*Journal of Bioenergetics and Biomembranes, Vol. 16, No. 3, 1984*

# Effects of Pyridoxal Phosphate Treatment on the (Na + K)-ATPase

Joseph D. Robinson[1]

*Received August 11, 1983; revised November 3, 1983*

## Abstract

Reaction of a dog kidney (Na + K)-ATPase with pyridoxal phosphate, followed by borohydride reduction, reduced the catalytic activity when measured subsequently. The time course of inactivation did not follow a first-order process, and certain characteristics of the residual enzymatic activity were modified. Moreover, various catalytic activities were diminished differently: Na-ATPase activity was largely spared, K-phosphatase activity was diminished only by half that of the (Na + K)-ATPase, whereas (Na + K)-CTPase and Na-CTPase activities were diminished more. ATP, ADP, CTP, nitrophenyl phosphate, and $P_i$ all protected against inactivation. Increasing salt concentrations increased inactivation, but KCl slowed and NaCl hastened inactivation when compared with choline chloride. Occupancy of certain substrate or cation sites seemed more crucial than selection of conformational states. For the residual (Na + K)-ATPase activity the $K_{0.5}$ for $K^+$ was lower and the $K_{0.5}$ for $Na^+$ higher, while the sensitivities to ouabain, oligomycin, and dimethylsulfoxide were diminished; for the residual K-phosphatase activity the $K_{0.5}$ for $K^+$ was unchanged, the sensitivity to ouabain and oligomycin diminished, but the stimulation by dimethylsulfoxide increased. These properties cannot be wholly accommodated by assuming merely shifts toward either of the two major enzyme conformations.

**Key Words:** (Na + K)-ATPase; pyridoxal phosphate; chemical modification; conformational changes; enzyme kinetics.

## Introduction

Current formulations of the reaction mechanism of the (Na + K)-ATPase depict a cyclical process passing through phosphorylated and dephosphory-

[1]Department of Pharmacology, State University of New York, Upstate Medical Center, Syracuse, New York 13210.



0145-479X/84/0600-0195$03.50/0 

lated states of two major conformational families

$$\begin{array}{ccc} E_1 & \rightleftharpoons & E_1 - P \\ \uparrow\downarrow & & \downarrow\uparrow \\ E_2 & \rightleftharpoons & E_2 - P \end{array}$$

with evidence for the conformational changes based on differential reactivity toward physiological ligands and experimental reagents and on fluorescence changes (Robinson and Flashner, 1979a; Cantley, 1981; Schuurmans Stekhoven and Bonting, 1981). Earlier studies on a rat brain enzyme showed that both $K^+$, considered to favor $E_2$ conformations, and ATP, considered to favor $E_1$ conformations, diminish enzyme inactivation by acetic anhydride and trinitrobenzene sulfonate (Robinson and Flashner, 1979b). Such analogous effects of ligands favoring alternative conformations clearly are not readily compatible with mechanisms based on selection of specific conformations. Moreover, although the modified enzyme had a decreased apparent affinity for $K^+$ as activator of the associated K-phosphatase activity, there was little change in apparent affinity for $Na^+$ or for $K^+$ as activators of the (Na + K)-ATPase activity. With this enzyme preparation ATP also diminished enzyme inactivation by pyridoxal phosphate, but $K^+$ had far less effect and the $K_{0.5}$ for $K^+$ as activator was unchanged (Robinson and Flashner, 1979b).

Recently, Skou (1982) showed that pyridoxal phosphate inactivation of a shark rectal gland enzyme was diminished both by ATP and by $K^+$, and that the modified enzyme appeared to be shifted toward the $Na^+$-selected $E_1$ conformations, as identified by fluorescence changes of the eosin-labeled enzyme. But, like the acetic anhydride-treated or trinitrobenzene sulfonate-treated rat enzymes, the K-phosphatase activity of the pyridoxal phosphate-treated shark enzyme was diminished less than the (Na + K)-ATPase activity, which seems inconsistent with a shift toward $E_1$ conformations since the K-phosphatase activity is catalyzed by $E_2$ forms (Robinson *et al.*, 1983). In addition, unlike the effects of those treatments on the rat enzyme, pyridoxal phosphate treatment of the shark enzyme appeared to increase the apparent affinity for $Na^+$ when $Na^+$ and $K^+$ were varied reciprocally together (Skou, 1982).

The experiments described here were designed to explore further the effects of pyridoxal phosphate, using a standard dog kidney enzyme preparation (Jorgensen, 1974), examining the effects (i) on inactivation of reagents presumed to favor certain conformational states, and (ii) on the properties of the modified enzyme, in terms of the presumed characteristics of those conformational states. Some differences in experimental results suggest alternative interpretations of the effects of pyridoxal phosphate treatment. In addition, the data emphasize difficulties in reconciling conflicting responses from different probes within the simple schemes proposed.

