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THALLIUM ACTIVATION OF THE (Na^+–K^+)-ACTIVATED ATPase OF RABBIT KIDNEY

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SUMMARY

Tl^+ has been found to replace K^+ in activation of the (Na^+–K^+)-sensitive ATPase of rabbit kidney. In comparison to the other known substitutes for K^+ in activating this ATPase, Tl^+ is unique in that it has an affinity approx 10 times greater than K^+ for the K^+ activating site

INTRODUCTION

In higher organisms it appears well established that there is an association between the active transport of Na^+ across cell membranes and the presence of a (Na^+–K^+)-stimulated, Mg^{2+}-dependent, ouabain-sensitive ATPase[1] in the particulate fractions of tissue homogenate. Rb^+, NH_4^+, Cs^+, and Li^+ may replace K^+ in the activation of this ATPase with affinities in the order $K^+ > Rb^+ > NH_4^+ > Cs^+ > Li^+$ (refs 1, 2). The extent of ATPase activation by the substitutes is less than the K^+ activation except in the case of NH_4^+ which may activate to a slightly greater extent[1,2] Somewhat more variably these same ions serve to replace the K^+ required in the metabolically dependent transport of Na^+ from the inside to the outside of cell membranes. During this Na^+ transport, K^+, or one of its substitutes, must be present in the extracellular fluid The substitutes show very roughly the same order of efficiency in replacing K^+ in the active transport of Na^+ as they do in affinity for ATPase activation[3–7]

The detailed nature of this biological ion transport system is not known. Polarographic techniques, used in the study of ion transport processes across interfacial films[8,9], would appear to be potentially useful for the study of the biological system But it is not possible to apply polarographic techniques to processes involving alkali metal ions under appropriate conditions For this reason we were prompted to look for ions which would be handled by the Na^+–K^+ transport system of cells and which could be analyzed polarographically As part of this search, we decided to test Tl^+ as a possible substitute for Na^+ or K^+ in the (Na^+–K^+)-sensitive ATPase. It was found that Tl^+ may replace K^+ in the activation of an ATPase of rabbit kidney In all respects tested, this (Na^+–Tl^+)-sensitive ATPase was found to behave like the (Na^+–K^+)-sensitive ATPase. The two enzymes are evidently identical and Tl^+ substitutes for

K^+ at the K^+ activation site of the rabbit kidney (Na^+–K^+)-sensitive ATPase with an affinity approx. 10 times greater than K^+

MATERIALS

Frozen rabbit kidneys were purchased from Pel Freez Biologicals, Rogers, Ark , thallium acetate, from Fisher Scientific Company, Tris and Na_2ATP from Sigma Chemical Company, EDTA, from Mallinckrodt Chemical Works

METHODS

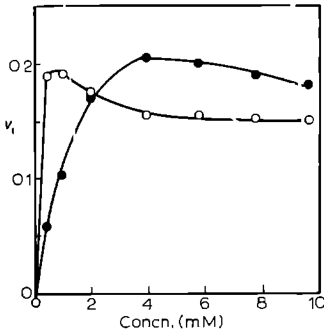
Tris–ATP was prepared by passage of a solution of Na_2ATP through a column of cation exchange resin in the H^+ form, followed by neutralization with Tris ATPase was prepared from the frozen rabbit kidneys by a modification of the method of KINSOLVING, POST AND BEAVER[10] Two frozen rabbit kidneys were thawed, decapsulated, and homogenized in 150 ml of 0.25 M sucrose, $2 \cdot 10^{-3}$ M Tris–EDTA, and $1.2 \cdot 10^{-2}$ M mercaptoethanol The homogenate was centrifuged at 0° for 20 min at $100 \times g$ Discarding the supernatant, the loose upper layer of sediment was resuspended in homogenizing solution and centrifuged as before, this time resuspending the loose upper layer of sediment in storage solution (1 3 M urea, 0.02 M Tris–HCl (pH 7.4), $2 \cdot 10^{-3}$ M Tris–EDTA, 10^{-4} M $MgCl_2$, $7.6 \cdot 10^{-3}$ M $(NH_4)_2SO_4$ and $1.2 \cdot 10^{-2}$ M mercaptoethanol) After standing for at least 10 h at 5°, the suspension was spun down at 10 000 $\times g$ for 10 min at 0° The sediment was twice resuspended in 0 02 M Tris–HCl (pH 7.4), $3 \cdot 10^{-4}$ M Tris–EDTA, and $1.2 \cdot 10^{-2}$ M mercaptoethanol and held at 5° in that solution until used

Protein was determined by the method of GORNALL, BARDAWILL AND DAVID[11], standardized on crystalline bovine albumin

ATPase activity was estimated by incubating enzyme and Tris–ATP in 1 ml of appropriate ionic composition at 38° for 15 min. The reaction was terminated with 0.5 ml of 15% (w/v) trichloroacetic acid and the inorganic phosphate assayed according to the method of TAUSSKY AND SHORR[12] Blanks with choline replacing Na^+–K^+, or Na^+–Tl^+, allowed determination of ion-dependent ATPase activity Enzyme concentration was adjusted so that the phosphate assayed represented the initial velocity of the reaction.

