ESTIMATIONS OF MEMBRANE POTENTIALS IN STREPTOCOCCUS FAECALIS BY MEANS OF A FLUORESCENT PROBE

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Received January 24, 1974

SUMMARY

Membrane potentials in Streptococcus faecalis (faecium) were estimated by means of the fluorescent probe, 1,1'-dihexyl-2,2'-oxycarbocyanine. In the absence of D-glucose the potential was -60 to -70 mV for normal cells suspended in 0.09 M NaCl + 0.01 M Tris-HCl at pH 7.5. When metabolism was initiated by the addition of D-glucose the cells became hyperpolarized (internal becomes more negative). The new potential, -130 to -140 mV, was fully manifested 35 seconds after the glucose was added. N,N'-dicyclohexylcarbodiimide, a membrane ATPase inhibitor prevented the hyperpolarization seen upon the addition glucose. The results are consistent with the view that glycolyzing cells generate a considerable electrical potential across the cell membrane.

INTRODUCTION

Recent investigations have indicated that the active transport of β-galactosides (1, 2) in Streptococcus lactis and neutral amino acids (3) in Streptococcus faecalis may be driven by an electrical potential difference across the membrane. The studies support the hypothesis that the active transport of these metabolites is dependent on a "proton motive force" generated by the activity of a membrane ATPase (4, 5). In S. faecalis it has been suggested (3) that the proton motive force consists predominantly of a membrane potential. An attempt to measure this potential was made using a method based on the distribution of lipid soluble cations (6-8). Although these studies indicated that there was a potential of the requisite order in Na-loaded cells, no potential was measured in normal K-loaded cells even though both kinds of cells were capable of the active transport of neutral amino acids. The discrepancy may be caused by limitations inherent in the use of lipid soluble

Abbreviations: CC_6, 1,1'-dihexyl-2,2'-oxycarbocyanine; DCCD, N,N'-dicyclohexylcarbodiimide; CCCP, carbonyl cyanide m-chlorophenylhydrazone.

cations to measure potentials (9). Since measurements of the electrical potentials are important in the critical evaluation of this hypothesis, we have employed fluorometric methods which have been used successfully in red blood cells (10) to measure them.

METHODS AND MATERIALS

KTY and NaTY cells (S. faecalis ATCC 9790) were prepared as described by Harold and Papineau (6). The method for measuring fluorescence has been previously described (10) and the compositions of the incubation mixtures are given in the figure legends. The cuvette contained 1.3 to 1.6 mg (dry weight) of cells in an initial volume of 3 ml (approximately a 0.6% suspension). The dye employed in these studies, 1,1'-dihexyl-2,2'-oxycarbocyanine (hereafter CC_6), was generously donated by Dr. Alan Waggoner of Amherst College. Final concentration of the dye was 2.9×10^{-6}M. Wherever noted valinomycin (final concentration 1×10^{-6}M) and DCCD (final concentration 2×10^{-4}M) were added dissolved in ethanol. The final ethanol concentration in the cuvette was never higher than 0.6%. DCCD was purchased from K and K Laboratories, and valinomycin from Calbiochem. Cellular K^+ was determined by flame photometry. Samples were digested in concentrated HNO_3 for 48 hours before analysis. The water space of S. faecalis was taken to be 2.43 ml/g (dry weight) of cells (6).