## Methods and Materials

The enzyme preparation was obtained from medullae of frozen canine kidneys, following the procedure of Jorgensen (1974). The specific activity of the (Na + K)-ATPase activity ranged from 16 to 22 $\mu$mol $P_i$ liberated/mg protein · min at 37°C.

Reaction of the enzyme with pyridoxal phosphate was for 15 min unless otherwise specified, at 0°C in the dark. The standard medium contained 40–50 $\mu$g/ml enzyme protein, 1 mM pyridoxal phosphate, 40 mM imidazole/HCl (pH 7.8), 80 mM sucrose, and 10 mM choline chloride; the reaction was terminated by addition at 0°C of freshly prepared 0.15 M sodium borohydride (in water) to give a final concentration of 15 mM, and 5 min later by dilution with 20 vol of 0.25 M sucrose and centrifugation for 40 min at 50,000 × $g$. The pellet was resuspended in 0.25 M sucrose and assayed within 4 hr; the enzyme was shielded from light until completion of the assay. In all cases activity is compared to control preparations treated identically except for the absence of pyridoxal phosphate.

(Na + K)-ATPase activity was measured at 37°C in terms of the production of $P_i$, as described previously (Robinson, 1967). The standard incubation medium contained 30 mM histidine · HCl/Tris (pH 7.8), 3 mM ATP, 3 mM $MgCl_2$, 90 mM NaCl, and 10 mM KCl. Na-ATPase activity was measured under the same conditions, except that KCl was omitted and the NaCl concentration was 100 mM. (Na + K)-CTPase and Na-CTPase activities were measured by substituting 3 mM CTP for ATP in the corresponding media. K-phosphatase activity was measured in terms of nitrophenol production from incubation at 37°C with nitrophenyl phosphate (NPP) as substrate (Robinson, 1969). The standard incubation medium contained 30 mM histidine · HCl/Tris (pH 7.8), 3 mM NPP, 3 mM $MgCl_2$, and 10 mM KCl. In all cases incubations were performed in duplicate or triplicate in parallel with control enzyme; data presented are averages of four or more such experiments and are presented ±S.E.M. where appropriate.

Frozen canine kidneys were obtained from Pel Freeze, and ATP, ADP, CTP, NPP, ouabain, oligomycin, and pyridoxal phosphate from Sigma.

## Results

Prior incubation of the (Na + K)-ATPase with pyridoxal phosphate markedly diminished enzyme activity measured subsequently. Incubation of the enzyme preparations at 0° with 1 mM pyridoxal phosphate, followed by addition of sodium borohydride, dilution, and washing by centrifugation, resulted in a time-dependent decrease in (Na + K)-ATPase activity that

deviated somewhat from a first-order course even in the initial 10–15 min of the prior incubation (Fig. 1).

Treatment with sodium borohydride alone had little effect on enzymatic activity assayed in the standard medium, averaging 93 ± 2% of a control preparation subjected to similar incubation, dilution, and centrifugation; however, despite the presence of the imidazole buffer, the pH after addition of sodium borohydryde rose to 8.1. [That sodium borohydride treatment itself had little effect on the kinetic properties is attested to by the similarities between the control values reported below and those previously described (Robinson, 1980a).]

