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Thallium activation of K^+-activated phosphatases from beef brain

The microsomal fractions of many tissues contain, in addition to the $(Na^+ + K^+)$-ATPase (ATP phosphohydrolase, EC 3.6.1.3), a group of neutral phosphatase activities which require K^+, are inhibited by Na^+ and are capable of hydrolyzing a variety of artificial substrates including acetyl phosphate[1–3], *p*-nitrophenyl phosphate[4–9] and carbamyl phosphate[1,3]. The ATPase is not a purified enzyme and therefore it has not been possible to determine conclusively whether the K^+-activated phosphatase activities are a manifestation of the same enzyme that is involved in the hydrolysis of ATP. The close relationship between the K^+-activated phosphatases and the $(Na^+ + K^+)$-ATPase with respect to cation activation, pH optimum and sensitivity to inhibitors[1–9], along with reports that the acid-stable phosphorylated intermediate of the ATPase is an acyl phosphate[10,11], have led to the suggestion that K^+-activated phosphatases may be the same as the K^+-dependent part of the $(Na^+ + K^+)$-ATPase system. It is therefore of interest to examine the relationship which exists between the activation of the ATPase and the activation of the phosphatases.

BRITTEN AND BLANK[12] have reported that Tl^+ can substitute for K^+ in the activation of the $(Na^+ + K^+)$-ATPase of rabbit kidney. A comparison of the concentrations necessary for half-maximal activation of ATPase revealed that Tl^+ has an affinity approx. 10 times greater than K^+. It has been observed by the author that the ATPase of beef brain microsomes, like the kidney ATPase, is activated by Tl^+ and that the affinity is about 10 times greater than the affinity of K^+. The purpose of these studies was to determine if Tl^+ would also substitute for K^+ in the activation of K^+-phosphatases.

The present communication demonstrates that Tl^+ can activate both the acetylphosphatase and *p*-nitrophenylphosphatase of beef brain microsomes. The affinity of Tl^+ is approx. 9–10 times greater than the affinity of K^+.

Beef brain microsomes containing $(Na^+ + K^+)$-ATPase and K^+-phosphatase activities were prepared from sucrose homogenates of grey matter as described by

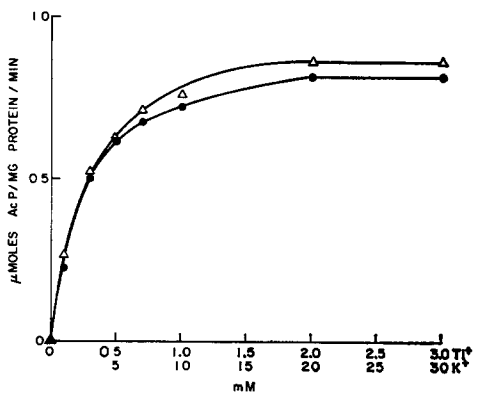


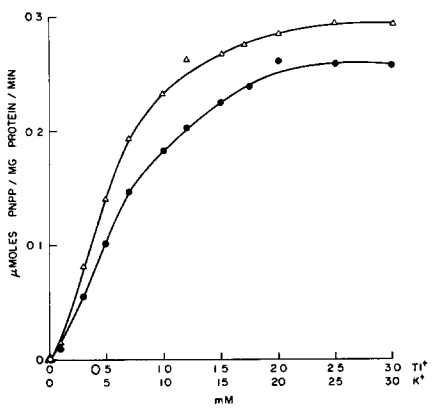
Fig. 1. The activation of acetylphosphatase by Tl^+ or K^+. Final concentrations were 6 mM Tris–acetyl phosphate, 15 mM $MgCl_2$, 50 mM Tris–HCl (pH 7.4), 0.1–3.0 mM thallium acetate (●) or 1–30 mM potassium acetate (△). Results are expressed as µmoles of acetyl phosphate (AcP) hydrolyzed per mg of protein per min.

SCHONER *et al.*[13]. Acetylphosphatase activity was determined by the hydroxamate method as described by ISRAEL AND TITUS[2]. *p*-Nitrophenylphosphatase activity was measured by the appearance of *p*-nitrophenol as described by INTURRISI AND TITUS[14]. The assay reaction was initiated by the addition of 0.070 mg of enzyme protein and the incubation time was 6 min at 37°. The hydrolytic activity in the presence of Mg^{2+} alone (2–5 % of total activity at maximal activation) was subtracted from the hydrolytic activity in the presence of Mg^{2+} *plus* Tl^+ or K^+ to give the phosphatase activity.

