MEMBRANE POTENTIAL GENERATION IN MUTANTS OF *ESCHERICHIA COLI* WITH ALTERATIONS IN THE MEMBRANE ATPase COMPLEX. STUDIES ON INTACT CELLS

L. GRINIUS and J. BRAŽENAITĖ

Department of Biochemistry and Biophysics and Laboratory of Growth Stimulators, Čiurlionio 21, Vilnius University, Vilnius 232031, Lithuanian S.S.R., USSR

Received 18 November 1975

1. Introduction

The use of the mutants of *Escherichia coli* with a defect in the membrane ATPase* has been extended to the study of the mechanism of energy conservation in bacterial membranes (for review see [1]). One class of mutants has been shown to lack ATPase activity [2–11], while a second class of mutants retains the ATPase activity [4,7,11–13], but in a form which is resistant to the ATPase inhibitor DCCD.

This report presents a comparative study of membrane potential generation in mutants of *E. coli* with alterated ATPase. The changes in the fluorescence intensity of ANS^- were used to detect the membrane potential. A characteristic ANS^- fluorescence change can be induced by the difference of electrostatic potentials imposed across membranes of mitochondria [20,21], *E. coli* [22], an electroplax cell of *Electrophorus electricus* [23] and phospholipid liposomes [24]. Changes in the fluorescence intensity were also observed during the application of voltage pulses across ANS^--stained phospholipid bilayers [25].

The experimental material presented in this report indicates that alterations in the membrane ATPase lead to the inactivation of ATP-dependent membrane potential generator.

Abbreviations: ATPase, Mg^{2+} + Ca^{2+} dependent ATPase (EC 3.6.1.3); DCCD, *N,N'*-dicyclohexylcarbodiimide; ANS^-, 1-anilino-8-naphthalenesulfonate; CCCP, carbonylcyanide *p*-trichloromethoxyphenylhidrazone.

2. Materials and methods

The bacterial strains used were *E. coli* K_{12} AN 180 (F^-, *argE3, thi*$^-$, *mtl*$^-$, *xyl*$^-$, *str-804*) AN 382 (F^-, *argE3, thi*$^-$, *mtl*$^-$, *xyl*$^-$, *str-704, uncB401*) and AN 120 (F^-, *argE3, thi*$^-$, *mtl*$^-$, *xyl*$^-$, *str-804, uncA401*), generous gifts of Professor F. Gibson, Australian National University. Stock cultures were maintained on solid medium, containing casein hydrolysate, yeast extract, agar and glucose (0.4%). The liquid minimal salt medium used for strains AN 382 and AN 120 was that of Davis and Mingioli [26] and contained (in 1 litre): 2 g of casein hydrolysate, 1 g of yeast extract and 5 g of glucose. Strain AN 180 was grown in medium where instead of glucose 2.5 g of succinic acid was added. Strains AN 382 and AN 120 were grown at 37°C for 7 h under aerated conditions and harvested by centrifugation at 4°C for 10 min at 10 000 *g*. Strain AN 180 was grown under analogous conditions for 8 h, then diluted 1:8 with fresh medium and aeration continued at 37°C for 2.5 h. The harvested cells of all strains were washed twice at 4°C with 0.1 M potassium phosphate buffer (pH 7.0), suspended in the same buffer and kept in ice. Measurements of ANS^- fluorescence intensity were carried out as described earlier [22] in the medium, containing 0.05 M potassium phosphate buffer (pH 6.0), $5 \cdot 10^{-5}$ M ammonium salt of ANS^-, catalase (0.05 mg/ml), $6 \cdot 10^{-3}$ M glucose or $5 \cdot 10^{-3}$ M succinate and *E. coli* cells. DCCD was obtained from Ferak, Berlin, ammonium salt of ANS^- from Sigma, USA. CCCP was kindly provided by Professor V.P. Skulachev, Moscow. Catalase (37400 units of activity) was purchased from Olaine, USSR.

North-Holland Publishing Company – Amsterdam

3. Results

The use of ANS^- for detection of the membrane potential in *E. coli* cells is based on the quenching of ANS^- fluorescence in response to the generation of an imbalance of electrical charge such that the interior of cell is negative [22].

