TETRAPHENYLBORON – A POTENT ACTIVATOR OF KIDNEY MITOCHONDRIAL GLUTAMINASE

Zoran KOVAČEVIĆ
Department of Biochemistry, Medical Faculty, 21 000 Novi Sad, Yugoslavia

Received 29 March 1976

1. Introduction

Kidney glutaminase (EC 3.5.1.2) is a mitochondrial enzyme [1,2] which plays a key role in the production of ammonia and the maintenance of acid–base, balance especially during metabolic acidosis [3]. Glutaminase is an allosteric protein that is activated by inorganic phosphate and by some other anions [4].

Tetraphenylboron is known as a specific analytical reagent for K^+ since it forms an insoluble salt with this cation. Its NH_4^+ salt is also an insoluble substance. Tetraphenylboron uncouples oxidative phosphorylation and, in higher concentration, causes deformation of cell membrane structure [5]. Skulachev and co-workers have introduced this anion (and some other chaotropic ions) as reagents for determining the direction of electron flow in mitochondrial membrane. For these reasons it was of interest to see if tetraphenylboron affects the activity of glutaminase in intact mitochondria. It was found that it activates the enzyme very strongly and that the activation is the result of a direct effect of the anion on glutaminase in terms of lowering K_A for phosphate.

2. Experimental

Tetraphenylboron (Na^+ salt) was from BDH Chemicals Ltd., England. The effect of tetraphenylboron on glutaminase activity was tested by using preparations of intact, frozen and thawed, and lysed mitochondria from pig kidney. The isolation procedure has been described [7,8]. The present experiments were performed with intact and frozen and thawed mitochondria.

Unless otherwise stated, the activity of glutaminase was estimated in a medium of the following composition: 100 mM Tris-HCl, 30 mM Tris-phosphate, 2.5 mM $MgCl_2$ and 10 mM [U-^{14}C]glutamine; final pH 7.4, temperature 30°C. The amount of mitochondrial protein was about 5–6 mg in 2 ml of the incubation mixture. The reaction was stopped by adding 0.6 ml of trichloroacetic acid (20% w/v). [^{14}C]Glutamate formed was isolated by ion exchange chromatography and the radioactivity measured with a Geiger–Müller counter.

3. Results and discussion

Fig.1 shows that tetraphenylboron has a dual effect on kidney glutaminase. In the range 0–2 mM, low concentrations strongly activate the enzyme with a maximum activation at 0.5 mM tetraphenylboron. Higher concentrations progressively inactivate the enzyme. There is linear relationship between glutaminase activity and the amount of protein in the presence of a constant tetraphenylboron concentration. This holds down to 70 nmol tetraphenylboron/mg protein. If the ratio is further decreased the effect of tetraphenylboron gradually disappears. The enzyme is activated in intact and also in frozen and thawed preparation of mitochondria. Tetraphenylboron activates glutaminase in the presence of Triton X-100 (0.04%) provided it is added before the detergent.

It can be seen from Fig.2 that tetraphenylboron strongly activates glutaminase even at pH 6.5 when the activity of the enzyme without the activator is virtually absent. The activation was observed at all pH's tested.

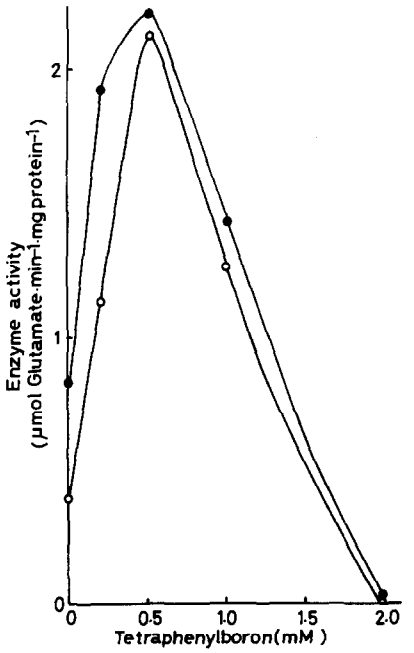

Fig.1. Effect of tetraphenylboron on the activity of pig kidney glutaminase. (○——○) 45 mM Tris-phosphate, (●——●) 90 mM Tris-phosphate.