RESULTS

Using a solution of 5 μmoles Tris–ATP, 5 μmoles magnesium acetate, 20 μmoles Tris–acetate buffer (pH 7 40), 0 165 mg of ATPase (diluted with 0.02 M Tris–acetate buffer) and variable sodium acetate–thallous acetate mixtures to a total of 96 μmoles in 1 ml, the activity of the ATPase was determined and compared to the ATPase activity when 96 μmoles of choline acetate replaced the sodium acetate–thallous acetate mixture. The additional ATPase activity found in the Na^+–Tl^+ system above that in the choline system was taken as (Na^+–Tl^+)-dependent ATPase activity. The results for various Na^+–Tl^+ ratios are plotted in Fig. 1 together with a similar curve where potassium acetate was used in place of thallous acetate Both a (Na^+–Tl^+)-sensitive and (Na^+–K^+)-sensitive ATPase are present with comparable maximal



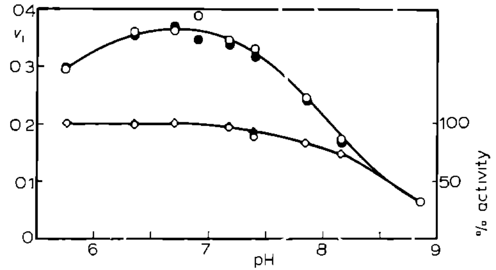
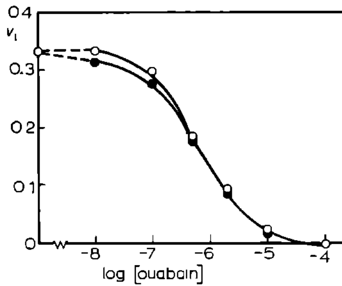


Fig. 1. Variation of the initial velocity, v_i (in μmoles phosphate produced/min per mg protein), with the concentration (mM) of K^+ (●) or Tl^+ (○). The reaction mixture contained Tris–ATP (5 μmoles), magnesium acetate (5 μmoles), Tris–acetate (20 μmoles, pH 7.4), enzyme (0.165 mg) and $Na^+ + K^+ = Tl^+ + Na^+ = 96$ μmoles in 1 ml, incubation at 38° for 15 min.

Fig. 2. Upper curve: Variation of the initial velocity, v_i (in μmoles phosphate produced/min per mg protein), with the pH of the assay medium for the (Na^+–Tl^+)-ATPase (○), and the (Na^+–K^+)-ATPase (●). The reaction mixture was that described in Fig. 1 with either Tl^+ (0.8 μmole), Na^+ (95.2 μmoles), or K^+ (8 μmoles), Na^+ (88 μmoles), and Tris–acetate (100 μmoles), incubated as before. Lower curve: Percent residual activity of (Na^+–Tl^+)-ATPase (◇) and the (Na^+–K^+)-ATPase (◆) following incubation as above but without ATP. Overlapping points are indicated as ◇.

activities. Repeating these experiments with Tris–acetate buffers at various pH's and expanded concentration ranges disclosed that for the (Na^+–Tl^+)-ATPase maximum activity was obtained at $0.5 \cdot 10^{-3}$–$0.9 \cdot 10^{-3}$ M Tl^+ with half maximal activity at $0.16 \cdot 10^{-3}$–$0.20 \cdot 10^{-3}$ M Tl^+ while for the (Na^+–K^+)-ATPase maximal activity was obtained at $6 \cdot 10^{-3}$–$10 \cdot 10^{-3}$ M K^+ and half maximal activity at $1.2 \cdot 10^{-3}$–$3.0 \cdot 10^{-3}$ M K^+. These values disclosed no consistent variation with pH over the range 6.0–8.6.



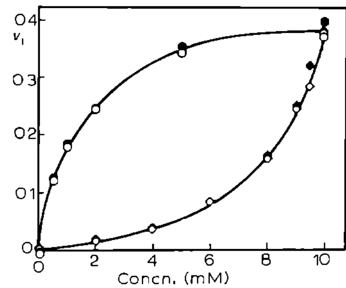


Fig. 3. Variation of (Na^+–Tl^+)- (○), or (Na^+–K^+)- (●) ATPase activity (v_i in μmoles of phosphate produced/min per mg protein) with log ouabain concn. (M). The reaction mixture contained Tris–ATP (5 μmoles), magnesium acetate (5 μmoles), Tris–acetate (20 μmoles, pH 6.9), enzyme (0.233 mg) and either thallium acetate (0.9 μmole) and sodium acetate (95.1 μmoles) or potassium acetate (9 μmoles) and sodium acetate (87 μmoles) in 1 ml, incubated at 38° for 15 min.