Fig. 1 depicts the time-course of the changes in the fluorescence intensity of ANS^- induced by the

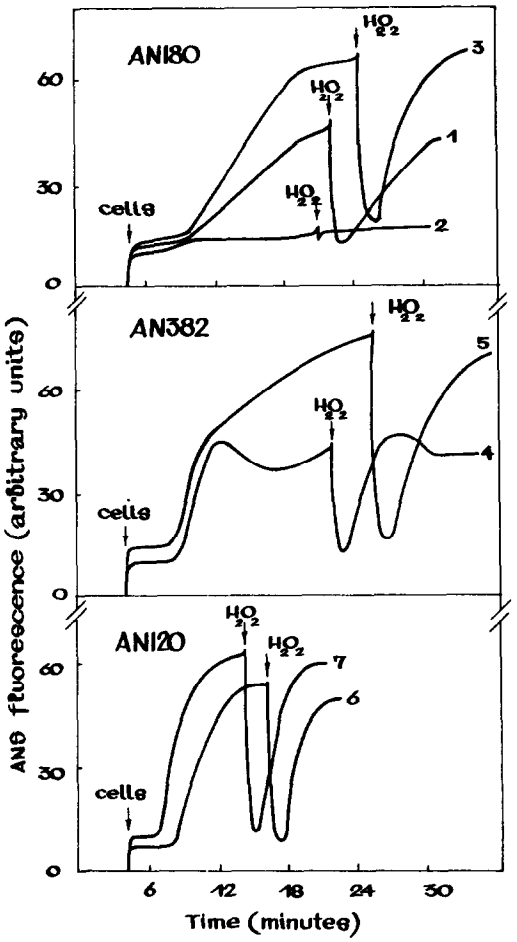

Fig.1. Changes in the fluorescence intensity of ANS-stained cells with normal and alterated membrane ATPase. The experiment was conducted as described in Materials and methods. The energy source used was succinate (curves 1 and 3), succinate plus glucose (curve 2) and glucose (curves 4–7. Curves 3, 5 and 7 were obtained in medium supplemented with 6×10^{-4} M DCCD. Additions: cells of AN 180, AN 382 and AN 120 strains (all up to 2.1 mg dry wt/ml) and 750 nmol of H_2O_2.

interaction between dye and cells. The strains used were: a wild-type strain AN 180, an *uncB* mutant (AN 328) retaining ATPase in DCCD-resistant form and an *uncA* mutant (AN 120) lacking ATPase activity (see [1]). It can be seen (curve 1, fig.1) that the interaction between wild-type cells and ANS^- in the succinate-containing medium is followed by a biphasic increase in the probe fluorescence. At first a rapid phase of increase is observed. The incubation of cells under conditions of limited exposure to the air results in the exhaustion of oxygen in the medium and leads to the enhancement of ANS^--stained cell fluorescence (curve 1, fig.1). The injection of a small amount of hydrogen peroxide, which is rapidly converted by catalase to oxygen and water, induces transitional quenching of ANS^- fluorescence and an exponential return of the intensity to the initial level (curve 1, fig.1). If wild-type cells were added to the glucose-containing medium (curve 2 fig.1) the large-amplitude increase in the fluorescence intensity of ANS^- under anaerobic conditions as well as the fluorescence response to oxygen injection were no longer observable. The incubation of wild-type cells in the medium supplemented with ATPase inhibitor DCCD (curve 3, fig.1) causes an additional increase in the fluorescence intensity of ANS^- under anaerobic conditions.

A biphasic increase in probe fluorescence is observed when cells of the mutant strains are added to the glucose-containing medium. It can be seen (curves 4 and 6, fig.1) that the exhaustion of oxygen in glycolyzing suspensions of mutant cells leads to an increase in the fluorescence intensity of ANS^-. The injection of oxygen causes a characteristic transitional quenching of dye fluorescence.