A comparison of the activation of acetylphosphatase by Tl^+ with the activation by K^+ (Fig. 1) shows that in concentrations from 0.1 to 3.0 mM, Tl^+ produces nearly the same activation curve as from 1 to 30 mM K^+. BADER AND SEN[1] have reported that acetylphosphatase from guinea-pig kidney cortex was activated by K^+, Rb^+, Cs^+, NH_4^+ or Li^+ (in order of decreasing effectiveness). The K^+:Tl^+ concentration ratio at half-maximal acetylphosphatase activity is 2.33 mM/0.23 mM, approx. 10. Tl^+ is clearly the activating cation with the highest affinity for the acetylphosphatase.

The maximal activation of *p*-nitrophenylphosphatase by Tl^+ was 85 % of the maximal activation induced by K^+ (Fig. 2). The K^+:Tl^+ concentration ratio at half-maximal *p*-nitrophenylphosphatase activation is 5.42 mM/0.62 mM, approx. 9. In addition to K^+, the *p*-nitrophenylphosphatase of beef brain microsomes is also activated by Rb^+ and NH_4^+ (E. Titus and P. Roddy, unpublished observation).

Na^+-inhibited the *p*-nitrophenylphosphatase activity induced by either 3 mM Tl^+ or 30 mM K^+. As the concentration of Na^+ was increased, the Tl^+- and K^+-induced *p*-nitrophenylphosphatase activities fell with nearly identical slopes. The concentration of Na^+ required for half-maximal inhibition was 85 mM when 3 mM Tl^+ was present and 80 mM when 30 mM K^+ was present. The mechanism of the Na^+ inhibition of K^+-phosphatases appears to involve more than a simple competition between Na^+



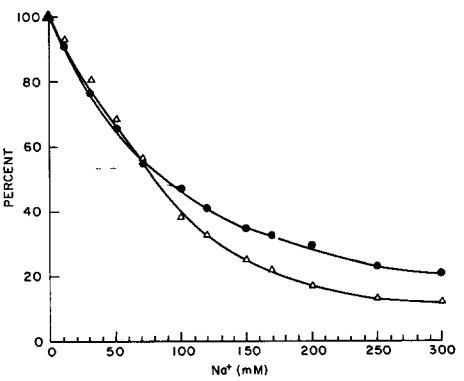


Fig. 2. The activation of *p*-nitrophenylphosphatase by Tl^+ or K^+. Final concentrations were 6 mM Tris–*p*-nitrophenyl phosphate, 15 mM $MgCl_2$, 20 mM Tris–HCl (pH 7.4), 0.1–3.0 mM thallium acetate (●) or 1–30 mM potassium acetate (△). Results are expressed as μmoles of *p*-nitrophenyl phosphate (PNPP) hydrolyzed per mg of protein per min.

Fig. 3. The inhibitory effect of Na^+ on the activation of *p*-nitrophenylphosphatase by Tl^+ or K^+. Final concentrations were as in Fig. 2 except that Tl^+ (●) and K^+ (△) were constant at 3 and 30 mM, respectively. Results are expressed as the percent activity in the presence of sodium acetate, taking the activity in the absence of Na^+ as 100 %.

and K^+ for the K^+ site[4]. NAGAI, IZUMI AND YOSHIDA[15] have suggested that Na^+ may induce an alteration in the affinity of substrate binding to the enzyme.

The physiochemical properties of Tl^+ which may be important in allowing Tl^+ to substitute for K^+ have been discussed by BRITTEN AND BLANK[12]. The cause of the apparently unique ability of Tl^+ to activate the ATPase and phosphatases with an affinity 10 times greater than K^+ remains unknown.

The results suggest that Tl^+ and K^+ act at a common site in the activation of acetylphosphatase or *p*-nitrophenylphosphatase. These studies also provide further evidence for a close association between the properties of the K^+-activation site of the microsomal $(Na^+ + K^+)$-ATPase and K^+-phosphatases.

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Laboratory of Chemical Pharmacology,
National Heart Institute,
National Institutes of Health,
Bethesda, Md. 20014 (U.S.A.)

CHARLES E. INTURRISI

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Evidence against the involvement of the carbonyl group in the glucose transport mechanism of human erythrocytes

The nature of the interaction between sugars and the carrier which has been postulated to facilitate their transport across the erythrocyte membrane is obscure. LANGDON AND SLOAN[1] studied the incorporation of [^{14}C]glucose which occurred upon borohydride reduction and proposed an imine as an intermediate. ROSE *et al.*[2] have more recently suggested that a carbinolamine may be involved. However, reduction under the conditions described by LANGDON AND SLOAN[1] fails to inhibit glucose