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EFFECT OF TETRAPHENYLBORON UPON THE UPTAKE OF THE LIPOPHILIC CATION DIBENZYLDIMETHYLAMMONIUM BY YEAST CELLS

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SUMMARY

The rate of uptake of the lipophilic cation dibenzyldimethylammonium by yeast cells is increased by tetraphenylboron. However, tetraphenylboron increases also the equilibrium partition of dibenzyldimethylammonium between cells and medium, probably because a complex between tetraphenylboron and dibenzyldimethylammonium is trapped inside the cells. Accumulation of dibenzyldimethylammonium in the presence of tetraphenylboron is not reversed by dinitrophenol, whereas accumulation of the lipophilic cation in the absence of tetraphenylboron appears to be almost completely reversible.

One of the main means of getting information about the membrane potentials of small cells in which it is difficult to measure the membrane potential directly by means of microelectrodes is the determination of the partition coefficient of a lipophilic cation between cells and medium. Frequently the lipophilic quaternary base dibenzyldimethylammonium, which is able to pass artificial membranes, is applied. This cation has been used with mitochondria [1, 2], bacteria [3, 4] and bacterial vesicles [5, 6]. According to Bakeeva et al. [2] addition of small amounts of tetraphenylboron increases considerably the rate of dibenzyldimethylammonium uptake by mitochondria and thus causes a decrease in the equilibration time of this cation between mitochondria and medium. This is also found for *Streptococcus faecalis* [3] and for *Escherichia coli* vesicles [6]. The results presented here show that addition of tetraphenylboron may lead to wrong values of the computed membrane potential.

2.2 % yeast, *Saccharomyces cerevisiae* Delft 2, is preaerated for 1 night in distilled water in order to deplete the internal substrate of the cells. Then the yeast is washed twice by centrifuging and resuspending the cells in 45 mM Tris/succinate buffer of pH 4.5. The cells are kept anaerobically by bubbling nitrogen through the suspension. ^{14}C-labelled dibenzyldimethylammonium is added, together with non-labelled dibenzyldimethylammonium, to the yeast cell suspension to a final concentration of 0.1 mM, whereby the concentration of the yeast is reduced to 2 % w/v. When adding tetraphenylboron to the yeast suspension, this is done together with dibenzyldimethylammonium. Uptake of dibenzyldimethylammonium is determined

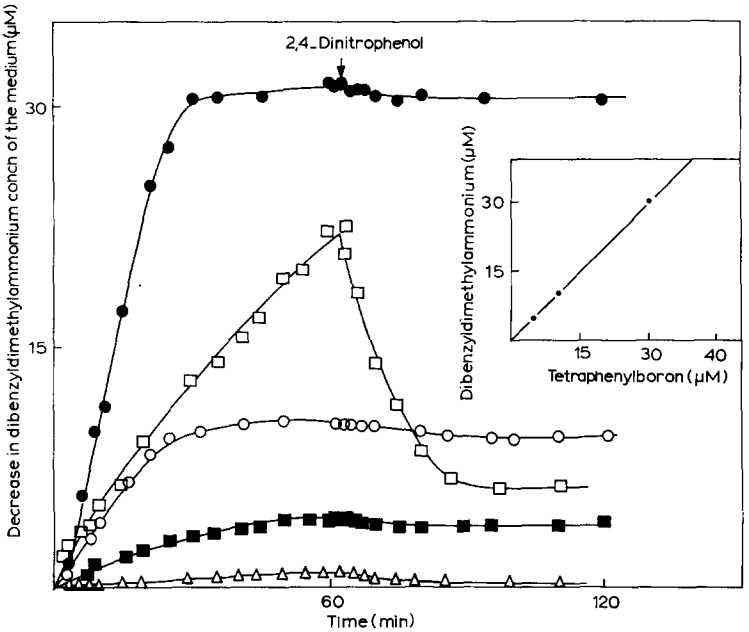

Fig. 1. Effect of varying amounts of tetraphenylboron upon the uptake of 0.1 mM dibenzyldimethylammonium by non-metabolizing cells, reversal of dibenzyldimethylammonium uptake induced by adding 1 mM 2,4-dinitrophenol and the relation between the decrease in the concentration of dibenzyldimethylammonium in the medium and the concentration of tetraphenylboron (inset). On the ordinate is indicated the decrease in concentration of dibenzyldimethylammonium in the medium. ■, ○, ●, 5, 10 and 30 μM tetraphenylboron, respectively. □ refers to 20 times the blank (△). The arrow indicates the time at which 2,4-dinitrophenol to a final concentration of 1 mM is added.

with the same procedure as that for examining the uptake of radioactive inorganic cation [7], except that washing with acetone is omitted and the radioactivity on the filter papers is measured by means of liquid scintillation according to Borst-Pauwels [8]. ^{14}C-labelled dibenzyldimethylammonium is prepared according to Lombardi et al. [5]. Tetraphenylboron is obtained from Sigma chemical company and non-radioactive dibenzyldimethylammonium from K and K Laboratories.

