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Thallium-induced dephosphorylation of a phosphorylated intermediate of the (sodium + thallium-activated) ATPase

It is generally agreed that the ($Na^+ + K^+$)-ATPase (ATP phosphohydrolase, EC 3.6.1.3) is the same as, or represents an important component of, the system responsible for the active transport of Na^+ and K^+ through the plasma membranes of many types of cells[1,2]. Several laboratories[3–9] have reported a Na^+-induced transfer of the terminal phosphate of [^{32}P]ATP to microsomal protein. The subsequent addition of K^+ produced a rapid loss of P_i from the protein. Based on these observations the hydrolysis of ATP by the ($Na^+ + K^+$)-ATPase is believed to involve at least two steps. The initial step consists of the Na^+-induced phosphorylation of the protein by ATP, followed by a K^+-induced dephosphorylation step. It is of interest to determine if other cations capable of activating the ATPase also have effects on the ^{32}P-labeled intermediate which are consistent with the currently held reaction sequence.

It has been reported that Tl^+ can replace K^+ in the activation of the ($Na^+ + K^+$)-ATPase of rat erythrocyte[10] and rabbit kidney[11]. BRITTEN AND BLANK[11] found that Tl^+ was unique in possessing an affinity for the K^+-activating site that was 10 times greater than K^+. I have recently observed[12] that Tl^+ can also activate the acetylphosphatase and *p*-nitrophenylphosphatase of beef brain microsomes with a 9–10-fold greater affinity than K^+. These results suggest that Tl^+ and K^+ act at a common site to activate the ATPase.

The purpose of the present communication is to describe some of the properties of a ($Na^+ + Tl^+$)-ATPase of beef brain microsomes and to demonstrate the relationship between the effect of Tl^+ on the ^{32}P-labeled intermediate of the ATPase and the mechanism of Tl^+ activation of the ATPase.

Beef brain microsomal ($Na^+ + K^+$)-ATPase was prepared from sucrose homogenates of grey matter as described by SCHONER *et al.*[13].

[^{32}P]ATP of a specific activity from 6 to 8 mC/μmole was prepared by a modification[5,14] of the method of PFLEIDERER[15].

The incorporation of [^{32}P]phosphate into microsomal protein was measured by trichloroacetic acid precipitation and filtration on Millipore filters as described by CHIGNELL AND TITUS[16].

Protein was determined by the method of LOWRY *et al.*[17] using bovine serum albumin as a standard.

ATPase activity was estimated by incubating 0.070 mg of microsomal protein for 4 min at 37° with 2 mM Tris–ATP, 5 mM $MgCl_2$, 100 mM Tris–HCl (pH 7.4), 120 mM sodium acetate and 0.05–1.5 mM thallium acetate. The ($Na^+ + Tl^+$)-ATPase activity was measured by the liberation of P_i in the presence of $Mg^{2+} + Na^+ + Tl^+$ *minus* that which occurred when Mg^{2+} and 0.1 mM ouabain were present. The liberation of P_i was determined by a modification of the procedure of MARTIN AND DOTY[18] as described by GIBBS *et al.*[5].

Rutamycin (mol. wt., 432) was dissolved in 95% ethanol and added to the tubes before the addition of the other reagents. Control tubes received an equal volume of ethanol. The ethanol was evaporated under a stream of N_2.

The addition of 0.05–1.5 mM Tl^+ produced activation of a ($Na^+ + Tl^+$)-ATPase

of beef brain microsomes (Fig. 1) in the presence of 120 mM Na^+. In the absence of Na^+ the ATPase was not activated by Tl^+. The apparent K_m for Tl^+ activation of the ($Na^+ + Tl^+$)-ATPase was 0.2 mM, a value approx. 0.1 of the apparent K_m (2.0 mM) for K^+ (unpublished observation) for this beef brain ($Na^+ + K^+$)-ATPase. These results suggest that the ATPases from beef brain and from rabbit kidney[11] are similar in that both have an affinity for Tl^+ that is 10 times greater than for K^+.

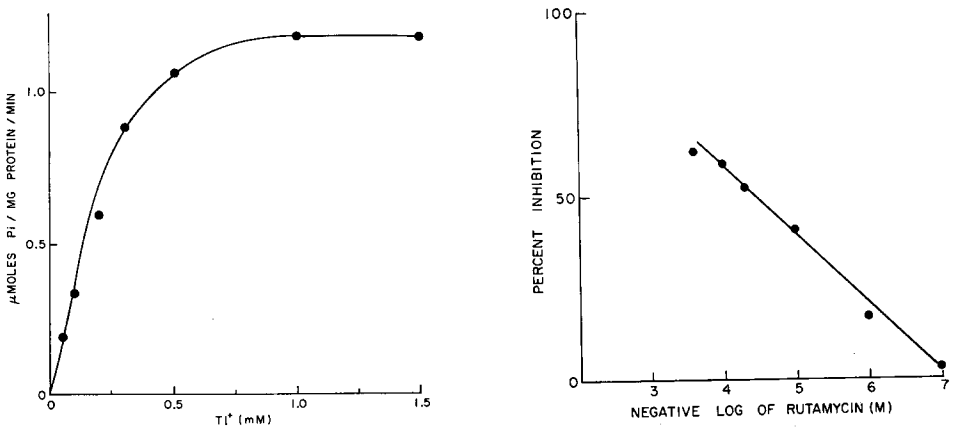

Fig. 1. Tl^+ activation of ($Na^+ + Tl^+$)-ATPase of beef brain microsomes. Final concentrations were 2 mM ATP, 5 mM $MgCl_2$, 100 mM Tris–HCl (pH 7.4), 120 mM sodium acetate and 0.05–1.5 mM thallium acetate. Results are expressed as µmoles of P_i released/mg of microsomal protein per min.

