SOLUBILIZATION AND RECONSTITUTION OF MEMBRANE ENERGY-TRANSDUCING SYSTEMS OF *ESCHERICHIA COLI*

Tomofusa TSUCHIYA, Atsuko MISAWA, Yumi MIYAKE, Kyoko YAMASAKI and Sumiko NIIYA
Department of Microbiology, Faculty of Pharmaceutical Sciences, Okayama University, Tsushima-naka, Okayama 700, Japan

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1. Introduction

For detailed studies of membrane processes such as energy transduction, it is important to purify the protein(s) involved in the process. One of the main problems in the purification of membrane proteins is the lack of an adequate procedure for solubilizing the proteins from membrane without loss of activity. Many attempts have been made to solve this problem. Several procedures have been developed and some successful results with respect to bacterial membrane proteins have been reported [1–4]. These methods, however, have not been found applicable for the purification of other membrane proteins in active form. Newman and Wilson [5] have developed a method by which they have succeeded in solubilizing and reconstituting the lactose carrier of *Escherichia coli*. They utilized the octylglucoside method in [6]. This octylglucoside technique has also been applied successfully to the solubilization and reconstitution of the melibiose transport system of *E. coli* [7].

Here, we describe solubilization and reconstitution of the respiratory chain, the H^+-translocating ATPase, the Na^+/H^+-antiporter and the glutamate transport carrier of *E. coli*.

2. Materials and methods

2.1. *Organism and membrane vesicles*

E. coli strain W3133-2S (a derivative of K12), which possesses elevated activity of Na^+/H^+-antiporter [17] was grown as in [8]. This strain was used for all experiments except the one on glutamate transport system. Strain 29-78 (a derivative of B) was grown as in [9], and used for the study on glutamate transport system. Membrane vesicles were prepared as in [7].

2.2. *Solubilization and reconstitution*

Membrane proteins were solubilized with octylglucoside and reconstituted into liposomes as in [5,7]. Phospholipids were prepared from *E. coli* strain W3133-2S as in [10].

2.3. *Assays*

Respiration was monitored with a Clark-type oxygen electrode. Fluorescence of acridine orange was measured using an excitation wavelength of 467 nm and emission of 527 nm. ATPase activity was measured as in [11]. Glutamate transport driven by membrane potential and Na^+ gradient was assayed as in [7]. Protein was determined by the method in [12].

3. Results and discussion

3.1. *The respiratory chain*

The respiratory chain extrudes H^+, and establishes an electrochemical potential difference of H^+ across membranes [13]. Membrane vesicles of *E. coli* utilize ascorbate plus *N,N,N',N'*-tetramethyl-*p*-phenylenediamine (TMPD) as respiratory substrates [14], and consume oxygen. This oxidase activity was sensitive to NaCN (table 1).

The membrane vesicles were treated with 1.25% octylglucoside and the solubilized membrane proteins reconstituted into liposomes by the octylglucoside dilution method [5–7]. The reconstituted proteoliposomes were collected by ultracentrifugation. The reconstituted proteoliposomes had 11-times higher specific activity of ascorbate–TMPD oxidase than membrane vesicles (table 1). This activity was strongly inhibited by NaCN. Since TMPD donate its reducing equivalent directly to cytochrome *b*-562 [14], the reconstituted proteoliposomes must contain at least

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Table 1
Solubilization and reconstitution of the respiratory chain

Activity of	O_2 consumption[a] (μg atom O . min^{-1} . mg $protein^{-1}$)	
	Control	+5 mM NaCN
Exp. 1[b]		
membrane vesicles	0.18	0.01
reconstituted proteoliposomes	2.0	0.01
Exp. 2[c]		
membrane vesicles	1.5	0.07
reconstituted proteoliposomes	2.7	0.20

[a] Oxygen consumption was assayed in 100 mM potassium phosphate buffer (pH 7.5) containing 2 mM $MgSO_4$ and either membrane vesicles (84 μg protein) or reconstituted proteoliposomes (8 μg protein), at 28°C
[b] Ascorbate (5 mM) and TMPD (5 mM) were added as substrates
[c] NADH (1 mM) was added as a substrate

cytochrome *b*-562 and cytochrome *o*. Cyanide sensitive NADH oxidase activity was also detected in the reconstituted proteoliposomes (table 1). The specific activity was 2-times higher than membrane vesicles. Lactate oxidase activity and succinate oxidase activity were not detected (not shown). These results indicate that the respiratory chain was unevenly reconstituted. Respiratory control (stimulation of respiration by uncouplers) was not observed with the reconstituted proteoliposomes (not shown).

