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Enzyme inhibition by tetraphenylboron

Tetraphenylboron is a powerful chelating agent for potassium[1]. It induces changes in the ultrastructure of mitochondria[2] and has been used in the assay of catecholamines[3]. In the course of studies on plasma membrane preparations from calf thyroids we observed that tetraphenylboron inhibited the Mg^{2+}-dependent ATPase activity. The inhibition is not specific, since tetraphenylboron was found also to inhibit several other enzymes, and inhibition was much reduced in the presence of albumin.

Thyroid membranes were prepared by a modification of the method of KAMAT AND WALLACH[4]. Calf thyroids were perfused with 0.25 M sucrose containing 5 mM Tris and 0.2 mM $MgCl_2$ (pH 7.4) per l to wash them free of red cells. They were cut with scissors into small pieces, saturated with nitrogen at a pressure of 800 lb/inch^2 for 20 min; by sudden release of pressure the pieces were then disrupted and the cells broken. EDTA was added to a final concentration of 1 mM and nuclei and cell debris were removed by centrifugation at $1.5 \cdot 10^5$ $g \cdot$min. The supernatant was centrifuged at $4.5 \cdot 10^6$ $g \cdot$min, and the pellet resuspended in 0.01 M Tris (pH 8.6); the sediment was resuspended in 1 mM Tris (pH 8.6), centrifuged, and resuspended finally in 1 mM Tris and 1 mM $MgCl_2$ (pH 8.6) and dialyzed for 2–4 h against the same buffer. The resultant homogenate was layered over Ficoll (mol. wt. 400 000 with a density of 1.096 at 4°), which contained 1 mM Tris and 1 mM $MgCl_2$. It was centrifuged in the SW 25.1 Spinco rotor at 24 000 rev./min for 16 h at 4°. The pellet was resuspended in 1 mM Tris, 0.01 M EDTA (pH 8.6), centrifuged at $4.5 \cdot 10^6$ $g \cdot$min at 4°, resuspended in 1 mM Tris and 0.01 M EDTA, and dialyzed in the same buffer for 30 min and then in 1 mM Tris (pH 8.6) for 1 h.

Protein content was determined by the method of LOWRY *et al.*[5]. ATPase activity was measured by the release of phosphate from ATP after incubation for 10 min at 37° (pH 7.4). The final concentrations in a volume of 1 ml, were: Mg^{2+}, $5 \cdot 10^{-3}$ M, and Tris

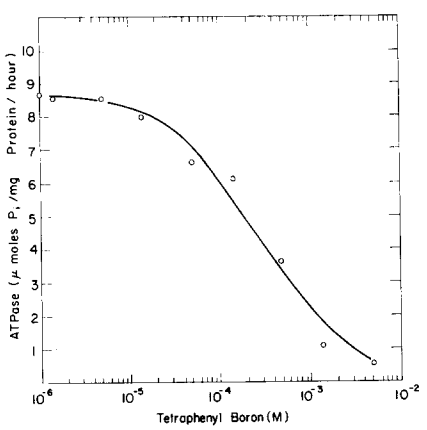

Fig. 1. Inhibition of ATPase activity of a calf thyroid cell membrane preparation by tetraphenylboron. Conditions of incubation appear in the text.

TABLE I

INHIBITION BY TETRAPHENYLBORON OF ATPASE ACTIVITY OF THYROID CELL MEMBRANES

The values are expressed in μg phosphate released/mg protein per h. The final concentration of tetraphenylboron where applicable was 5 mM. The incubation mixture for ATPase activity contained: ATP, 5 μmoles; EDTA, 1 μmole; $MgCl_2$, 5 μmoles; and Tris–HCl (pH 7.4), 50 μmoles. Incubation time was 60 min at 37°. Dialysis was at 4° for 24 h for Expts. 1–4 and for 48 h for Expt. 5.

Conditions	*Expt. 1*	*Expt. 2*	*Expt. 3*	*Expt. 4*	*Expt. 5*
Dialysis only	531	237	251	302	197
Tetraphenylboron; no dialysis	53.9	37.9	—	26.3	33.2
Dialysis; tetraphenyl boron added	9.3	14.4	—	2.2	—
Tetraphenyl boron; dialysis	164	72.1	117	132	133

(pH 7.4), 0.05 M, with approx. 100 μg protein per ml. EDTA (10^{-3} M) was present in all incubations.

