reversed.

RESULTS AND DISCUSSION

Reaction of E·Na$_3$ with ATP. The reaction of sheep kidney ATPase preincubated with 120 mM Na^+ and 3 mM Mg^{2+} (E·Na_3) with 0.01−2.0 mM [^{32}P]ATP at 25 °C and pH 7.4 results in the formation of [^{32}P]E-P·Na_3. Previous investigations of this behavior, under a variety of conditions (Hegyvary & Post, 1971; Froehlich *et al*., 1976a; Mårdh & Lindahl, 1977; Mårdh & Post, 1977), have reported equilibrium dissociation constants for ATP of 0.1−20 μM and rate constants for phosphorylation of 140−250 s^{-1} with saturating concentrations of ATP of less than 20−100 μM (Mårdh & Zetterqvist, 1974; Froehlich *et al*., 1976a; Mårdh & Lindahl, 1977; Hobbs *et al*., 1980). Although we also observe similar rate constants for phosphorylation at such concentrations of ATP (Figure 1 and supporting information), the first order rate constant increases to >250 s^{-1} at concentrations of ATP above 100 μM. The observed end points for these reactions (E-P_{max}/E_{tot}) were found to be stable for as long as 50 s, and the stoichiometry of phosphorylation was unimolar given the specific activity of the enzyme preparations (Jørgensen, 1974b).

Figure 1 shows that the first order rate constant for phosphorylation of E·Na_3 (k_{obsd}) increases hyperbolically with increasing ATP concentration, with a maximum value of k_{obsd} = (4.6 ± 0.9) × 10^2 s^{-1} and $K_{0.5}$ = 75 ± 25 μM for ATP. Phosphorylation proceeds to completion, because hydrolysis of the phosphoenzyme is inhibited in the absence of potassium. The back-reaction with ADP (k_r, eq 1) is not significant under these conditions because the maximum concentration of ADP produced in the reaction is less than 2.5 μM.

$$\text{E·Na}_3 + \text{ATP} \rightleftharpoons \text{E·Na}_3\text{·ATP} \underset{k_r}{\overset{k_f}{\rightleftharpoons}} \begin{matrix}\text{E~P·Na}_3\text{·ADP} \\ \Updownarrow \\ \text{E~P·Na}_3 + \text{ADP}\end{matrix} \qquad (1)$$

Also shown in Figure 1 (open symbols) are the pseudo-first order rate constants for phosphorylation of the ATPase

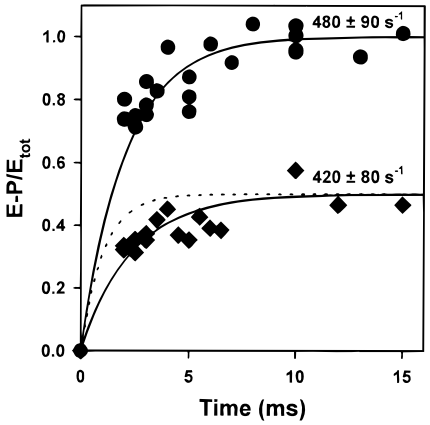


FIGURE 2: Phosphorylation of E·Na_3 with ATP ± ADP. The final conditions were 30 mM Tris buffer, pH 7.4, 120 mM NaCl, 3 mM $MgCl_2$, 1.0 mM [γ-^{32}P]ATP (◆, ●), and 2.0 mM ADP (◆) at 25 °C. Reactions were carried out essentially as described in Figure 1. The solid lines were drawn for first order rate constants of 480 s^{-1} (●, no ADP) and 420 s^{-1} (◆, 2.0 mM ADP). The dashed line is drawn for the first order rate constant of 960 s^{-1} that would be expected for approach to equilibrium if the phosphorylation step were rate-limiting [Note that, although the rate constants of 480 and 420 s^{-1} have half-times which approach the limit of detection for this system, the amount of phosphorylation at such times (~2 ms) is still significantly distinguishable (within 2σ) from that expected for phosphorylation with a first order rate constant of 960 s^{-1}].