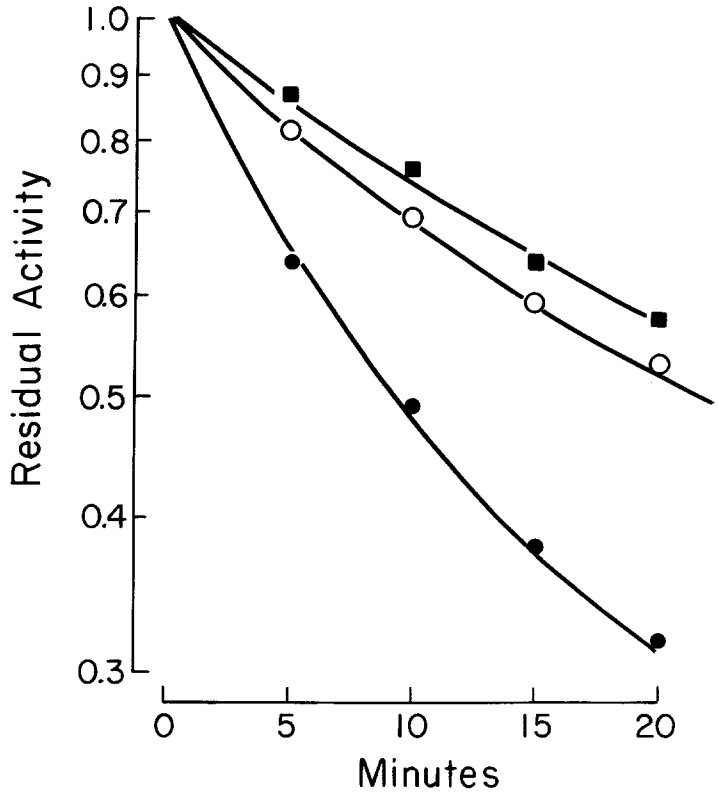


**Fig. 1.** Loss of (Na + K)-ATPase activity during treatment with pyridoxal phosphate. The remaining (Na − K)-ATPase activity following treatment with pyridoxal phosphate is plotted against the duration of the incubation of 0°C in the standard inactivation medium (●), as described under Methods, or in that medium modified by substituting 10 mM KCl for 10 mM choline chloride (○) or by adding 10 μM ATP (■). In all cases residual activity is compared to control activity of enzyme treated identically except for the absence of pyridoxal phosphate.

**Table I.** Effects of Pyridoxal Phosphate Treatment on Catalytic Activities of the (Na + K)-ATPase[a]

| Catalytic activity | % Loss of activity |
|---|---|
| (Na + K)-ATPase | 62 ± 1 |
| Na-ATPase | 6 ± 2 |
| (Na + K)-CTPase | 83 ± 4 |
| Na-CTPase | 66 ± 6 |
| K-Phosphatase | 27 ± 3 |

[a]The enzyme preparation was first reacted with pyridoxal phosphate in the standard inactivation medium for 15 min at 0°C, and the residual catalytic activities then assayed in the standard incubation media at 37°C, as described under Methods; loss of activity is compared to concurrent controls without pyridoxal phosphate.

The various reactions catalyzed by this enzyme were affected by pyridoxal phosphate treatment differently (Table I). Na-ATPase activity, measured with 100 mM NaCl and no KCl, was scarcely inhibited after initial incubation with pyridoxal phosphate for 15 min, whereas both (Na + K)-CTPase and Na-CTPase activities were diminished more than the corresponding ATPase activities. The enzyme also catalyzes a K-dependent phosphatase reaction, and this activity measured with NPP as substrate was diminished by only half as much as the (Na + K)-ATPase.

The rate of inactivation by pyridoxal phosphate could be modified by including in the initial incubation medium various agents known to affect enzyme activity. Low concentrations of ATP severely slowed the decline in (Na + K)-ATPase activity (Fig. 2), with a $K_{0.5}$ near 10 $\mu$M (Fig. 2). ADP was equally effective, although with CTP the $K_{0.5}$ was near 0.5 mM (Fig. 2). Both NPP and $P_i$ could also protect against the loss of activity, although the concentrations required were still greater (Fig. 2; Table II). Adding EDTA, either alone or with the nucleotides, did not appreciably alter the loss in (Na + K)-ATPase activity; correspondingly, adding $MgCl_2$ did not improve the protection by ADP or by $P_i$ (Table II).

The rate of inactivation by pyridoxal phosphate could also be sharply curtailed by substituting 10 mM KCl for 10 mM choline chloride in the initial incubation medium (Fig. 1). Under these conditions of substituting $K^+$ for choline the $K_{0.5}$ for $K^+$ as a protector against enzyme inactivation was roughly 0.2 mM (Fig. 3). On the other hand, substituting 10 mM NaCl for choline chloride led to further enzyme inactivation (Fig. 4). Although inactivation increased with salt concentration, KCl consistently preserved and NaCl consistently diminished enzyme activity over the range 1 to 100 mM, when compared to choline chloride (Fig. 4).

$K^+$ is thought to affect the (Na + K)-ATPase not only by activating dephosphorylation of the enzyme but also by favoring the $E_2$ enzyme conformations; similarly, $Na^+$ not only by activating phosphorylation but also

