

# Tetraphenylborate-Sensitive Electrode for Measuring Membrane Potential

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**ABSTRACT.** The paper describes the construction of a new type of ion-selective electrode sensitive to tetraphenylborate (TPB<sup>-</sup>) and its electric characteristics. The electrode responds to increasing concentrations of the TPB<sup>-</sup> anion in accordance with the Nernst equation and can be used down to 0.1  $\mu$ M concentration. The applicability of the electrode for measuring the membrane potential (positive inside) was proved in inside-out oriented membrane vesicles derived from *Paracoccus denitrificans*. The calculated values were  $175 \pm 12$  mV with NADH and  $180 \pm 6$  mV with succinate.

**Abbreviations:** TPP<sup>+</sup> tetraphenylphosphonium, SCN<sup>-</sup> thiocyanate, TPB<sup>-</sup> tetraphenylborate.

For measuring the membrane potential (negative inside) with the aid of permeant ion distribution the most suitable and commonly applicable method is the use of a TPP<sup>+</sup>-sensitive electrode (Kamo *et al.* 1979), the reliability of which for bacterial preparation has recently been proved (Felle *et al.* 1980; Singh and Bragg 1979). Of the anion-selective electrodes required for membrane potential measurements (positive inside) the electrode sensitive to SCN<sup>-</sup> has been widely used. The disadvantage of the type commercially available (Orion 94-58) is the relatively low sensitivity to SCN<sup>-</sup> ions; therefore, the sensibilized nitrate module of the type Orion 93-07 was introduced for SCN<sup>-</sup> ions measurement (Singh and Bragg 1979). This, however, makes it impossible to add nitrate or nitrite as terminal electron acceptors or cyanide as respiratory inhibitor to the reaction mixture. In this report we describe the construction of an electrode sensitive to TPB<sup>-</sup> which is a structural analog of TPP<sup>+</sup> and as such it easily penetrates through biological membranes (Grinius *et al.* 1970). The construction makes use of the fact that TPB<sup>-</sup> effectively precipitates tetraalkylammonium ions (Narasimhan *et al.* 1972). The practical applicability of the electrode was already proved on membrane vesicles from *Paracoccus denitrificans* (Karlovský *et al.* 1982).

## MATERIALS AND METHODS

Hexadecyltrimethylammonium bromide (pure) and sodium tetraphenylborate (p.a.) were obtained from Lachema, Brno; tetraphenylphosphonium chloride from Fluka, Switzerland; tetrahydrofuran was purified by leaving it in the presence of solid KOH and by redistillation with sodium. Other chemicals were of analytical grade.

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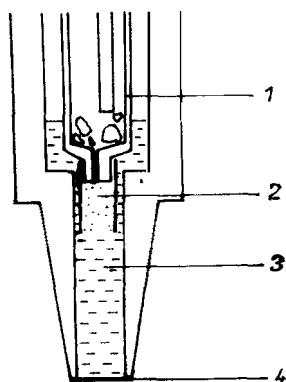


FIG. 1. Diagram of  $\text{TPB}^-$ -sensitive electrode: 1 reference calomel electrode, 2 3 % agar + 17 % LiCl, 3 1 mM NaTPB, 4 PVC membrane.

**Preparation of the  $\text{TPB}^-$ -sensitive electrode.** Hexadecyltrimethylammonium bromide (12 mg) was dissolved in 0.5 mL of absolute methanol and diluted with 12 mL of tetrahydrofuran. Then 0.5 g polyvinyl chloride and 1.5 mL dioctylphthalate were added and dissolved under continuous stirring. The mixture was poured on a Petri dish of 60 cm<sup>2</sup> area and left to dry at room temperature. After a temporary opacity a clear membrane was obtained. A disk cut from it was cemented to the end of a polymethylmethacrylate tube with a water-insoluble glue and the closed tube was filled with 1 mM  $\text{TPB}^-$ . The saturated calomel reference electrode was fitted with a polyethylene tube 7 mm long which was filled with warm 3.7 % agar solution containing 17 % (V/W) LiCl. After the agar had solidified the calomel electrode was introduced into the inner solution of  $\text{TPB}^-$  (see Fig. 1.). Before the first measurement the electrode was immersed for 12 h in 1 mM tetraborate; the same medium was used for its storage.