Fig. 4. Upper curve: Variation in (Na^+–Tl^+)- (○), or (Na^+–K^+)- (●) ATPase activity (v_i in μmoles phosphate produced/min per mg protein) with Mg^{2+} (mM). Reaction mixture contained Tris–ATP (5 μmoles), Tris–acetate (100 μmoles, pH 6.7), enzyme (0.233 mg), magnesium acetate as indicated, and either thallium acetate (0.9 μmole) and sodium acetate (95.1 μmoles) or potassium acetate (9 μmoles) and sodium acetate (87 μmoles) in 1 ml incubated at 38° for 15 min. Lower curve: Variation in (Na^+–Tl^+)- (◇), (Na^+–K^+)- (◆) ATPase activity, v_i, with Mg^{2+} when $Mg^{2+} + Ca^{2+}$ was constant at 10 mM. Reaction mixture was the same as above except for the addition of the required calcium acetate.

To obtain the curves of pH *versus* ATPase activity (Fig 2) the system was poised at Na^+ Tl^+ ($9.5 \cdot 10^{-2}$ $0.8 \cdot 10^{-3}$ M), or at Na^+ K^+ ($8.8 \cdot 10^{-2} \cdot 8$ 10^{-3} M), concentration ratios giving maximal ion-sensitive ATPase activity throughout the pH range Both ATPases were found to have maximal activity in the range of pH 6 5–7.0 The stability of the enzymes was determined by incubation at various pH's for 22 min as in the assay procedure, but without ATP, followed by adjustment to pH 6 7 and assay Comparison of the two curves of Fig 2 suggests that the peak in the variation of ion-sensitive ATPase activity with pH is not a reflection of enzyme stability even though loss in activity is encountered at a relatively low pH range

The similarities of the ouabain sensitivity and the Mg^{2+} dependence of the two enzymes are shown in Figs. 3 and 4, respectively The thallium enzyme was assayed in a 1-ml solution containing 0 9 μmole thallium acetate, 95 1 μmoles sodium acetate, 5 μmoles Mg^{2+}, 20 μmoles Tris–acetate buffer, pH 6 89, 0 167 mg protein, and various concentrations of ouabain The potassium enzyme was similarly assayed except that thallium was omitted and 9 μmoles potassium acetate and 87 μmoles sodium acetate were present These same two systems were used to contrast the behavior of the enzymes when Mg^{2+} was progressively replaced by Ca^{2+} or when Mg^{2+} was varied alone

With these similarities to suggest the identity of the two enzymes, further

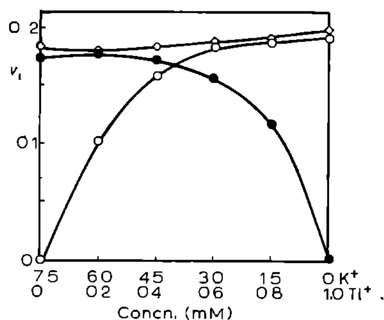


Fig 5 Variation in ion-sensitive ATPase activity, v_i (in μmoles phosphate produced/min per mg protein) with $Na^+ + Tl^+ + K^+$ (mM) The reaction mixture contained magnesium acetate (5 μmoles), Tris–ATP (5 μmoles), Tris–acetate (20 μmoles, pH 6 8) and either potassium acetate without thallium acetate (●), thallium acetate without potassium acetate (○), or potassium acetate and thallium acetate (◇) so that in all cases $Na^+ + Tl^+ + K^+$ was 96 μmoles in 1 ml, incubation at 38° for 15 min

support of this hypothesis was sought Heating the enzyme preparation to progressively higher temperatures before assay resulted in equal losses of activity of both the K^+-stimulated and the Tl^+-stimulated APTase. Moreover, progressive change of the Na^+ Tl^+ K^+ concentrations in the assay from Na^+ $9.5 \cdot 10^{-2}$ M Tl^+ 10^{-3} M no K^+ (which should yield maximum $(Na^+–Tl^+)$-ATPase activity) to Na^+ $8.8 \cdot 10^{-2}$ M : no Tl^+ : K^+ $7.5 \cdot 10^{-3}$ M (which should give maximal $(Na^+–K^+)$-ATPase activity) resulted in essentially constant ion-sensitive activity and certainly did not represent the algebraic sum of the separate ion-sensitive activities (Fig 5).