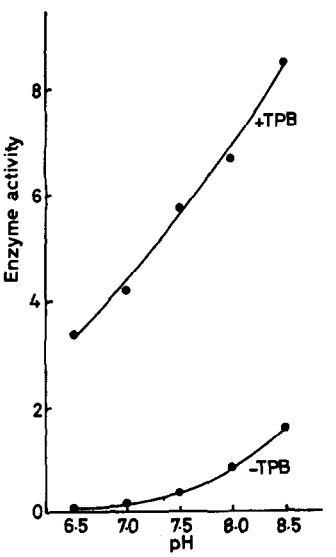

Fig.2. Effect of tetraphenylboron (0.5 mM) on the activity of glutaminase at different pHs. Enzyme activity, μmoles glutamate/min/mg protein.

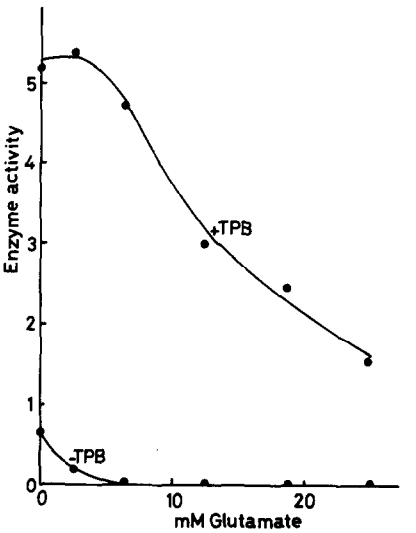

Fig.3. Inhibition of glutaminase by glutamate (Tris salt) in the presence and absence of 0.5 mM tetraphenylboron. Enzyme activity, μmoles glutamate/min/mg protein.

Glutamate is known as a potent inhibitor of glutaminase [9,10]. The inhibition by glutamate is non-competitive in respect to phosphate. Fig.3 shows that tetraphenylboron effectively removes or prevents the inhibition by glutamate. Moreover, tetraphenylboron strongly activates the enzyme even in the presence of such glutamate concentrations that completely inhibit glutaminase.

Further investigation showed that the activatory effect of tetraphenylboron is especially pronounced at lower pHs and at lower phosphate concentrations (figs.4 and 5). When the optimum pH and phosphate concentration were approached, the effect of tetraphenylboron virtually disappeared. This suggests that the main effect of the activator is to increase the affinity of glutaminase for phosphate so that the K_A for this anion decreases to unmeasurable values. It was found that tetraphenylboron stimulates glutaminase even in preparations of frozen and thawed mitochondria that were dialysed against 100 mM Tris-HCl, pH 7.4, in order to remove endogenous phosphate.

Since regulation of glutaminase activity in intact mitochondria is of special importance, it was interesting to see whether tetraphenylboron activates the enzyme at concentrations that do not damage the

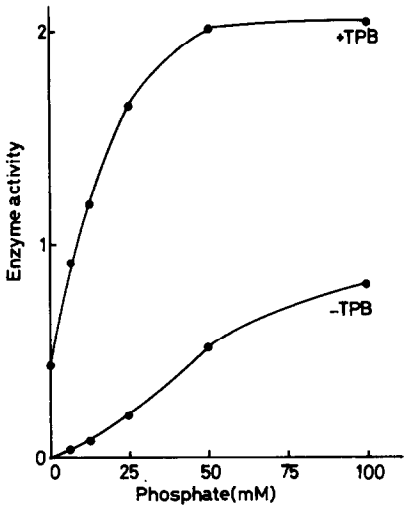

Fig.4. Effect of 0.5 mM tetraphenylboron on glutaminase activity as a function of phosphate concentration. Enzyme activity expressed as in fig.1.