3.2. *The H^+-ATPase (F_1–F_0 complex)*

The H^+-translocating ATPase (F_1–F_0 complex) catalyzes reversible reaction between ATP hydrolysis (or formation) and H^+ translocation across the membrane [13]. The ATPase is composed of two portions, F_1 (extrinsic membrane component) and F_0 (intrinsic membrane component). F_1 can be solubilized without detergent, but adequate detergent is required to solubilize F_1–F_0 complex [4].

Octylglucoside at 1.25% efficiently solubilized the ATPase activity (>90%) (table 2). Increase of the specific activity was 3.4-fold at this step. At the reconstitution step, however, only 25% of the ATPase activity was recovered in the proteoliposomes, and the specific activity was reduced to some extent. The ATPase activity of the solubilized fraction and of the

Table 2
Solubilization and reconstitution of F_1–F_0 ATPase[a]

Step	Protein (mg)	ATPase activity (units/mg protein)		
		Units[b]	Control	+DCCD[c]
Membranes	2.0	3.4	1.7	0.3
Octylglucoside extract	0.55	3.2	5.8	2.5
Reconstituted proteoliposomes	0.23	0.8	3.5	1.4

[a] In these experiments, Tris–HCl buffer was used instead of phosphate buffer
[b] One unit equals 1 μmol phosphate released/min
[c] DCCD (30 μM) was preincubated for 20 min

reconstituted proteoliposomes was partially sensitive to dicyclohexylcarbodiimide (DCCD) which is an inhibitor of F_0 (table 2). This may indicate that some portions of F_1 were dissociated from F_0. To confirm the reconstitution of F_1–F_0 into liposomes, we tested whether or not H^+ translocation takes place as a result of ATP hydrolysis. Following the addition of ATP, quenching of acridine orange took place, both with membrane vesicles and with the reconstituted proteoliposomes. This indicates proton pumping and the formation of a pH gradient (fig.1). Addition of DCCD inhibited the quenching. The fluorescence quenching was also sensitive to a proton conductor (not shown). Thus F_1–F_0 complex was satisfactorily reconstituted into proteoliposomes.

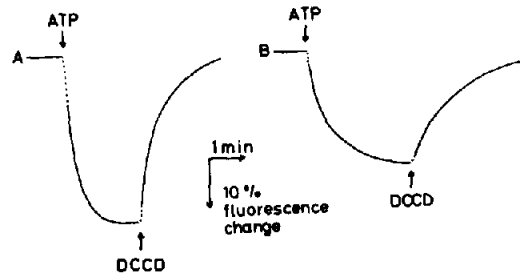

Fig.1. ATP-dependent quenching of acridine orange fluorescence by the reconstituted proteoliposomes. Assay mixture (1.5 ml) contained 10 mM Tris–HCl (pH 8.0), 100 mM KCl, 2 mM $MgSO_4$, 1 μM acridine orange and either membrane vesicles (43 μg protein) (A) or the reconstituted proteoliposomes (3.3 μg protein) (B). The final concentrations of ATP and DCCD were 0.25 mM and 30 μM, respectively.

3.3. *The Na^+/H^+ antiporter*

The protonmotive force, which is established by the respiratory chain in *E. coli*, must be converted to an electrochemical potential difference for Na^+ to energize Na^+-coupled transport systems such as that for glutamic acid [9]. The Na^+/H^+ antiporter performs this energy conversion. We tested the Na^+/H^+ antiporter activity of the reconstituted proteoliposomes by measuring fluorescence quenching [15]. Addition of ATP to the proteoliposomes quenched acridine orange fluorescence. Li^+, which possesses higher affinity for the Na^+/H^+ antiporter than Na^+ in strain W3133-2S [17] (submitted) was added to the assay mixture. The inward movement of Li^+ on the antiporter results in a loss of H^+ from the membrane vesicles with a consequent reduction in the fluorescence quenching (rise in the curve). Reversal of the fluorescence intensity on the addition of Li^+ was less pronounced with the reconstituted proteoliposomes than with membrane vesicles (fig.2). Addition of Na^+ elicited only a very small reversal of the quenching (not shown). This suggests that only a fraction of the antiporter was reconstituted or that this antiporter is unstable when solubilized.