$\text{TPP}^+$ -sensitive electrode was prepared as described by Kamo *et al.* (1979).

**Microorganisms.** *Paracoccus denitrificans* Davis, strain NCIB 8944, was cultivated anaerobically as described previously (Štros *et al.* 1982). Membrane vesicles were prepared according to Burnell *et al.* (1975), but the concentration of lysozyme was raised to 200 mg/L (Karlovský *et al.* 1982).

## RESULTS AND DISCUSSION

### *Characteristics of the $\text{TPB}^-$ -sensitive electrode*

The  $\text{TPB}^-$ -sensitive electrode developed in this laboratory responded to increasing concentrations of  $\text{TPB}^-$  ions almost exactly in accordance with the Nernst equation. In Fig. 2 the calibration curves for  $\text{TPP}^+$  and  $\text{TPB}^-$  electrodes are compared. From the view of membrane potential estimation the most important characteristic is the response of the electrode to 10  $\mu\text{M}$  and lower ion concentrations. The calibration curves of both electrodes are nearly linear for 1–10  $\mu\text{M}$  concentrations with slopes of about 50 mV for the  $\text{TPP}^+$  electrode and of 60–68 mV for the  $\text{TPB}^-$  electrode. Both electrodes are applicable down to 0.1  $\mu\text{M}$  concentrations, the  $\text{TPB}^-$  electrode being more sensitive in the range from 1 to 10  $\mu\text{M}$ . Below that value the linearity is not ideally fulfilled. This requires to work in a relatively narrow concentration range, i.e., to use relatively low concentrations of membrane vesicles. This condition does not mean a serious limitation to the applicability of the electrode because of its high sensitivity. When the concentration of  $\text{TPB}^-$  was increased twofold, i.e.

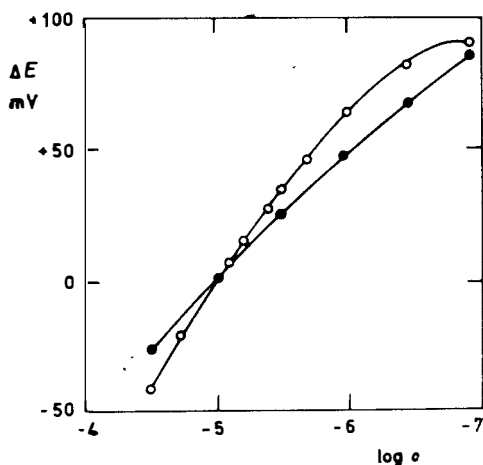


Fig. 2. Calibration curves of TPP<sup>+</sup>-sensitive (closed symbols) and TPB<sup>-</sup>-sensitive (open symbols) electrodes. Measurements were performed in a vessel equipped with magnetic stirring. The potential of either type of electrode was measured versus a saturated calomel electrode connected with the solution by LiCl (TPB<sup>-</sup> electrode) or KCl (TPP<sup>+</sup> electrode) bridge. Both electrodes were charged with such a polarity that they responded to a drop of ion concentration by an increase of potential. They were prepolarized to a zero potential at 10  $\mu$ M concentration. The difference of potential was monitored by a millivoltmeter OP-208 (Radelkis).

from 1  $\mu$ M to 2  $\mu$ M the electrode potential stabilized in 3 s; this interval is the common response time in buffered media. The slope of the curve is constant for several hours of an experiment, there being a deflection of the slope of 9 mV after 3 d which is obviously due to time changes occurring in the membrane. Renewal of the membrane and of the inner electrode solution after a week is recommendable, the dry membrane can be stored for a least 6 weeks. The slope of the curve drops with increasing ionic strength by 7–10 % in 0.1 M and 0.3 M NaCl, after aging of the membrane by 21 % and 29 %, respectively. The standard electrode potential is affected even more substantially which makes it necessary to perform measurements at a constant ionic strength as is commonly done.