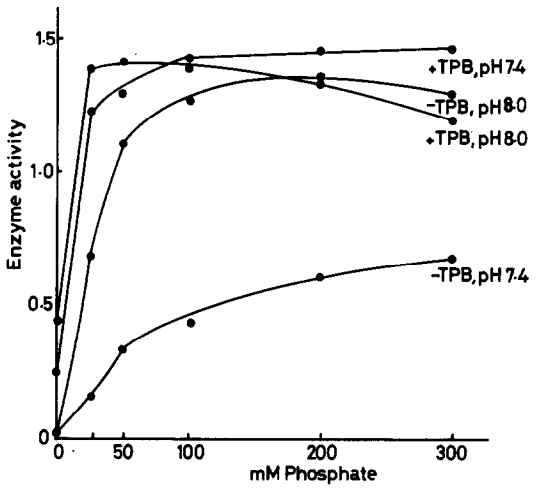

Fig.5. Effect of 0.5 mM tetraphenylboron on the activity of glutaminase at different pHs and different concentrations of Tris-phosphate. $MgCl_2$ was omitted. Ionic strength of the medium was kept relatively constant by adding appropriate amounts of Tris-HCl. Enzyme activity was expressed as c.p.m. $\times 10^{-3}$ of glutamate formed.

membrane. It was found that 100 to 200 μM tetraphenylboron did not inhibit respiration with glutamine or pyruvate as substrate but the mitochondria were uncoupled (table 1). The same concentrations of tetraphenylboron, however, strongly stimulated glutaminase activity. The uncoupling effect can be partly removed by adding bovine serum albumin. It is interesting to note that 100 μM tetraphenylboron strongly inhibits respiration if glutamate is substrate probably due to inhibition of the transport of the anion, whereas the addition of glutamine completely abolishes the inhibition. All these data suggest that tetraphenylboron, at the concentration which strongly stimulates glutaminase in intact mitochondria, does not seriously damage the mitochondrial membrane. Further increase in tetraphenylboron concentration induces a large swelling of the mitochondria and complete inhibition of their respiration due to severe damage of the membrane. These results suggest that under controlled experimental conditions this activator might be a useful tool in the study of regulation of glutaminase activity in intact mitochondria.

Table 1

Experimental conditions	Enzyme activity (%)	Rate of O_2 uptake (%)	Respiratory control
Control	100	100	7
+ 40 μM tetraphenylboron	125	100	5
+ 100 μM tetraphenylboron	458	100	uncoupled
+ 200 μM tetraphenylboron	1350	91	uncoupled
+ 400 μM tetraphenylboron	2010	4	–

The mitochondria (3.5 or 7 mg protein) were incubated in a medium of the following composition: 100 mM Tris-HCl, 4 mM Tris-phosphate and 2.5 mM $MgCl_2$; pH 7.25. Final volume 2.5 or 5 ml; temperature, 30°C. Oxygen uptake was estimated with a Clark oxygen electrode. Respiration was stimulated by adding ADP (0.4 mM) or carbonyl cyanide *m*-chlorophenylhydrazone (0.5 μg/ml).

The effect of tetraphenylboron is very much the same as the effect of Bromothymol blue on the same enzyme [11]. Glutaminase is known as a polymeric protein which occurs in different interconvertible molecular forms. Activation is connected with aggregation of protomers. According to Kvamme and co-workers the enzyme has at least two regulatory phosphate binding sites so that the binding of the activator to the one of these sites has a positive cooperative effect on the binding of the ligand to the other site [12]. The activation by tetraphenylboron may be caused by its binding to some other allosteric site which results in lowering the K_A for phosphate, whereas the inhibition might be the result of competition between tetraphenylboron and phosphate for the same anionic binding site.

Although tetraphenylboron is not a physiological substance, its potent activatory effect may be utilized as a tool for the study of molecular properties and regulatory mechanisms of glutaminase. The allosteric nature of the enzyme and its strong activation by tetraphenylboron and some other anions suggest that a physiological substance with a similar effect might exist especially during metabolic acidosis when production of ammonia is greatly increased.

References

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