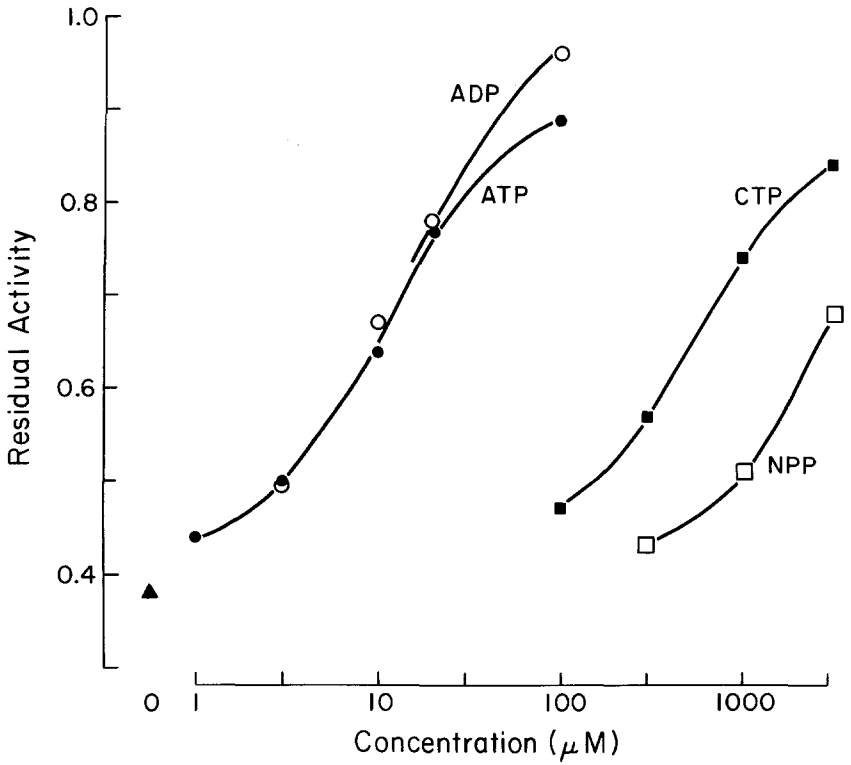


**Fig. 2.** Protection against pyridoxal phosphate-induced loss of activity. Residual (Na + K)-ATPase activity of enzyme reacted with pyridoxal phosphate for 15 min in the standard medium containing the additions shown is plotted against the concentrations of the additions: ATP (●), ADP (○), CTP (■), and NPP (□).

**Table II.** Effects of Various Agents on the Loss of (Na + K)-ATPase Activity During Incubation with Pyridoxal Phosphate[a]

| Addition to inactivation medium | % Loss of activity |
|---|---|
| None | 62 ± 1 |
| Triton X-100, 0.08 μl/ml | 66 ± 2 |
| Oligomycin, 10 μg/ml | 65 ± 2 |
| Dimethylsulfoxide, 100 μl/ml | 61 ± 1 |
| $MgCl_2$, 0.05 mM | 63 ± 2 |
| 1.0 mM | 61 ± 2 |
| EDTA, 0.1 mM | 67 ± 2 |
| ATP, 0.01 mM | 36 ± 1 |
| plus EDTA, 0.1 mM | 36 ± 3 |
| ADP, 0.01 mM | 31 ± 2 |
| plus EDTA, 0.1 mM | 34 ± 2 |
| plus $MgCl_2$, 0.05 mM | 37 ± 1 |
| NPP, 1 mM | 49 ± 2 |
| $P_i$ 1 mM | 41 ± 2 |
| plus $MgCl_2$, 1 mM | 53 ± 3 |

[a]Experiments were performed as in Table I, except that the additions noted were made to the standard inactivation medium; comparison in activity is made to controls containing identical additions except for pyridoxal phosphate.

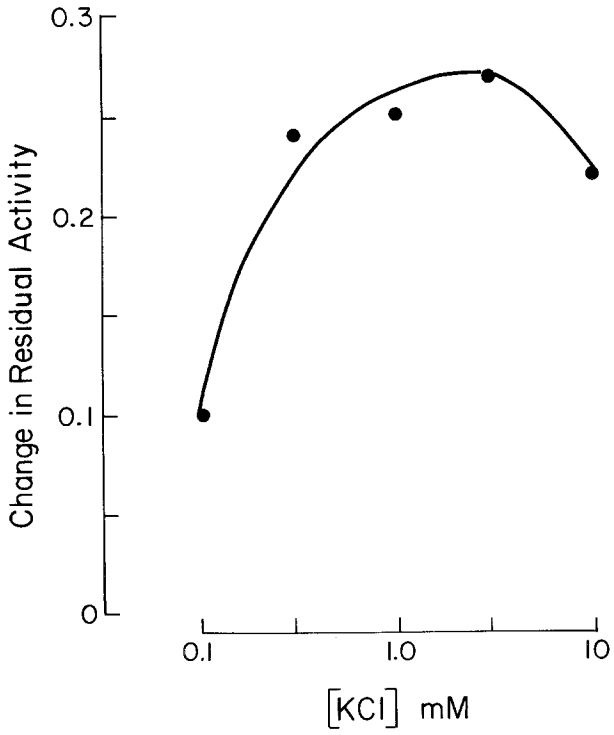


**Fig. 3.** Effect of KCl on pyridoxal phosphate-induced loss of activity. Residual (Na + K)-ATPase activity is plotted against the concentration of KCl substituted for choline chloride, on an equimolar basis, in the standard inactivation medium.

