PHYSIOLOGICAL CHANGE IN THE IONOPHORE-PORTION OF PROTON-TRANSLOCATING ATPase IN AN UNCOUPLED MUTANT OF *ESCHERICHIA COLI*

Hiroshi KANAZAWA and Masamitsu FUTAI
Department of Microbiology, Faculty of Pharmaceutical Sciences, Okayama University, Okayama 700, Japan

Received 17 July 1979

1. Introduction

The proton-translocating ATPase complex (F_1–F_0) has essentially the same structure and function in chloroplasts, mitochondria and bacterial membranes (reviewed [1–3]). This complex is composed of two portions: a catalytic portion, F_1, and a proton pathway, F_0. Use of *Escherichia coli* as a source of F_1–F_0 has the advantages that this organism is well characterized genetically, and that it is possible to study the physiological regulation of the complex in the bacteria.

Differences have been found in the ionophoric properties of F_0 from cells of the F_1 mutant (NR 70, DL54) of *E. coli* [4,5] grown aerobically and anaerobically. The sensitivity of strain RF7 to DCCD (dicyclohexylcarbodiimide) was shown [6] to vary with growth conditions: when this strain is grown on a respiratory substrate its ATPase is resistant to DCCD, because of a mutational alteration in the DCCD binding protein in F_0, whereas when the cells are grown on glucose or glycerol the ATPase is significantly less resistant. Their results suggest that a functional change of the ATPase complex may occur when the growth conditions are varied.

The purpose of the present communication is to provide further evidence that the F_0 portion of the complex changes when the growth conditions are varied in aerobic culture. As shown below the proton permeability of membranes of the F_1 mutant DL54 [7] varies with the physiological conditions under which the cells are grown.

2. Materials and methods

Escherichia coli strain DL54 (F_1 mutant) and its parent ML308-225 were grown to the late logarithmic phase in a synthetic medium [8] containing 0.5% glucose or 0.5% glycerol as the sole carbon source or in a rich medium (phosphate-buffered) containing 5.0 g beef extract and 15 g peptone (Daigo Eiyo Co., Tokyo) per liter [9]. In some experiments final concentrations of 0.15% peptone and 0.05% beef or yeast extract were also added to the synthetic medium. Inverted membrane vesicles were prepared as in [9] from cells disrupted in a French pressure cell (400 kg/cm^2). Formation of a proton gradient was estimated by the quenching of quinacrine [10]. Other procedures and materials were as in [9].

3. Results

3.1. *Membranes from cells grown in rich medium*

Quinacrine can be used as a probe for measuring the ability of membranes to form a proton gradient with a respiratory substrate or ATP [10], since the magnitude of the quenching of quinacrine is qualitatively proportional to the proton gradient. As shown in fig.1A, membranes from DL54 (grown in rich medium) showed essentially the same magnitude of quenching as those from wild-type cells (ML308-225) (fig.1D). In both cases the quenching did not increase on addition of DCCD. These results suggest that wild