Fig. 2. Inhibition of ($Na^+ + Tl^+$)-ATPase by rutamycin. Final concentrations were as in Fig. 1 except that Tl^+ concentration was constant at 1.5 mM. Results are expressed as the percent inhibition of ($Na^+ + Tl^+$)-ATPase in the presence of rutamycin.

INTURRISI AND TITUS[19] have reported that rutamycin, a macrolide antibiotic with properties similar to oligomycin[20], can inhibit the ($Na^+ + K^+$)-ATPase. Fig. 2 indicates that rutamycin can inhibit the ($Na^+ + Tl^+$)-ATPase. At the highest concentration of rutamycin used ($2.5 \cdot 10^{-4}$ M) approx. 65% of the total ($Na^+ + Tl^+$)-ATPase activity was inhibited. These results are similar to those previously reported[19] for inhibition by rutamycin of the ($Na^+ + K^+$)-ATPase.

In the presence of $Mg^{2+} + Na^+$ the incorporation of [^{32}P]phosphate from [^{32}P]ATP into beef brain microsomes at 15 sec is approx. 3 times that seen in the presence of Mg^{2+} (Fig. 3). The Na^+-induced phosphorylation remains fairly constant at 30 sec. When 0.2 mM Tl^+ is added at 15 sec in the presence of Na^+ and Mg^{2+} a rapid dephosphorylation of the microsomes occurs; between 5 and 15 sec after the addition of Tl^+ the level of ^{32}P labeling has been reduced to that seen with only Mg^{2+} present. The addition of 0.2 mM Tl^+ does not alter the level of ^{32}P labeling when only Mg^{2+} is present (not shown in Fig. 3). When the concentration of Tl^+ is reduced 100-fold to 0.002 mM, only a small dephosphorylation has occurred at 15 sec after the addition of Tl^+.

The Tl^+-induced dephosphorylation of ^{32}P-labeled microsomes is accompanied by an increase in the liberation of $^{32}P_i$ from [^{32}P]ATP (Fig. 4). The addition at 15 sec of Tl^+, 0.2 mM, produces a prompt increase in $^{32}P_i$ liberation that indicates a Tl^+-

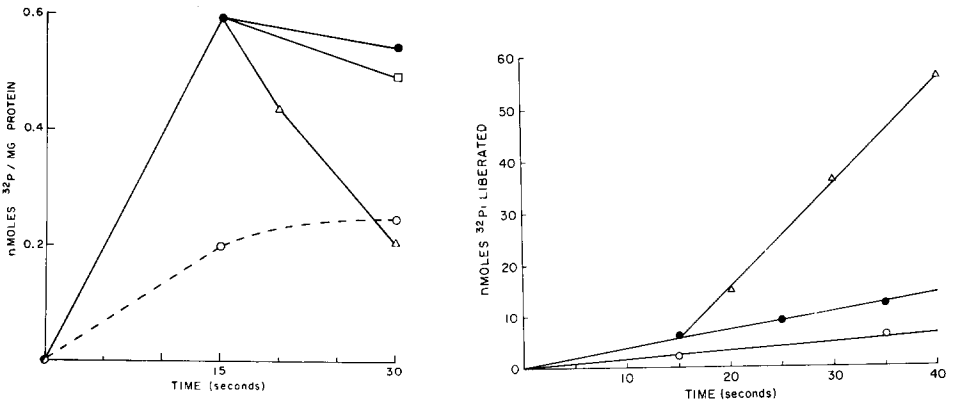

Fig. 3. The effect of Tl^+ on the incorporation of [^{32}P]phosphate from [^{32}P]ATP into beef brain microsomes. Final concentrations were 0.5 mM [^{32}P]ATP ($2.3 \cdot 10^6$ counts/min), 1.25 mM $MgCl_2$, 100 mM Tris–HCl (pH 7.4). All tubes, with the exception of those designated (○) also contained 120 mM sodium acetate. Beef brain microsomes (0.18 mg) were added at zero time and at 15 sec additions were made to give the following final concentrations: ●, water; □, 0.002 mM thallium acetate; △, 0.2 mM thallium acetate. Results are expressed as nmoles ^{32}P/mg of microsomal protein.

Fig. 4. The effect of Tl^+ on the release of P_i from [^{32}P]ATP in the presence of beef brain microsomes. These results were obtained using the conditions described in Fig. 3. The $^{32}P_i$ liberated from [^{32}P]ATP was extracted as described in ref. 5 and the radioactivity counted.

induced increase in [^{32}P]ATP hydrolysis. The rates of hydrolytic activity calculated from the data of Fig. 4 in μmoles of ATP hydrolized per mg of protein per min are 0.053 for Mg^{2+}, 0.100 for $Mg^{2+} + Na^+$ and 0.684 for $Mg^{2+} + Na^+ + Tl^+$.

These experiments indicate that Tl^+, like K^+, can produce a decrease in Na^+-induced phosphorylation of microsomes and an increase in the hydrolysis of ATP. It appears likely that the Tl^+-induced activation of a ($Na^+ + Tl^+$)-ATPase is due to a K^+-like dephosphorylation mechanism.

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