by favoring the $E_1$ conformations (Karlish, 1980). Dimethylsulfoxide is also thought to favor the $E_2$ conformations and Triton X-100 the $E_1$ conformations (Robinson, 1980a), whereas oligomycin is thought to inhibit the conversion of $E_1$ conformations to $E_2$ (Fahn *et al.*, 1966; Hobbs *et al.*, 1983). However, unlike $K^+$ and $Na^+$, none of these had appreciable effects on enzyme inactivation by pyridoxal phosphate (Table II).

Initial incubation with pyridoxal phosphate not only led to a loss of enzymatic activity, inactivation, but it also modified certain properties of the remaining enzymatic activity. For the residual (Na + K)-ATPase activity the $K_{0.5}$ for $Na^+$ as an activator nearly doubled, rising from 8 to 13 mM (Fig. 5A). Conversely, the $K_{0.5}$ for $K^+$ as an activator decreased, from 0.8 to 0.5 mM (Fig. 5B). The $K_m$ for ATP as substrate did not change detectably (data not presented). Sensitivity to certain inhibitors was, however, altered. Inhibition by ouabain was diminished somewhat (Fig. 6), as was inhibition by both oligomycin and dimethylsulfoxide (Table III).

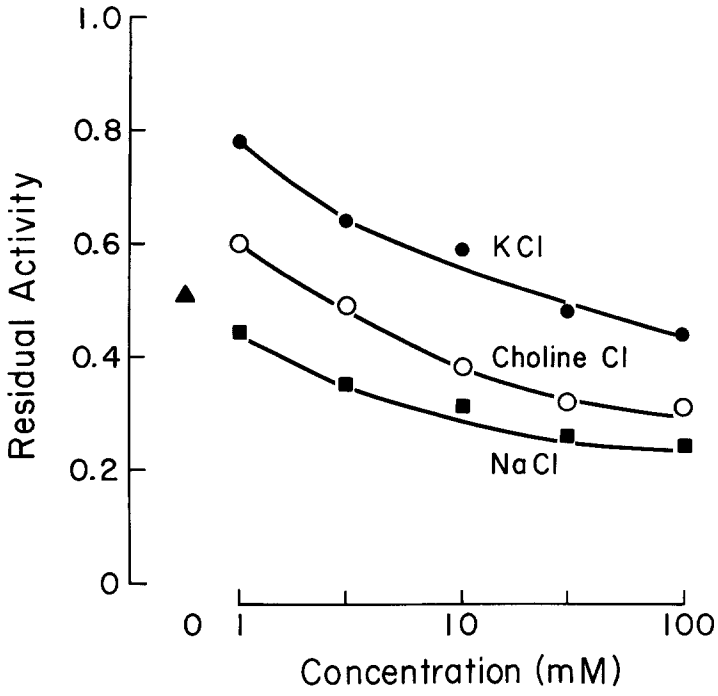


**Fig. 4.** Effects of salts on pyridoxal phosphate-induced loss of activity. Residual (Na + K)-ATPase activity is plotted against the concentrations of KCl (●), NaCl (■), or choline chloride (O) present in the inactivation medium. Residual activity with no monovalent cations added to the inactivation medium (▲) is also plotted.

Unlike the residual (Na + K)-ATPase activity, the residual K-phosphatase activity did not have an altered $K_{0.5}$ for $K^+$ as activator, but like the ATPase the $K_m$ for the substrate, in this case NPP, was also unaffected (data not presented).

With the K-phosphatase activity the effects of two inhibitors, ouabain and oligomycin, were diminished, as in the case of the (Na + K)-ATPase (Table III); oligomycin inhibits K-phosphatase activity only at quite low KCl concentrations, such as with the 0.3 mM KCl shown. On the other hand, dimethylsulfoxide stimulates rather than inhibits the K-phosphatase activity, and this effect was augmented in the residual activity after pyridoxal phosphate treatment.