RESULTS

Valinomycin Addition. The intensity of fluorescence of CC_6 in a suspension of S. faecalis cells in 0.1M NaCl-Tris (0.09M NaCl + 0.01M Tris-HCl, pH 7.5) was about 2 times higher than that seen in the absence of cells. When valinomycin, an ionophore known to induce a marked K permeability in these cells (11), was added to cells suspended in 0.1M NaCl-Tris, there was a rapid drop in fluorescent intensity of 25-30% (Fig. 1). In contrast the addition of valinomycin to cells in 0.1M KCl-Tris (0.09M KCl + 0.01M Tris-Cl, pH 7.5) resulted in an increase of about 10%. The steady level of fluorescence attained was the same whether valinomycin was added before or after the dye (Fig. 1). The addition

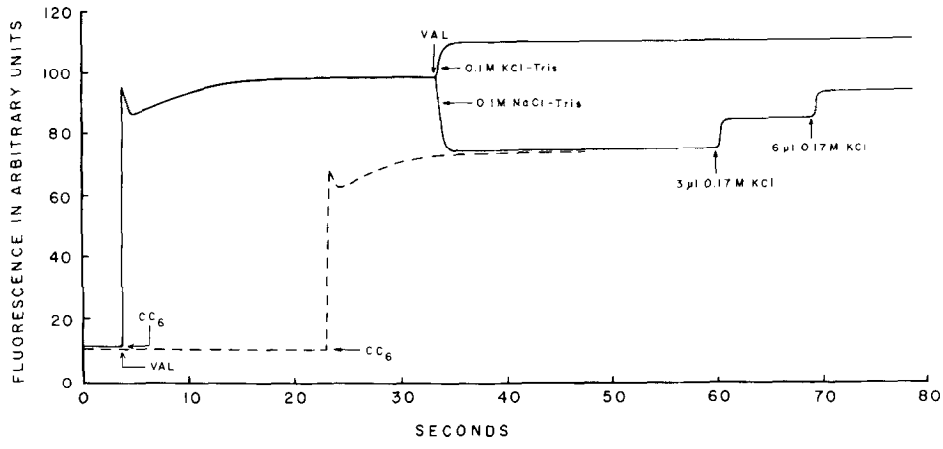

Fig. 1. Fluorescent intensity of the dye CC_6 (excitation 460 nm; emission 505 nm) in a 0.6% suspension of S. faecalis cells in 0.1M NaCl-Tris or 0.1M KCl-Tris media, pH 7.5, as a function of time. CC_6 and valinomycin (VAL) added to final concentrations 2.9×10^{-6}M and 10^{-6}M respectively. 0.17M KCl was added to cells in 0.1M NaCl-Tris as indicated. In the curve with dashed line valinomycin was added before CC_6.

of the same amount of ethanol alone did not change the fluorescent intensity. When the selective integrity of the permeability barrier of the cells was disrupted by treatment with toluene (12), sodium dodecyl-sulphate (13) or cetyltrimethylammonium bromide (14), the addition of valinomycin did not change the fluorescent intensity.

After treatment with valinomycin, the fluorescent intensity of cells suspended in 0.1M NaCl-Tris increased upon addition of KCl (Fig. 1). The level of fluorescence was found to be related to the amount of KCl added and was directly proportional (Fig. 2) to the logarithm of the external K concentration in the range of K = 0.6mM to 8mM. At higher or lower concentrations of KCl the intensity appeared to approach upper and lower limits.

Glucose Addition. When metabolism was initiated in KTY or NaTY cells with D-glucose (final concentration 0.5mM) there was a decrease in fluorescent intensity (Fig. 3). This decrease was seen in cells suspended in either 0.1M NaCl-Tris or 0.1M KCl-Tris media. However, since the decrease was most rapid and largest in the case of KTY cells in 0.1M NaCl-Tris, this combination of cells and medium was used in subsequent experiments involving glucose.