Tetraphenylboron proved to be an effective inhibitor of the ATPase activity of these membranes at concentrations well below those of other constituents of the reaction mixture. Thus, the inhibition could not be attributed to direct interaction with the Mg^{2+} or with the substrate ATP. Inhibition was observed at a final concentration as low as $5 \cdot 10^{-5}$ M and was essentially complete at 10^{-3} M (Fig. 1). Increasing the Mg^{2+} concentration did not prevent the inhibition.

Membranes were incubated at room temperature for 10 min with 5 mM tetraphenylboron and then dialyzed in 0.01 M Tris (pH 7.4) with four changes of buffer for 24 or 48 h. Control samples were kept frozen or dialyzed without the addition to tetraphenylboron. Samples were then pelleted, resuspended to original volume, and ATPase activity measured. Samples containing tetraphenylboron were fully inhibited. Samples dialyzed after incubation with tetraphenylboron showed from approximately one-third to one half the ATPase activity of dialyzed controls (Table 1).

Inhibition of ATPase activity appeared to be competitive in type (Fig. 2). Other

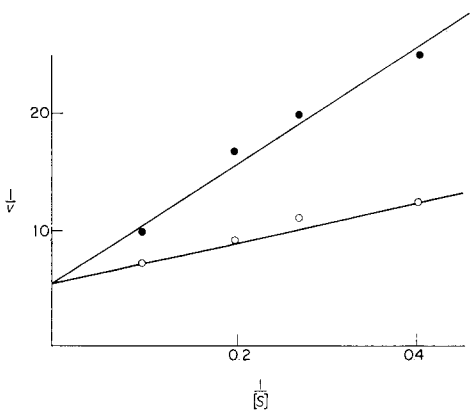

Fig. 2. Inhibition of ATPase activity of a calf thyroid cell membrane preparation by tetraphenylboron at varying substrate concentrations of ATP. Conditions of incubation as described in the text, except the incubation time was 15 min and 2 mg protein per tube. v: rate of formation of phosphate in mmoles phosphate per min; [S]: ATP concentration (mM). ●—●, with tetraphenylboron; ○—○, no tetraphenyl boron.

enzymes were also inhibited by tetraphenylboron. Lactate dehydrogenase was tested by the method of BERGMEYER *et al.*[6] using the enzyme obtained from Boehringer, Mannheim. The enzyme was half inhibited at $5 \cdot 10^{-4}$ M tetraphenylboron. Addition of albumin to a final concentration of 0.8% just prior to addition of enzyme almost completely prevented inhibition by the tetraphenylboron. NADH diaphorase activity using sodium ferricyanide as acceptor and the thyroid membrane preparation was 85% inhibited at $1.6 \cdot 10^{-3}$ M. The malic enzyme was fully inhibited at $5 \cdot 10^{-4}$ M and not appreciably at $5 \cdot 10^{-5}$ M. This assay was kindly performed by Dr. J. P. Flatt using an enzyme preparation obtained from rat liver by an adaptation of the method of HSU AND LARDY[7]. Glucose-6-phosphatase activity determined on a rat liver homogenate was approximately half inhibited at a concentration of $2 \cdot 10^{-4}$ M. Glucose-6-phosphate dehydrogenase activity using an enzyme preparation obtained from Boehringer, Mannheim was half inhibited at $5 \cdot 10^{-4}$ M and fully inhibited at $1.3 \cdot 10^{-3}$ M. A dialyzed crude alkaline phosphatase obtained from Sigma was not inhibited by $5 \cdot 10^{-3}$ tetraphenylboron using β-glycerophosphate as substrate.

These observations indicate that the inhibition of membrane ATPase activity is not specific. Partial reversibility by dialysis suggests that inhibition is not a result of chelation of a small amount of a metal ion required for activity and carried through the purification because of tight binding to the membrane fraction. The strong interaction of tetraphenylboron with K^+ made it difficult to determine any inhibitory effect on the (Na^+-K^+)-activated fraction of ATPase activity: persistence of ATPase activity after the addition of K^+ could be attributed to precipitation of the tetraphenylboron by the K^+. If the tetraphenylboron was incubated with the membranes for 10 min before addition of K^+, the membranes failed to show ATPase activity. Thus, K^+ failed to reverse tetraphenylboron inhibition if the tetraphenylboron was first permitted to interact with the membranes. The fact that inhibition of several other enzymes occurs at approximately the same concentration (except in the case of the crude alkaline phosphatase) and the protective effect of albumin indicates a type of inhibition due presumably to a nonspecific interaction of tetraphenylboron with proteins.

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