## Discussion

Inactivation of the (Na + K)-ATPase followed a time-dependent course, and the curvatures of the semilogarithmic plots of residual activity vs. time

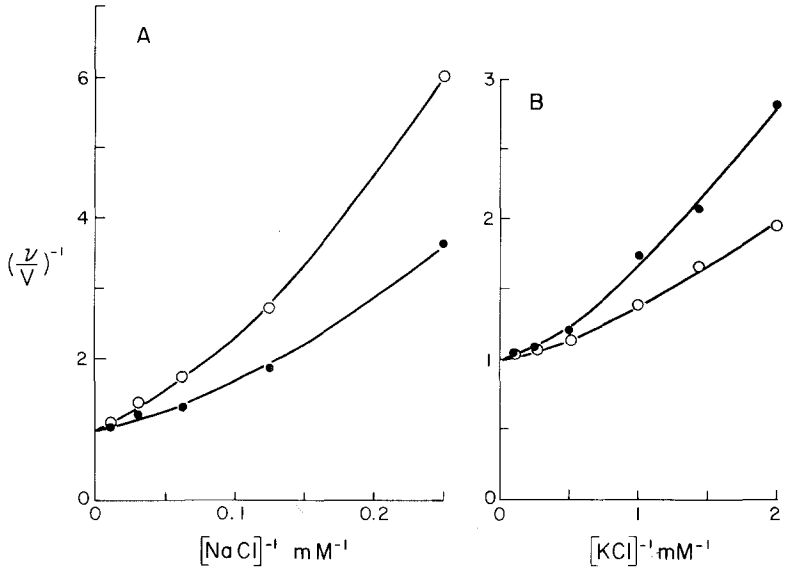


**Fig. 5.** Modification of cation activation of the (Na + K)-ATPase activity after treatment with pyridoxal phosphate. (A) $Na^+$ activation plotted in double-reciprocal form for experiments with control (●) and pyridoxal phosphate-treated (○) enzyme incubated in the standard medium modified to contain the concentrations of NaCl shown; the KCl concentration was 10 mM. (B) $K^+$ activation of control (●) and treated (○) enzyme plotted similarly for experiments in the standard medium modified to contain the concentrations of KCl shown; the NaCl concentration was 90 mM.

(Fig. 1) indicate that more than one class of sites on the enzyme (presumably lysine groups) are reacting with pyridoxal phosphate. This conclusion is supported by the finding that not only was catalytic activity lost, but that the residual activity was also modified. Distinction between these classes of sites was not possible since modification proceeded in parallel with inactivation, suggesting analogous effects on the enzyme; nevertheless, the possibility remains that various ligands and reagents may produce apparently similar changes in enzymatic properties through modification of different classes of groups.

Three major factors affecting the rate of inactivation were, however, distinguishable: substrates (and products), ionic strength, and specific monovalent cations. Protection against inactivation by micromolar ATP and ADP and by millimolar CTP (Fig. 2) is consistent with their action through the high-affinity substrate sites, in accord with their relative affinities for those sites (Robinson, 1976). The lack of effect of divalent cations on the protection is also consistent with their minimal effects on nucleotide binding (Robinson, 1980b). Such occupancy of the high-affinity substrate sites by the nucleotides would require the enzyme to be in the $E_1$ conformation that displays those sites; however, protection by NPP and $P_i$ (Fig. 2; Table I) at concentrations

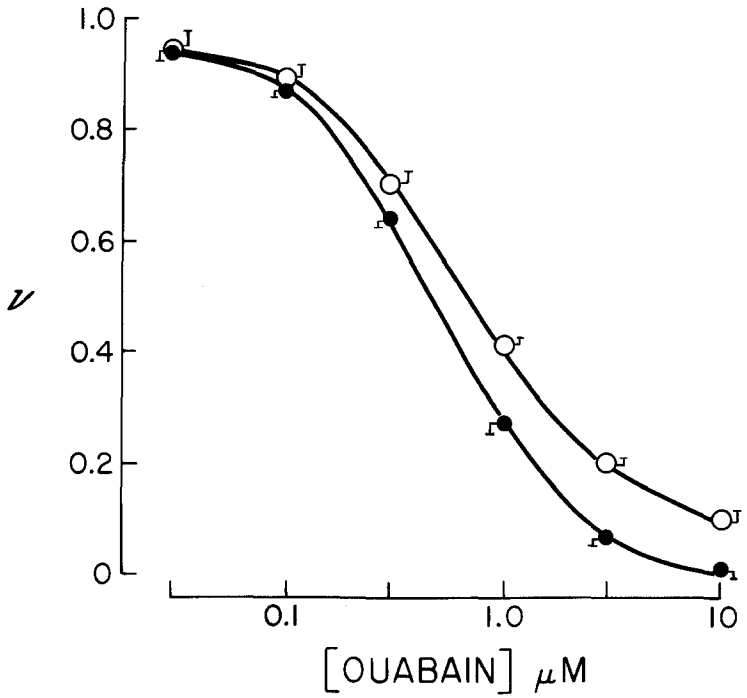


**Fig. 6.** Inhibition by ouabain. The decrease in (Na + K)-ATPase activity, for both control (●) and pyridoxal phosphate-treated enzyme (○), is plotted against the concentrations of ouabain added to the standard medium. In these experiments the enzyme was first incubated for 5 min at 37°C in the standard medium containing ouabain but lacking ATP, and the reaction then initiated by adding ATP.