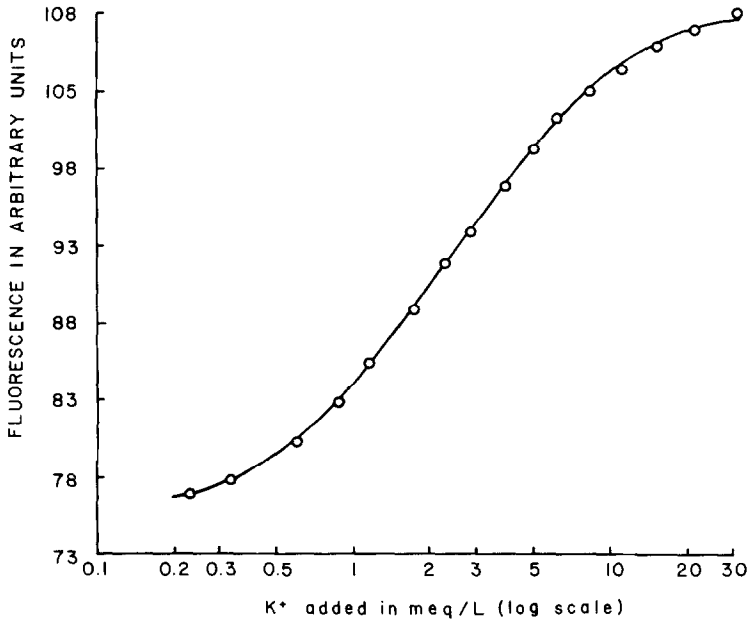

Fig. 2. Fluorescent intensity of the dye CC_6 (excitation 460 nm; emission 505 nm) in valinomycin-treated KTY cells in 0.1M NaCl-Tris following additions of KCl (see Fig. 1) and plotted as a function of log K^+_{ex}. Final concentrations of valinomycin and CC_6 were 10^{-6}M and 2.9 x 10^{-6}M respectively.

As shown in Figure 3, the decline began a few seconds after the addition of D-glucose and was complete in approximately 40 seconds. The same level of fluorescence was approached directly when glucose (final concentration 0.5mM) was added 40 seconds before the dye. When a lower concentration (final concentration 0.05mM) of D-glucose was added, the decrease in fluorescence was temporary. No change in fluorescence was seen when L-glucose (final concentration 0.5mM) was added. The possibility that pH change or lactate production were responsible for the observed change in fluorescence was easily eliminated, since: (i) there was no perceptible change in pH during the 40 second interval following glucose addition, and (ii) the addition of Tris-lactate (final concentration 0.1mM, pH 7.5) had no effect on fluorescence.

After the fluorescent intensity of cell suspensions in 0.1M NaCl-Tris plus glucose had reached a steady level (Fig. 3), the addition of valinomycin led to a further decline which was smaller than that recorded in the absence

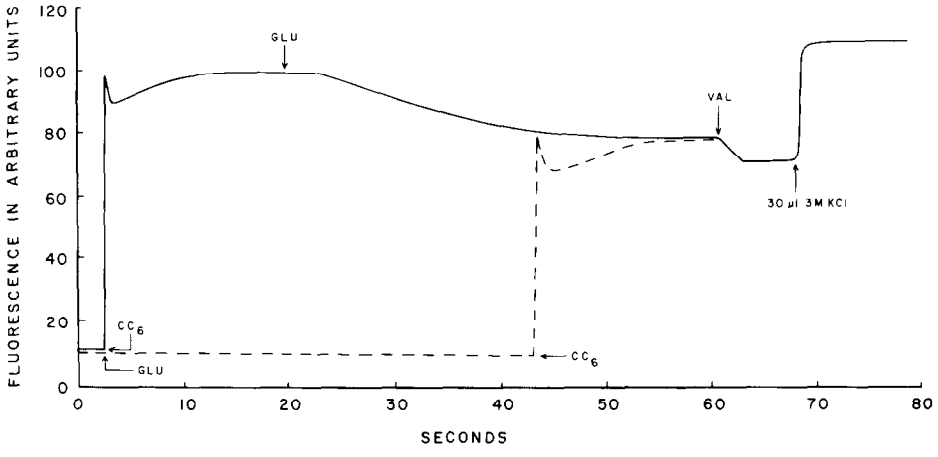

Fig. 3. Fluorescent intensity of the dye CC_6 (excitation 460 nm; emission 505 nm) in a 0.6% suspension of KTY cells in 0.1M NaCl-Tris following the addition of D-glucose (GLU) to final concentration 0.5mM. Final concentrations of valinomycin (VAL) and CC_6 were 10^{-6}M and 2.9 x 10^{-6} respectively. KCl was added as indicated. In the curve with dashed line glucose (final concentration 0.5mM) was added before CC_6.

of glucose. The addition of KCl to these suspensions again led to an increase in intensity.