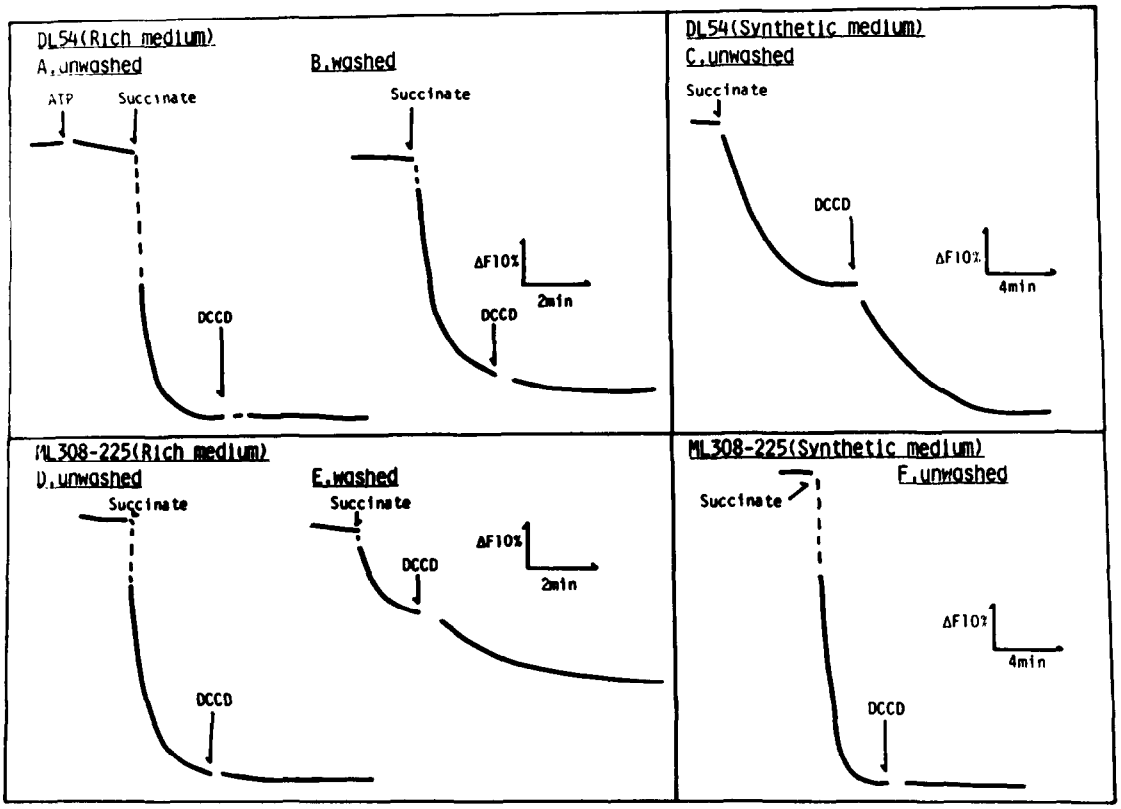

Fig.1. Formation of proton gradient in membrane vesicles from mutant (DL54) and wild-type (ML308-225) cells. Membrane vesicles from strain DL54 and ML308-225 (A,B,D,E, 150 μg protein; C,F, 230 μg protein) in 2.0 ml 20 mM Tricine buffer containing 100 mM KCl and 2.0 mM $MgCl_2$ were mixed with 4 μl 50 μM quinacrine and fluorescence (emission, 500 nm; excitation, 420 nm) was monitored in a Hitachi MFP-4 fluorophotometer. At the indicated point, 2 μl 0.2 M ATP, 10 μl 1.0 M potassium succinate, or 2 μl 10 mM DCCD was added. Cells grown in rich medium (A,B,D,E) and synthetic medium with glucose (C,F) were used for preparation of membranes. In experiments B,E, membranes were washed with 1.0 mM Tris–HCl (pH 7.0) containing 0.5 mM EDTA. Other procedures were as described in [10].

type and mutant membranes do not show proton leakage and that they are equally capable of forming a respiratory proton gradient.

It must be noted that membranes of DL54 did not show proton leakage even after being washed with dilute buffer containing EDTA (fig.1B). As shown [10], membranes of the wild-type became leaky to protons after the same treatment (fig.1E), because the treatment removes F_1 and protons can then pass freely through the F_0 portion. The leakage could be blocked by addition of DCCD, which binds to the proteolipid [10] of the F_0 portion. On washing the membranes with EDTA only the α subunit of F_1 was solubilized from membranes of DL54 (data not shown), whereas the entire F_1 molecule was released from those of the wild type [11].

3.2. *Membranes from cells grown in synthetic medium*

Membranes of DL54 behaved differently when the strain was grown in a synthetic medium supplemented with glucose. As shown in fig.1C, membrane vesicles showed quenching of low magnitude which was enhanced by the addition of DCCD and finally reached the level of wild type membranes (fig.1F). These results indicate that mutant membranes leak protons and that this leakage could be prevented by the binding of DCCD to F_0, confirming the results in [12]. This membrane leakage is probably not due to the release

of F_1, because subunits of F_1 were found immunochemically in membranes of DL54 from cells grown in either synthetic or rich medium, and because on immunodiffusion assay antibodies against isolated α,β,γ formed qualitatively similar precipitin lines against both types of membranes suspended in 0.2% sodium dodecyl sulfate (not shown).

Membranes of DL54 from cells grown in a synthetic medium with glycerol, glycerol plus peptone, glycerol plus beef extract, glucose plus peptone were also leak protons, and the leakage was prevented by DCCD. Furthermore, cells grown in a synthetic medium with glycerol plus beef extract and peptone had membrane leakage. It must be pointed out that rich medium discussed above [3,1] contains beef extract and peptone together with NaCl and potassium phosphate, although cells grown in this medium had membranes which did not leak protons.

4. Discussion

These results suggest that the proton pathway (F_0) of the ATPase complex changes depending on the physiological conditions in which the cells are grown. Some factor or factors in the synthetic medium may be responsible for making mutant membranes permeable to protons, because membranes from cells grown in the medium always leaked protons regardless of other supplements (beef extract and/or peptone). Inorganic ions with concentrations higher in the synthetic than rich medium may be these factors. The actual physiological change in F_0 may be explained in two ways.

(i) The proton pathway itself in F_0 may change with the growth conditions.

(ii) The interaction of F_0 and F_1 may change so that F_1 molecules cannot seal the proton pathway in cells grown on glucose or glycerol in synthetic medium.

These changes may not be due to alteration of F_0 polypeptides: they could be due to alteration of phospholipids.

Acknowledgements

This study was supported in part by grants from the Ministry of Education, Science and Culture of Japan and the Naito Foundation (Naito Research grant for 1978).

References

[1] Racker, E. (1976) A New Look at Mechanisms in Bioenergetics, Academic Press, New York.
[2] Harold, F. M. (1977) Curr. Top. Bioenerg. 6, 83–119.
[3] Kagawa, Y. (1978) Biochim. Biophys. Acta 505, 45–93.
[4] Hasan, S. M. and Rosen, B. P. (1977) Biochim. Biophys. Acta 459, 225–240.
[5] Boonstra, J., Gutnick, D. L. and Kaback, H. R. (1975) J. Bacteriol. 124, 1248–1255.
[6] Fillingame, R. H. and Wopat, A. F. (1978) J. Bacteriol. 134, 687–689.
[7] Simoni, R. D. and Shallenberger, M. K. (1972) Proc. Natl. Acad. Sci. USA 69, 2663–2667.
[8] Tanaka, S., Lerner, S. A. and Lin, E. C. C. (1969) J. Bacteriol. 93, 643–648.
[9] Kanazawa, H., Miki, T., Tamura, F., Yura, T. and Futai, M. (1979) Proc. Natl. Acad. Sci. USA 76, 1126–1130.
[10] Tsuchiya, T. and Rosen, B. P. (1975) J. Biol. Chem. 256, 8409–8415.
[11] Futai, M., Sternweis, P. C. and Heppel, L. A. (1974) Proc. Natl. Acad. Sci. USA 71, 2725–2729.
[12] Altendorf, K., Harold, F. M. and Simoni, R. D. (1974) J. Biol. Chem. 249, 4587–4593.