As was shown above (curve 2, fig.1) no large-amplitude changes in ANS^- fluorescence were observed in the glycolyzing wild-type cell suspension. Thus, the increase in ANS^- fluorescence intensity under conditions of anaerobic glycolysis is a specific feature of mutant cells. The time-course of the changes in the fluorescence of ANS^- depends on the type of mutant cells used. In the suspension of *uncB* mutant cells the oscillatory character of the time-course of these changes is observed (curve 4, fig.1). This effect is absent in the suspension of *uncA* mutant cells (curve 6, fig.1). The incubation in DCCD-containing medium induces a remarkable increase in the fluorescence intensity of ANS^--stained *uneB* mutant cells

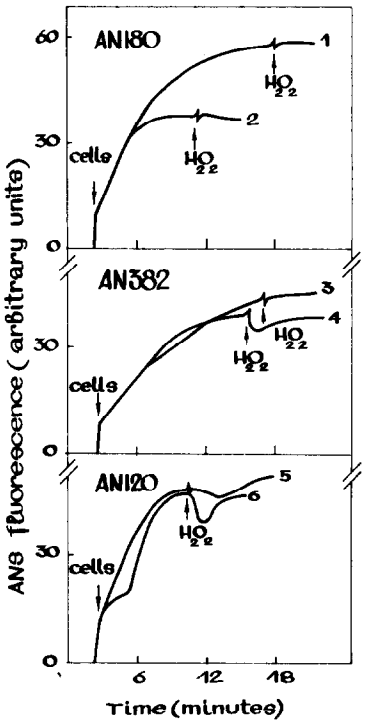

Fig.2. Changes in the fluorescence intensity of ANS-stained de-energized cells. The experiment was conducted as described in Materials and methods. The energy source used was succinate (curves 1 and 2) or glucose (curves 3–6). Curves 1, 3 and 5 were obtained in medium supplemented with 6×10^{-4} M DCCD and 1.6×10^{-4} M KCN; curves 2, 4 and 6 in medium supplemented with 4×10^{-6} M CCCP. Additions as in fig.1.

(curve 5, fig.1), while in the cell suspension of *uncA* mutant the effect of DCCD is considerably less pronounced (curve 7, fig.1).

The next set of experiments was carried out in medium supplemented either with respiratory inhibitor cyanide and ATPase inhibitor DCCD (curves 1,3 and 5, fig.2) or with uncoupler CCCP (curves 2,4 and 6, fig.2). It can be seen that the de-energization of cells abolishes the biphasic increase in probe fluorescence. The addition of cells is immediately followed by the increase in the fluorescence intensity of ANS^- in spite of the presence of oxygen in the medium. The inhibition of DCCD-treated cell respiration by cyanide or the increase of membrane permeability for H^+ ions caused by CCCP inhibits the respiration-coupled changes in the fluorescence intensity of ANS^- in the suspensions of wild-type cells (curves 1 and 2, fig.2) as well as in the suspensions of mutant cells (curves 3–6, fig. 2).

4. Discussion

In the anaerobic suspension of glycolyzing cells ATP hydrolysis is the sole energy source for membrane potential generation. The changes in the fluorescence intensity of ANS^- are a reliable index of this charge imbalance generated by the membrane ATPase. The experimental material presented above indicates that alterations in the membrane ATPase lead to impairment of ATP hydrolysis-coupled generation of the membrane potential. The *uncB* mutant differs from the *uncA* mutant in the mode of impairment. ATPase of the former mutant, but not of the *uncA* mutant, generates a membrane potential, while the value of this charge imbalance is considerably lower than in the parental strain (compare curves 2 and 4, fig.1).

Under aerobic conditions cells of the parental strain as well as cells of both types of mutants exhibit (see fig.1) almost identical low levels of ANS^- fluorescence, indicating effective membrane potential generation by the respiratory chain.

Consideration of these facts and recently established potency of the charge imbalance to drive the transport of certain metabolites and synthetic ions in *E. coli* cells and membrane vesicles [27–29], leads to the view that well known inhibition of the metabolite transport in mutants under anaerobic conditions [15–19] is due to the impairment of membrane potential generation by altered ATPase. Observation of the transport of metabolites in the cells of mutant and parental strains under aerobic conditions [15,19] is consistent with the conclusion that the respiratory chain acts as effective charge imbalance generator in these cells.