Table 1: Rate Constants and the Fraction of *E-P*/E_{tot} Formed in the Reaction of E·Na_3 with ATP ± ADP at pH 7.4 (30 mM Tris Buffer) and 25 °C

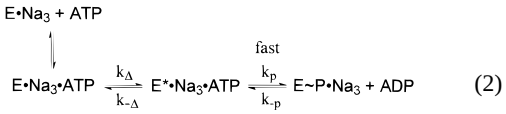
[ATP] (mM)	[ADP] (mM)	[NaCl] (mM)	[$MgCl_2$] (mM)	k_{obsd} ($\times 10^2$ s^{-1})	*E-P*/E_{tot}
0.50	0	120	3	3.3	1.0
0.50	0	120	3	3.8	0.82
0.50	0	150	3	4.2	0.91
0.50	0	200	3	3.2	0.96
0.50	0	120	5	4.8	0.87
0.50	0	120	10	4.1	0.91
0.50	0.1	120	3	3.3	0.69
0.50	0.2	120	3	2.5	0.66
0.50	0.5	120	3	2.3	0.46
1.0	0	120	3	4.8	1.0
1.0	0.5	120	3	4.8	0.82
1.0	0.5	120	3	5.0	0.80
1.0	1.0	120	3	4.2	0.68
1.0	1.0	120	3	4.5	0.70
1.0	2.0	120	3	4.2	0.50

with a saturating concentration of 0.5 mM ATP and increasing concentrations of Na^+ and Mg^{2+}. Since the rate constant for phosphorylation does not increase significantly in the range of 3−10 mM Mg^{2+} and 120−200 mM Na^+ (Table 1), the concentrations of 0.5 mM ATP, 120 mM Na^+, and 3 mM Mg^{2+} were concluded to be saturating. Campos and Beaugé (1992) have reported that the K_M for Mg^{2+} is 38 μM. Mårdh and Post (1977) have reported that the K_M for Na^+ is 8 mM.

Reaction of E·Na₃ with ATP plus ADP. The following experiment was performed in order to identify the rate-limiting step for the phosphorylation of E·Na_3 by ATP. In the absence of ADP, phosphorylation by ATP proceeds to 100% E-P of E_{tot} with an observed rate constant of $k_{obsd} = k_f = (4.8 \pm 0.9) \times 10^2$ s^{-1} (Figure 2). If the rate-limiting step with saturating ATP were phosphoryl transfer, then the observed rate constant for approach to the equilibrium concentration of phosphoenzyme in the presence of ADP would equal the sum of the first order rate constants for the forward and the reverse phosphoryl-transfer steps; i.e., $k_{obsd} = k_f + k_r$. Phosphorylation of the enzyme in the presence of ADP at a concentration sufficient to lower the equilibrium concentration of E-P to 50% of E_{tot} would then require that $k_f = k_r = 480$ s^{-1} and $k_{obsd} = 480 + 480 = 960$ s^{-1} according to this model. However, if a conformational change is rate-limiting and is followed by fast phosphorylation by ATP, then k_{obsd} is expected to be 480 s^{-1} in the presence and absence of ADP.

Table 1 shows that the observed rate constant for phosphorylation does not increase in the presence of added ADP. Furthermore, Figure 2 shows that phosphorylation in the presence of 1.0 mM ADP proceeds to equilibrium to give ~50% of the maximum phosphoenzyme with an observed rate constant of $(4.2 \pm 0.8) \times 10^2$ s^{-1}. This does not approach the rate constant of 960 s^{-1} that is expected for approach to equilibrium if phosphoryl transfer were rate-limiting (Figure 2, dashed line). Note that the observed rate constants for phosphorylation in the presence and absence of added ADP have half-times that approach the limit of detection for a rapid mix−quench kinetic study (Mårdh & Zetterqvist, 1974; Froehlich *et al*., 1976a,b; Mårdh & Lindahl, 1977; Mårdh & Post, 1977; Hobbs *et al*., 1980; Stahl & Jencks, 1984; Johnson, 1986). This is reflected in the relative error of the reported rate constants. However, the observed early data points are nevertheless reproducibly statistically distinguishable from those that would be observed if the rate constant were 2-fold larger, as expected for a rate-limiting phosphoryl transfer (Figure 2, dashed line). The decrease in k_{obsd} from 4.8×10^2 to 4.2×10^2 s^{-1} in the presence of ADP, if it is real, may arise from competition with ATP for binding to E·Na_3; such inhibition does occur at lower concentrations of ATP (Table 1).

We conclude that the rate-limiting step for phosphorylation is a conformational change of the initially formed E·Na_3·ATP complex to give an active species, E*·Na_3·ATP, that catalyzes phosphoryl transfer (k_Δ, eq 2) at a rate that is too fast to measure. Simulations of the time course show that



(2)

the absence of a detectable induction period for phosphorylation requires that the rate constant for the phosphoryl-transfer step after the conformational change (k_p, eq 2) be >3000 s^{-1}.