**Table III.** Effects of Inhibitors and Activators on Residual (Na + K)-ATPase and K-Phosphatase Activities[a]

| Activity and reagent | Effects of reagent on | |
|---|---|---|
| | Control enzyme | Treated enzyme |
| (Na + K)-ATPase | | |
| None | (100) | (100) |
| Oligomycin, 10 μg/ml | 41 ± 2 | 53 ± 3 |
| Dimethylsulfoxide, 100 μl/ml | 69 ± 3 | 85 ± 2 |
| K-Phosphatase with 10 mM KCl | | |
| None | (100) | (100) |
| Ouabain, 3 μM | 23 ± 2 | 36 ± 1 |
| Oligomycin, 10 μg/ml | 101 ± 1 | 105 ± 3 |
| Dimethylsulfoxide, 100 μl/ml | 132 ± 3 | 150 ± 3 |
| K-Phosphatase with 0.3 mM KCl | | |
| None | (100) | (100) |
| Oligomycin, 10 μg/ml | 72 ± 3 | 107 ± 2 |

[a]The enzyme preparation was first reacted with pyridoxal phosphate in the standard inactivation medium, and the residual catalytic activity then assayed in the standard incubation media modified to contain the reagents listed; activity is presented relative to that in the absence of the reagents, set at 100 for both the control and the pyridoxal phosphate-treated enzyme. K-phosphatase activity was measured both with the standard 10 mM Kcl and with 0.3 mM KCl.

consistent with their acting through the low-affinity substrate sites (Robinson 1976; Robinson *et al.*, 1983) requires the enzyme to be in the $E_2$ conformation that displays those sites. Occupancy of the substrate sites, which may be transformations of the same structural entity (Smith *et al.*, 1980), would thus seem more important than the conformational state favored by the ligand.

Increasing salt concentration increased inactivation by pyridoxal phosphate, regardless of the cation (Fig. 4); this relationship makes earlier studies comparing 150 mM KCl with 3 mM ATP (Skou, 1982) less easily interpreted. Nevertheless, KCl, over the range 1 to 100 mM, protected against inactivation, when compared to equimolar choline chloride, whereas NaCl potentiated inactivation. The concentration dependence for $K^+$ is consistent with its occupying the moderate-affinity $\alpha$-sites, in light of their increase in apparent affinity with decrease in temperature (Robinson, 1977; Swann, 1983).

Since $K^+$ favors $E_2$ conformations and $Na^+$ favors $E_1$, these results suggest a relationship between conformational state and reactivity toward pyridoxal phosphate. Attempts to increase inactivation by reagents favoring the $E_1$ conformations, Triton X-100 and oligomycin, or to decrease inactivation by a reagent favoring the $E_2$ conformations, dimethylsulfoxide, did not, however, have distinguishable effects (Table II), suggesting that the relevant reactive groups are equally exposed under $E_1$ and $E_2$ conformations. Again, occupancy may be more important than conformational selection, although the $Na^+$ and $K^+$ sites may be transformations of the same structural entities (e.g., Glynn and Lew, 1969).

The lack of effect of reagents presumed to favor alternative conformations might be explained instead by the expected change in poise toward $E_2$ conformations (Swann, 1983) during incubation at 0°C with pyridoxal phosphate, and the possibility of altered efficacy of these reagents at reduced temperatures. During incubations at 10°C, however, these concentrations of Tritox X-100 and dimethylsulfoxide exerted readily-detectable effects similar to those at 37°C (J. D. Robinson, unpublished experiments), whereas at 10°C the effect of oligomycin is accentuated (Robinson, 1971). On the other hand, the possibility remains that these reagents may affect the $E_1$ and $E_2$ conformations differently, so that some groups on $E_1$ conformations react with pyridoxal phosphate preferentially while with $E_2$ conformations other groups are favored; that such diverse effects could produce such similar responses, however, seems unlikely.