Turning now to the membrane potential generation by ATPase of *uncB* mutant discussed above it must be pointed out that no transport of metabolites has been observed [15,19] in anaerobic cell suspensions of this type of mutant. Therefore, further study of the quantitative correlation between the membrane potential value in the cell and the transport of the metabolite is necessary.

Acknowledgement

The authors are greatly indebted to Professor V.P. Skulachev for stimulating advice and to Professor F. Gibson for a generous gift of mutant strains and interest in our work.

References

[1] Cox, G. B. and Gibson, F. (1974) Biochim. Biophys. Acta 346, 1–25.
[2] Butlin, J. D., Cox, G. B. and Gibson, F. (1971) Biochem. J. 124, 75–81.
[3] Cox, G. B., Newton, N. A., Butlin, J. D. and Gibson, F. (1971) Biochem. J. 125, 489–493.
[4] Cox, G. B., Gibson, F. and McCann, L. (1973) Biochem. J. 134, 1015–1021.
[5] Cox, G. B., Gibson, F. and McCann, L. (1974) Biochem. J. 138, 211–215.
[6] Kanner, B. I. and Gutnick, D. L. (1972) J. Bacteriol. 111, 287–289.
[7] Kanner, B. I. and Gutnick, D. L. (1972) FEBS Lett. 22, 197–199.
[8] Simoni, R. A. and Shallenberger, M. K. (1972) Proc. Natl. Acad. Sci. US 69, 2263–2267.
[9] Bragg, P. D. and Hou, C. (1973) Biochem. Biophys. Res. Commun. 50, 729–736.
[10] Schairer, H. U. and Haddock, B. A. (1972) Biochem. Biophys. Res. Commun. 48, 544–551.
[11] Daniel, J., Roisin, M. P., Burstein, C. and Kepes, A. (1975) Biochim. Biophys. Acta 376, 195–209.
[12] Butlin, J. D., Cox, G. B. and Gibson, E. (1973) Biochim. Biophys. Acta 366–375.
[13] Gutnick, D. L., Kanner, B. I. and Postma, P. W. (1972) Biochim. Biophys. Acta 283, 217–222.
[14] Nieuwenhuis, F. J. R. M., Kanner, B. I., Gutnick, D. L., Postma, P. W. and Van Dam, K. (1973) Biochim. Biophys. Acta 325, 62–71.
[15] Or, A., Kanner, B. I. and Gutnick, D. L. (1973) FEBS Lett. 35, 217–219.
[16] Berger, E. A. (1973) Proc. Natl. Acad. Sci. US 70, 1514–1518.
[17] Pames, J. R. and Boos, W. (1973) J. Biol. Chem. 248, 4429–4435.
[18] Rosen, B. P. (1973) Biochim. Biophys. Res. Commun. 53, 1289–1296.
[19] Rosenberg, H., Cox, G. B., Butlin, J. D. and Gutowski, S. J. (1975) Biochem. J. 146, 417–423.
[20] Jasaitis, A. A., Kulienė, V. V. and Skulachev, V. P. (1971) Biochim. Biophys. Acta 234, 177–181.
[21] Azzi, A., Gherardini, P. and Santato, M. (1971) J. Biol. Chem. 246, 2035–2042.
[22] Griniuvienė, B., Dzheia, P. and Grinius, L. (1975) Biochem. Biophys. Res. Commun. 64, 790–796.
[23] Patrick, J., Valeur, B., Monneric, L. and Changeux, J. P. (1971) J. Membrane Biol. 5, 102–120.
[24] Bakker, E. P. and Van Dam, K. (1974) Biochim. Biophys. Acta 339, 157–163.
[25] Conti, F. and Malerba, F. (1972) Biophysik 8, 326–332.
[26] Davis, B. D. and Mingioli, G. S. (1950) J. Bacteriol, 60, 17–28.
[27] Altendorf, K., Hirata, H. and Harold, F. M. (1975) J. Biol. Chem. 250, 1405–1412.
[28] Hirata, H., Altendorf, K. and Harold, F. M. (1974) J. Biol. Chem. 249, 2939–2945.
[29] Griniuvienė, B., Chmieliauskaitė, V., Melvydas, V., Dzheia, P. and Grinius, L. (1975) J. Bioenergetics 7, 17–38.