Although it cannot be excluded that this observed kinetic behavior reflects the properties of a partially damaged (≤25% active) enzyme, it seems reasonable to use a partially active enzyme since phosphorylation is what is measured, and inactive enzyme is assumed not to phosphorylate. Yet the recent results of Peluffo and co-workers (Peluffo *et al*., 1992) suggest that an inactive enzyme, under certain circumstances, may also be phosphorylated. In their investigation, enzyme exhibiting both high- and low-affinity ATP binding sites underwent rapid (370 s^{-1}) "superphosphorylation" to 2.4-fold over the stoichiometric level, followed by dephosphorylation to return to the stoichiometric level within 40 ms. However, the purified enzyme used in this study displayed a linear affinity for ATP (Figure 1) over the concentration range studied and was rapidly (460 s^{-1}) and stoichiometrically (Jørgensen, 1974b) phosphorylated, showing no evidence

of biphasic kinetics, to an end point that was stable for at least 50 s.

Experiments similar to ours with a related P-type ATPase, the Ca^{2+}-ATPase from sarcoplasmic reticulum, have shown that the rate-limiting step for phosphorylation is a conformational change giving the activated enzyme−substrate complex, which is phosphorylated at a rate that is too fast to measure with rapid-mixing techniques (Petithory & Jencks, 1986).

Additional evidence for a conformational change of the E·Na_3·ATP complex prior to phosphoryl transfer has been obtained indirectly by Taniguchi and co-workers (Taniguchi *et al*., 1984, 1986, 1991). They initially interpreted fluorescence changes upon formation of E-P from the free enzyme in terms of a pathway that proceeds through an "activated" enzyme−substrate complex, analogous to our E*·Na_3·ATP. Later they suggested that the change in fluorescence reflects steps in the transit of Na^+ through the enzyme.

Finally, Tonomura and co-workers have also proposed the existence of an activated enzyme−substrate complex. They observed that, upon interruption of enzyme phosphorylation through the addition of excess EDTA or unlabeled ATP, the rate of appearance of labeled P_i from phosphoenzyme hydrolysis was greater than the rate of disappearance of labeled E-P (Kanazawa *et al*., 1970). This was accounted for through the proposal of a pathway for phosphorylation from [γ-^{32}P]ATP which proceeds through the irreversible conformational change of one enzyme−substrate complex, E·ATP, into another, E′·ATP, which is able to form phosphoenzyme even after the addition of excess EDTA or unlabeled ATP (eq 3).

$$E + ATP \rightleftharpoons E{\cdot}ATP \rightarrow E'{\cdot}ATP \rightleftharpoons E\text{-}P \qquad (3)$$

The Post−Albers scheme has been proven to be very useful for the visualization of the different conformations of Na^+,K^+-ATPase and their participation in cation transport. Unfortunately, use of the E_1-E_2 nomenclature implies that the phosphoenzyme and free enzyme can exist in only two conformational states and that these two states account for the coupling of Na^+ and K^+ transport to the hydrolysis of ATP. In the present work, we provide evidence for a conformational change (of the E_1 enzyme) other than the single conformational change described by a strict interpretation of the E_1-E_2 model. We therefore agree with others (Nørby, 1988; Glynn & Karlish, 1990; Pratap & Robinson, 1993) that there are several conformational changes in the reaction cycle of the sodium−potassium ATPase and that the mechanism of the coupling of ATP hydrolysis to the transport of sodium and potassium ions is not adequately described by nomenclature derived from a simple two-state model.

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SUPPORTING INFORMATION AVAILABLE

A table containing the rate constants and end points for the phosphorylation of sodium−potassium adenosinetriphosphatase by 0.03−2.0 mM ATP in the presence of 120 mM NaCl and 3 mM $MgCl_2$ at pH 7.4 (30 mM Tris buffer) and 25 °C (1 page). Ordering information is given on any current masthead page.