The alterations in residual activity after treatment with pyridoxal phosphate do, however, seem to reflect an altered disposition between conformational states. But, unlike the findings of Skou (1982) using an eosin fluorescent probe with an enzyme from shark rectal gland, the current studies on kinetic properties of the dog kidney enzyme suggest a shift toward $E_2$ conformations. Thus: (i) the apparent affinity for $Na^+$ as activator of the

(Na + K)-ATPase was decreased whereas that for $K^+$ was increased; (ii) activity with CTP as substrate was decreased more than activity with ATP, and CTP is a less effective selector of $E_1$ conformational states (Robinson, 1982), a necessary property in the cyclic reaction scheme; (iii) Na-ATPase activity at high $Na^+$ concentrations was diminished less than (Na + K)-ATPase activity, whereas a normally inhibitory aspect of the former reaction is Na-stimulated conversion of $E_2$-P to $E_1$-P (Hara and Nakao, 1981); and (iv) the K-phosphatase activity was diminished less than the (Na + K)-ATPase activity, and the former reaction seems to proceed entirely with $E_2$ conformations (Robinson *et al.*, 1983), whereas the ATPase cycles between $E_1$ and $E_2$. Possibly consistent with this formulation is diminished sensitivity to oligomycin, which favors $E_1$ (Fahn *et al.*, 1966; Hobbs *et al.*, 1983), and perhaps to dimethylsulfoxide, which favors $E_2$ (Robinson, 1980a), if the poise is already far toward $E_2$. Nevertheless, a number of observations cannot be readily accommodated, including the diminished sensitivity to ouabain, since it appears to inhibit after binding to the $E_2$ conformation (Hansen, 1979), the increased stimulation of K-phosphatase activity by dimethylsulfoxide if the poise is already shifted by pyridoxal phosphate far toward $E_2$, and the lack of any change in $K_{0.5}$ for $K^+$ as activator of the K-phosphatase reaction, since this $K_{0.5}$ seems sensitive to the equilibria between $E_2$ and $E_1$ (Robinson *et al.*, 1983). The conclusion thus may be that enzyme modifications that favor a few of the many steps of the reaction sequence may not be wholly accommodated within schemes depicting one or the other of two global alternatives, and that these difficulties for kinetic formulations may also apply to other indicators of conformational states as well.

## Acknowledgments

The careful technical assistance of Mrs. Grace Levine is gratefully acknowledged. This work was supported by U.S. Public Health Service research grant NS-05430.

## References

Cantley, L. C. (1981). *Curr. Top. Bioenerg.* **11,** 201–237.
Fahn, S., Koval, G. J., and Albers, R. W. (1966). *J. Biol. Chem.* **241,** 1882–1889.
Glynn, I. M., and Lew, V. L. (1969). *J. Gen. Physiol.* **54,** 289s–305s.
Hansen, O. (1979). In *Na,K-ATPase: Structure and Kinetics* (Skou, J. C., and Norby, J. G., eds.), Academic Press, London, pp. 169–180.
Hara, Y., and Nakao, M. (1981). *J. Biochem.* **90,** 923–931.
Hobbs, A. S., Albers, R. W., and Froehlich, J. P. (1983). *J. Biol. Chem.* **258,** 8163–8168.
Jorgensen, P. L. (1974). *Biochim. Biophys. Acta* **356,** 36–52.

Karlish, S. J. D. (1980). *J. Bioenerg. Biomembr.* **12,** 111–136.
Robinson, J. D. (1967). *Biochemistry* **6,** 3250–3258.
Robinson, J. D. (1969). *Biochemistry* **8,** 3348–3355.
Robinson, J. D. (1971). *Mol. Pharmacol.* **7,** 238–246.
Robinson, J. D. (1976). *Biochim. Biophys. Acta* **429,** 1006–1019.
Robinson, J. D. (1977) *Biochim. Biophys. Acta* **482,** 427–437.
Robinson, J. D. (1980a). *Biochim. Biophys. Acta* **598,** 543–553.
Robinson, J. D. (1980b). *J. Bioenerg. Biomembr.* **12,** 165–174.
Robinson, J. D. (1982). *Arch. Biochem. Biophys.* **213,** 650–657.
Robinson, J. D., and Flashner, M. S. (1979a). *Biochim. Biophys. Acta* **549,** 145–176.
Robinson, J. D., and Flashner, M. S. (1979b). *Arch. Biochem. Biophys.* **196,** 350–363.
Robinson, J. D., Levine, G. M., and Robinson, L. J. (1983). *Biochim. Biophys. Acta* **731,** 406–414.
Schuurmans Stekhoven, F., and Bonting, S. L. (1981). *Physiol. Rev.,* **61,** 1–76.
Skou, J. C. (1982). *Biochim. Biophys. Acta* **688,** 369–380.
Smith, R. L., Zinn, K., and Cantley, L. C. (1980). *J. Biol. Chem.* **255,** 9852–9859.
Swann, A. C. (1983). *Arch. Biochem. Biophys.* **221,** 148–157.