DISCUSSION

Most studies of the $(Na^+–K^+)$-activated ATPase have been made on cell fractions

prepared by differential centrifugation In these preparations the ATPase remains insoluble, bound to a fragment of cell membrane. While it seems reasonable to assume that only one enzyme is responsible for the splitting of the ATP observed with this preparation, no direct evidence has been advanced to show that the associated biological transport system consisted of one enzyme only The conventional model of Shaw, quoted by GLYNN[4], proposed the existence of at least three functional units, an ion carrier, an enzyme supplying energy, and a matrix holding the system in proper orientation But MITCHELL[13] has indicated that the carrier function may be a property of the orientation of an enzyme in the cell membrane matrix Moreover, the success of MEDZIHRADSKY, KLINE AND HOKIN[14] in obtaining a soluble preparation of (Na^+–K^+)-activated ATPase implies that all three functional units in the sodium transport system may exist in one protein of molecular weight approx 670 000. The large size of this protein in terms of the 100-Å thickness of the usual cell membrane, together with the functional response of the biological transport system to alterations of bathing medium both inside and outside the cell membrane, make it likely that the ATPase extends entirely through the lipid membrane The number of such proteins per unit area of membrane must be quite small as there is no discernible structure to the membrane bilayer on electron microscopy. An estimate by SOLOMON, GILL AND GOLD[15] for the red blood cell is 100 sites/μ^2 of membrane The discovery that Tl^+, atomic weight 203, substitutes for K^+ at the K^+ activation site offers the possibility of actually identifying these sites *in vivo* by electron microscopy and so establishing the number and location of such sites It is because of the potential use of Tl^+ in the study of the biological Na^+–K^+ transport system that we have attempted to offer the strongest possible evidence that the (Na^+–K^+)-activated ATPase and the (Na^+–Tl^+)-ATPase are the same. Since the ATPase is not a pure protein, the evidence necessarily must consist of an array of similar properties (Figs 2, 3 and 4) and functional interdependence (Fig 5)

The fact that the inhibition of the (Na^+–K^+)-ATPase by ouabain when K^+ activates requires higher concentrations of inhibitor than when Tl^+ activates, follows from the current interpretation of the action of ouabain This glycoside is known to inhibit both the Na^+-dependent formation and the K^+-dependent breakdown of a membrane bound phosphorylated intermediate[16]. However at low concentration ouabain may be considered to compete in some indirect fashion with K^+ at the K^+ activation site[17] Since thallous ion has a higher affinity than K^+ at that site, more ouabain is required to displace Tl^+ than an equivalent amount of K^+

If the only biological action of thallous salts is due to competition with K^+, any toxic manifestations of Tl^+ would be expected to affect all tissues The toxic effects have been noted in the neurological system, cardiovascular system, liver, kindeys, and intestinal tract. Only the blood element seems to be spared[18,19] The possible therapeutic benefit of measures designed to increase K^+ turnover in cases of Tl^+ poisoning does not appear to have been explored

No direct clues to the mechanism of action of the K^+ substitutes in activation of the (Na^+–K^+)-ATPase appears from a consideration of the physicochemical properties of Tl^+ Its crystal radius is apparently very close to that of K^+ and it tends to replace K^+ in a number of compounds including complex salts[20]. Tl^+ also replaces Rb^+ and Cs^+ in a number of compounds, but it does not replace Na^+. TlCl has the "CsCl" structure rather than the "rock salt" crystalline form of the sodium salt The limiting

ionic conductances in water at 25° of Tl^+ is 74.7, K^+ is 73 54 and Na^+ is 50.11 (ref 21), indicating that the hydrated Tl^+ is almost identical in size to the hydrated K^+, both of which are considerably smaller than the hydrated Na^+. These properties make it likely that Tl^+ would mimic the biological behavior of K^+ more closely than that of Na^+ But these results do not allow us to identify a unique characteristic of the ions known to substitute for K^+ in K^+ activation of the (Na^+–K^+)-activated ATPase. For example, it is not possible to decide whether the substitution is by hydrated ions at a solvated site or by free ions interacting with an enzyme site Although the results give no further information about the basic biological difference between Na^+ and K^+, nevertheless, they do reinforce the prevailing notions about the size and charge requirements for activation of the ATPase by ions

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It has come to our attention that GEHRING AND HAMMOND[22] have reported the activation of rat erythrocyte ATPase by Tl^+ in the presence of Na^+ and have compared this to similar activation obtained with K^+ Earlier work of these authors[23] demonstrated metabolically dependent accumulation of Tl^+ by rabbit erythrocytes that was inhibited by ouabain and K^+. Moreover, MULLINS AND MOORE[24] have shown that Tl^+ is handled like K^+ by rat sartorius muscle and also that Tl^+ behaves like K^+ during electrical excitation of that tissue. These studies establish that Tl^+ is a substitute for K^+ *in vivo* and serve to strengthen the circumstantial implication of the (Na^+–K^+)-ATPase with the Na^+–K^+ exchange pump *in vivo*.

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