REFERENCES

Abeles, R. H., Frey, P. A., & Jencks, W. P. (1992) in *Biochemistry*, pp 791−822, Jones and Bartlett Publishers, Inc., Boston.
Albers, R. W. (1967) *Annu. Rev. Biochem. 36*, 727−756.
Campos, M., & Beaugé, L. (1992) *Biochim. Biophys. Acta 1105*, 51−60.
Cantley, L. C. (1981) *Curr. Top. Bioenerg. 11*, 201−237.
Froehlich, J. P., Albers, R. W., Koval, G. J., Goebel, R., & Berman, M. (1976a) *J. Biol. Chem. 251*, 2186−2188.
Froehlich, J. P., Sullivan, J. V., & Berger, R. L. (1976b) *Anal. Biochem. 73*, 331−341.
Glynn, I. M. (1985) in *The Enzymes of Biological Membranes* (Martonosi, A. M., Ed.) pp 35−114, Plenum Press, New York.
Glynn, I. M., & Karlish, S. J. D. (1990) *Annu. Rev. Biochem. 59*, 171−205.
Hegyvary, C., & Post, R. L. (1971) *J. Biol. Chem. 246*, 5234−5240.
Hobbs, A. S., Albers, R. W., & Froehlich, J. P. (1980) *J. Biol. Chem. 255*, 3395−3402.
Jencks, W. P. (1980) *Adv. Enzymol. 51*, 75−106.
Jencks, W. P. (1982) in *Membranes and Transport* (Martonosi, A. M., Ed.) pp 515−520, Plenum Press, New York.
Jencks, W. P. (1983) *Curr. Top. Membr. Transp. 19*, 1−19.
Jencks, W. P. (1989) *Methods Enzymol. 171*, 145−164.
Johnson, K. A. (1986) *Methods Enzymol. 134*, 677−705.
Jørgensen, P. L. (1974a) *Biochim. Biophys. Acta 356*, 36−52.
Jørgensen, P. L. (1974b) *Biochim. Biophys. Acta 356*, 53−67.
Kanazawa, T., Saito, M., & Tonomura, Y. (1970) *J. Biochem. 67*, 693−711.
Lowry, D. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265−275.
Mårdh, S., & Zetterqvist, Ö. (1974) *Biochim. Biophys. Acta 350*, 473−483.
Mårdh, S., & Lindahl, S. (1977) *J. Biol. Chem. 252*, 8058−8061.
Mårdh, S., & Post, R. L. (1977) *J. Biol. Chem. 252*, 633−638.
Nørby, J. G. (1988) *Braz. J. Med. Biol. Res. 21*, 1251−1259.
Peluffo, R. D., Garrahan, P. J., & Rega, A. F. (1992) *J. Biol. Chem. 267*, 6596−6601.
Petithory, J. R., & Jencks, W. P. (1986) *Biochemistry 25*, 4493−4497.
Post, R. L. (1989) *Annu. Rev. Physiol. 51*, 1−15.
Post, R. L., & Sen, A. K. (1965) *J. Histochem. 13*, 105−112.
Post, R. L., & Kume, S. (1973) *J. Biol. Chem. 248*, 6993−7000.
Post, R. L., Kume, S., Tobin, T., Orcutt, B., & Sen, A. K. (1969) *J. Gen. Physiol. 54*, 306S−326S.
Post, R. L., Hegyvary, C., & Kume, S. (1972) *J. Biol. Chem. 247*, 6530−6540.
Pratap, P. R., & Robinson, J. D. (1993) *Biochim. Biophys. Acta 1151*, 89−98.
Robinson, J. D., & Pratap, P. R. (1993) *Biochim. Biophys. Acta 1154*, 83−104.
Rossi, B., Leone, F. de A., Gache, C., & Lazdunski, M. (1979) *J. Biol. Chem. 254*, 2302−2307.
Skou, J. C. (1965) *Physiol. Rev. 45*, 596−617.
Skou, J. C., & Esmann, M. (1992) *J. Bioenerg. Biomembr. 24*, 249−261.
Stahl, N., & Jencks, W. P. (1984) *Biochemistry 23*, 5389−5392.
Taniguchi, K., Suzuki, K., Kai, D., Matsuoka, I., Tomita, K., & Iida, S. (1984) *J. Biol. Chem. 259*, 15228−15233.
Taniguchi, K., Suzuki, K., Sasaki, T., Shimokobe, H., & Iida, S. (1986) *J. Biochem. 100*, 1231−1239.
Taniguchi, K., Sasaki, T., Shinoguchi, E., Kamo, Y., & Ito, E. (1991) *J. Biochem. 109*, 299−306.
Verjovski-Almeida, S., Kurzmack, M., & Inesi, G. (1978) *Biochemistry 17*, 5006−5013.

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