DCCD Addition. The effects of DCCD, which is known to inhibit the membrane ATPase in S. faecalis (15) were also tested on the system. Although no change in fluorescent intensity of the dye was observed when DCCD (final concentration 2 x 10^{-4}M) was added to cells suspended in 0.1M NaCl-Tris, this concentration of DCCD completely inhibited the decrease observed with glucose. When DCCD was added after the change with glucose had reached a steady level, the fluorescent intensity increased steadily and returned after about 45 seconds to the level seen before the addition of glucose. DCCD had no effect on the changes seen with valinomycin.

DISCUSSION

Previous studies (10, 16) have demonstrated that a hyperpolarization (internal becomes more negative) of the membrane of the human erythrocyte, the Amphiuma erythrocyte and the squid axon resulted in a decrease in the fluorescent intensity of CC_6 while depolarization led to an increase. Since S.

faecalis cells are high in K content, the increase in K permeability which results from the addition of valinomycin would be expected to cause a hyperpolarization. The decrease in fluorescent intensity observed with the addition of valinomycin to S. faecalis cells in K free media then is consistent with the results obtained using other cells. Also, additions of KCl to cells in K free media with valinomycin should depolarize the membrane. The anticipated increase in fluorescent intensity was observed. If we make two assumptions, first that the membrane potential after the addition of valinomycin is essentially equivalent to the K equilibrium potential, and second that equal levels of fluorescent intensity reflect equivalent membrane potentials, then it is possible to estimate the potential. The first assumption is reasonable since valinomycin is known to induce a high K permeability in these cells (11). In addition, our studies demonstrated that fluorescent intensity of CC_6 in the presence of valinomycin was a linear function of external K over at least a ten-fold range of K concentration. This linearity, of course, would be expected if the potential was equivalent to the K equilibrium potential. The second assumption is well supported by findings in human erythrocytes (10). In order to make this estimation, the fluorescent intensity given by an unknown potential must be matched by that of a known potential, that is, one induced by a particular external K concentration, in the presence of valinomycin. The Nernst equation can then be used to calculate the unknown potential from this external K concentration and the K concentration in the cells. The estimations for KTY cells indicated that the potential was between -60 and -70 mV before glucose addition and between -130 and -140 mV after. Previously Harold and Papineau (6) had estimated that the potential in glycolyzing Na-loaded cells reached a level of -155 mV based on the distribution of dibenzyldimethylammonium (DDA^+) or -195 mV when the potential was based on the distribution of K in the presence of valinomycin. However, they were unable to measure the development of a hyperpolarization during glycolysis in normal K-loaded cells. Since these cells were capable of the active transport of neutral amino acids (3), the

failure to measure a potential was certainly at variance with an hypothesis involving the membrane potential as a major factor in the active transport of these amino acids. Harold and co-workers (3) were forced to assume that a potential was developed in these cells but limitations of their method prevented measurement of the potential. Our measurements are in agreement with their assumption and hence support the hypothesis developed by Mitchell (4) and Harold (5). Furthermore, the prediction that DCCD, an inhibitor of the membrane ATPase, would inhibit the hyperpolarization induced by glucose metabolism was confirmed.

It would have been of interest to test another prediction of the hypothesis, namely that proton conductors should also inhibit the generation of the hyperpolarization (5). However, our attempts thus far have not been successful because the conductors tried, e.g., CCCP, interacted with the dye directly, making the interpretation of the results difficult. We are currently pursuing this problem.

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