Fig. 1 shows the kinetics of uptake of dibenzyldimethylammonium by non-metabolizing cells and the effect of tetraphenylboron upon the kinetics of uptake. Tetraphenylboron increases both the rate of uptake and the equilibrium concentration of dibenzyldimethylammonium in the cells. The amount of dibenzyldimethylammonium absorbed by the cell in the presence of tetraphenylboron appears to be almost linearly related to the amount of tetraphenylboron added. As a matter of fact, at an initial concentration of 30 μM tetraphenylboron, the decrease in dibenzyldimethylammonium concentration in the medium was also 30 μM. This indicates that dibenzyldimethylammonium is trapped in the cells as a stoichiometric 1:1 complex with tetraphenylboron. This view is supported by the fact that the accumulation of dibenzyldimethylammonium in the cells is no longer reversible in the presence of tetraphenylboron as shown by the fact that 1 mM 2,4-dinitrophenol does not much affect the dibenzyldimethylammonium concentration in the cells, whereas in the

absence of tetraphenylboron 1 mM 2,4-dinitrophenol leads to an almost quantitative reversal of dibenzyldimethylammonium uptake.

The uptake of dibenzyldimethylammonium by metabolizing cells preincubated for 1h with 3% glucose is also accelerated by tetraphenylboron. The uptake found in the presence of 10 μM tetraphenylboron is biphasic, consisting of a rapid uptake induced by tetraphenylboron, which is already complete within 1 min, and a slow uptake proceeding at the same rate as the uptake of dibenzyldimethylammonium occurring in the absence of added tetraphenylboron. The latter uptake is completed after about 30 min, much faster than the uptake by non-metabolizing cells which is completed after about 3 h. Table I shows the effect of 10 μM tetraphenylboron upon the equilibrium distribution ratios of both metabolizing cells and non-metabolizing cells. The equilibrium distribution ratios represent the quotients of the concentration of dibenzyldimethylammonium in the cell water (0.44 ml/g of pressed yeast [9]) and the concentration of the cation in the medium.

It is seen that the additional effect upon the distribution ratios excerted by tetraphenylboron does not depend upon the metabolic state of the cells.

There are indications that dibenzyldimethylammonium forms a complex with tetraphenylboron already in the medium. A solution of 0.1 mM dibenzyldimethylammonium becomes slightly opalescent on adding tetraphenylboron to a final concentration of 10 μM or higher. However, it is not probable that the extra amount of dibenzyldimethylammonium accumulated in the presence of tetraphenylboron consists of adsorption of a complex of tetraphenylboron and dibenzyldimethylammonium at the outerside of the yeast, because the kinetics of the tetraphenylboron dependent uptake of dibenzyldimethylammonium differs largely for non-metabolizing and for metabolizing cells. The half value times are 15 and $\leqslant$ 1 min, respectively, for the two kind of cells.

It may be concluded from the results represented in this article that one should be very careful in using tetraphenylboron as a means for accelerating the rate of dibenzyldimethylammonium uptake. The apparent partition ratio of dibenzyldimethylammonium is increased by this compound and too high values for the calculated membrane potential may be found on applying the Nernst equation to the equilibrium distribution data found. There are indications that tetraphenylboron increases the partition ratio of dibenzyldimethylammonium with other type of cells as well [6, 10]. An increase in dibenzyldimethylammonium partition ratio is also found with mitochondria when adding an other lipophilic anion, namely phenyldicarbaundecaborate, together with dibenzyldimethylammonium to the mitochondrial suspension [11]. On the other hand, for *Streptococcus faecalis* it is shown that

TABLE I

EQUILIBRIUM DISTRIBUTION RATIOS OF DIBENZYLDIMETHYLAMMONIUM FOUND IN THE ABSENCE (−) AND IN THE PRESENCE (+) OF 10 μM TETRAPHENYLBORON

	(−)	(+)
Non-metabolizing cells	1.7	11.5
Metabolizing cells	10.0	20.1

tetraphenylboron does not affect appreciably the dibenzyldimethylammonium partition ratio [3].

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