3.4. *The glutamate transport system*

Glutamate transport in *E. coli* strain 29-78 is driven by an electrochemical potential difference of Na^+ [9].

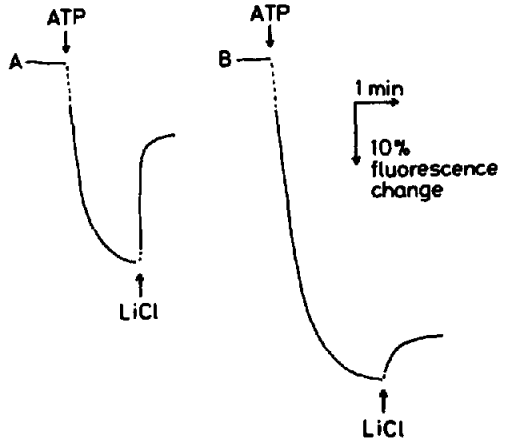

Fig.2. The $Na^+(Li^+)/H^+$ antiporter activity of the reconstituted proteoliposomes. Fluorescence quenching was measured as under fig.1. Membrane vesicles (43 μg protein) (A) or the reconstituted proteoliposomes (12 μg protein) (B) were added. The final concentrations of ATP and LiCl were 0.25 mM and 2.5 mM, respectively.

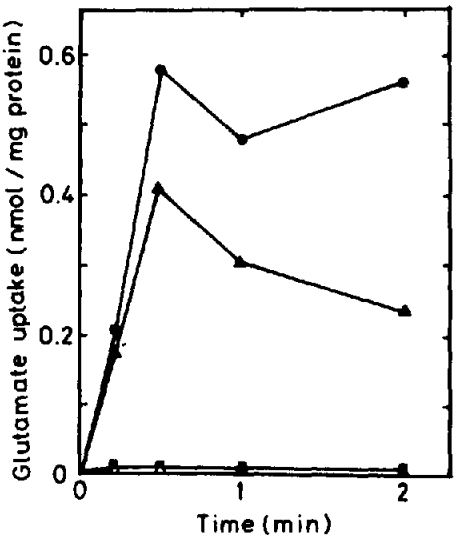

Fig.3. Glutamate transport driven by a membrane potential and Na^+ gradient in the reconstituted proteoliposomes. Potassium phosphate-loaded proteoliposomes (5,6) were diluted into 50 mM sodium phosphate buffer (pH 7.5) containing 1 μM [^{3}H]glutamate in the absence of ionophore (▲), in the presence of 2 μM valinomycin (●) or in the presence of 2 μM dianemycin (■). In one experiment, the proteoliposomes were diluted into 50 mM potassium phosphate buffer (pH 7.5) containing 1 μM [^{3}H]glutamate (○).

We were able to solubilize and reconstitute the glutamate-transport carrier. Glutamate accumulation in the reconstituted proteoliposomes was energized by a combination of membrane potential and Na^+ gradient or Na^+ gradient alone (fig.3). No glutamate accumulation was observed in the absence of a driving force or in the presence of dianemycin, a Na^+ ionophore [16]. These results indicate that the Na^+–glutamate cotransport carrier was reconstituted.

4. Conclusion

Octylglucoside was used to solubilize and reconstitute membrane energy transducing systems of *E. coli*. The respiratory chain, the H^+-translocating ATPase, the Na^+/H^+-antiporter and the Na^+–glutamate cotransport carrier were all solubilized and reconstituted. The extent of the reconstitution varied from system to system. Although it may be necessary to alter the conditions of reconstitution for each membrane protein, we believe that the octylglucoside procedure may be widely applicable for other membrane proteins.

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