The effect of interfering substances was followed on electric potential estimations in solution of 10  $\mu$ M sodium tetraphenylborate and 0.3 M NaCl, with various concentrations of tested substances, *i.e.* by the direct method (Punger and Tóth 1970). Sodium nitrate, sodium phosphate (pH 7.0), Tris.HCl (pH 7.0) and sodium sulphate were taken to represent the composition of biochemical media. The first three substances at concentrations up to 0.1 M caused electrode potential alterations of less than 2 mV, the potential being changed by the last compound by 4 mV. The sensitivity of the electrode to these substances expressed usually by selectivity coefficients is therefore negligible. Of the usual cations, potassium and ammonium ions form precipitates with tetraphenylborate. Under given conditions the alteration of electrode potentials for both was less than 3 mV, for up to 30 mM KCl and for up to 50 mM NH<sub>4</sub>Cl. When the actual membrane potential is estimated the concentrations of all the tested substances can be substantially higher than the described limits provided they stay constant during measurement and calibration is performed in a medium of corresponding composition.

#### *Estimation of membrane potential in vesicles*

Before quantitative interpretations of the data obtained with membrane preparations can be made it is necessary to consider whether the extent of uptake of TPB<sup>-</sup> anions really represents the size of electric potential across the bacterial membrane. Two criteria should be fulfilled: (1) the anion must follow Nernstian

equilibrium distribution and not bind irreversibly to cellular components; (2) it must not interfere with cellular energy metabolism. Furthermore, there must be a reliable estimate for the internal aqueous phase of vesicles. The addition of membrane vesicles of *P. denitrificans*, which were 82 % of inside-out orientation (Burnell *et al.* 1975; Karlovský *et al.* 1982), brought about the decrease of TPB<sup>-</sup> concentration in the suspension medium. The TPB<sup>-</sup> electrode responded to additions of respiratory substrates and inhibitors in a way that was expected from the generation of membrane potential. Thus the TPB<sup>-</sup> movement complied with the requirement stated above as was the case with TPP<sup>+</sup>. Yet the value of calculated membrane potential could be markedly overestimated by not subtracting the part of the TPB<sup>-</sup> uptake which was not fully reversible. In order to be rigorous we should introduce a correction for the amount of TPB<sup>-</sup> which was irreversibly adsorbed on membrane constituents. A correction method worked out for the use of TPP<sup>+</sup> in our previous work was also found to be applicable to TPB<sup>-</sup> (Karlovský *et al.* 1982).

The respiratory activity of intact cells of *P. denitrificans* was not influenced in the presence of TPB<sup>-</sup> from 1 to 10  $\mu\text{M}$  concentration. When oxygen consumption of vesicles with the inside-out membrane orientation was measured using succinate as substrate a TPB<sup>-</sup>-dependent inhibition was observed. In media containing 10 mM Tris-acetate (pH 7.3) addition of TPB<sup>-</sup> to a final concentration of 30  $\mu\text{M}$  lowered the oxidation by 21 %, whereas 10  $\mu\text{M}$  and 5  $\mu\text{M}$  TPB<sup>-</sup> inhibited by only 13 and 8 %, respectively. It therefore seemed unlikely that estimates of the membrane potential with the aid of TPB<sup>-</sup>-sensitive electrode would be significantly altered when working with a low concentration of the TPB<sup>-</sup> anion.

Estimation of the volume of the internal aqueous phase of vesicles with  $^3\text{H}$ -H<sub>2</sub>O and  $^{14}\text{C}$ -methoxyinulin according to Rottenberg (1979) led to values of  $2.6 \pm 0.4$  mL per g protein (Karlovský *et al.* 1982). Using this value membrane potentials of  $+175 \pm 12$  mV with NADH and  $+180 \pm 6$  mV with succinate were calculated, these being markedly higher than values found by Kell *et al.* (1978) but in agreement with values found in inside-out oriented submitochondrial particles (Azzi *et al.* 1971).

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