FEBS Letters 374 (1995) 72–76 FEBS 16151

Reconstitution of the metal-tetracycline/H^+ antiporter of *Escherichia coli* in proteoliposomes including F_0F_1-ATPase

Yuichi Someya[a,**], Yoshinori Moriyama[b,***], Masamitsu Futai[b], Tetsuo Sawai[a], Akihito Yamaguchi[a,*]

[a]*Division of Microbial Chemistry, Faculty of Pharmaceutical Sciences, Chiba University, Chiba 263, Japan*
[b]*Department of Organic Chemistry and Biochemistry, Institute of Scientific and Industrial Research, Osaka University, Ibaraki, Osaka 567, Japan*

Received 22 August 1995

Abstract **The tetracycline resistance gene (*tet*A) was cloned downstream of the *lac* promoter. When expression of the *tet*A gene in *E. coli* cells carrying the *lac* I^q gene was induced with isopropyl β-D-thiogalactopyranoside, the tetracycline resistance protein (TetA) was overproduced, amounting to about 30% of the integral cytoplasmic membrane protein. Essentially pure TetA protein could be obtained by solubilization with 1.25% *n*-octyl-β-D-glucopyranoside and one-step purification by DEAE Sepharose CL-6B column chromatography. The TetA protein was incorporated into proteoliposomes with F_0F_1-ATPase. The proteoliposomes exhibited [^{3}H]tetracycline transport dependent on ATP hydrolysis. The specific activity was about 2 nmol/mg protein/min. The proteoliposomes also showed H^+ efflux coupled with tetracycline influx. Tetracycline/H^+ antiport by proteoliposomes reconstituted with the Ser-65 → Cys mutant TetA protein was inhibited by *N*-ethylmaleimide. These results proved for the first time that the tetracycline/H^+ antiport is only mediated by the TetA protein.**

Key words: Tetracycline; Antiporter; Tetracycline/H^+ antiporter; H^+-ATPase; Reconstitution; Proteoliposome

1. Introduction

The metal-tetracycline/H^+ antiporter (TetA) [1,2] encoded by transposon Tn10 is the cytoplasmic membrane protein responsible for high level tetracycline resistance in Gram-negative bacteria [3,4]. It is composed of 401 amino acid residues [5,6]. Based on the hydropathy profile of its amino acid sequence, and the results of protease digestion [7] and antibody binding [8], a secondary structure model has been proposed in which the TetA protein consists of 12 transmembrane segments, connected by 11 hydrophilic loops, with the N- and C-terminals on the cytoplasmic surface. Recent studies on a series of pBR322-encoded TetA-alkaline phosphatase fusions also supported such a structure [9]. Through our site-directed mutagenesis studies [10–17], several amino acid residues essential or important for substrate translocation and/or H^+ translocation have been identified. However, because of a lack of information on its three-dimensional structure, the spatial and functional relationships of these residues remain unknown. Therefore, the purification of and a reconstitution system for the TetA protein are obligatory.

Despite its deleterious effect [18], some attempts to overproduce the TetA protein have been made [18–20]. Hickman et al. [19] constructed a clone in which the *tetA* gene was placed downstream of the λPL promoter, but the overproduction was unsuccessful. Alternatively, they overproduced a TetA-collagen-β-galactosidase fusion protein. After collagenase digestion of the purified fusion protein, the TetA protein was purified, but transport activity was not observed [19]. Eckert and Beck [18] also cloned the *tetA* gene behind the *tac* promoter. However, overproduction of the TetA protein was not detected, probably due to its lethal effect [18,20]. We also attempted to construct a TetA overproducing system in which the *tetA* gene is located downstream of the *lac* promoter in a multicopy plasmid vector in a previous study [21]. The use of *E. coli* cells carrying the constitutive *lac* repressor gene enabled us to overproduce the TetA protein [21]. This system is very useful for the purification and reconstitution of the functional TetA protein.

In order to assay the active tetracycline transport into reconstituted proteoliposomes, it is necessary to produce an interior-acidic proton gradient as a driving force. An artificial ΔpH imposed through ionophore-mediated K^+/H^+ exchange is too weak and transient to drive tetracycline/H^+ antiport even in inverted membrane vesicles [22]. Recently, Moriyama et al. [23] used F_0F_1-ATPase for the reconstitution of active transport of neurotransmitter transporters. ATP hydrolysis by F_0F_1-ATPase in proteoliposomes provides a large and durable interior-acidic proton gradient, which is adequate as a driving force for tetracycline/H^+ antiport in reconstituted proteoliposomes.

This is the first report of the functional reconstitution of a metal-tetracycline/H^+ antiporter using F_0F_1-ATPase as an energy generator. Reconstituted proteoliposomes exhibited ATP-dependent tetracycline/H^+ antiport.

2. Materials and methods

2.1. Materials

[7-^{3}H(N)]Tetracycline was purchased from Du Pont-New England Nuclear. DEAE-Sepharose CL-6B was from Pharmacia. Soybean L-α-phosphatidylcholine (type II-S) was from Sigma. All other chemicals were of reagent grade and obtained from commercial sources.

2.2. E. coli *strains and plasmids*

E. coli RB791 (W3110 *lac*I^q*L8*) [24] was used as the host strain for plasmids pSYTET and pSYS65C, which carry the wild-type and Cys65 mutant *tetA* genes [10], respectively, downstream of the *lac* promoter

*Corresponding author.

**Research Fellow of the Japan Society for the Promotion of Science.

***Present address: Mukaishima Marine Biological Laboratory, Hiroshima University, Hiroshima 722, Japan.

Abbreviations: TetA, tetracycline resistance protein; MOPS, 3-(*N*-morpholino)propanesulfonic acid; MES, 2-(*N*-morpholino)ethanesulfonic acid; tricine, *N*-tris(hydroxymethyl)methylglycine; octylglucoside, *n*-octyl-β-D-glucopyranoside; NEM, *N*-ethylmaleimide; TC, tetracycline.

SSDI 0014-5793(95)01079-3

[21]. *E. coli* DK8 (*ΔuncB-C*, *ilv*::Tn10) [25] harboring plasmid pBWU13 carrying the entire *unc* operon [23] was also used.

2.3. Preparation of E. coli *phospholipids and F_0F_1-ATPase*

E. coli RB791 cells were grown at 37°C on minimal medium supplemented with 0.2% glucose and 0.1% casamino acids. Phospholipids were extracted according to Bligh and Dyer [26], suspended in 2 mM β-mercaptoethanol at 50 mg of lipid/ml, and stored at −20°C. F_0F_1-ATPase was prepared by one-step purification from *E. coli* DK8/pBWU13 cells [23].

2.4. Cell fractionation and EDTA-treatment of inner membranes

E. coli RB791 cells harboring plasmid pSYTET or pSYS65C were grown at 37°C on minimal medium supplemented with 0.2% glucose and 0.1% casamino acids. Expression of the *tetA* gene was induced with 0.1 mM isopropyl β-D-thiogalactopyranoside at the late log phase. After 2 h, the cells were harvested, and lysed by passage through a French pressure cell at 10,000 psi in 50 mM MOPS-KOH (pH 7.0) containing 10 mM $MgCl_2$, 0.5 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, 5% glycerol and 10 μg/ml deoxyribonuclease I at 4°C. All manipulations were performed at 4°C. French press vesicles were suspended in 50 mM MOPS-KOH (pH 7.0) containing 0.5 mM EDTA, 1 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride and 5% glycerol. The inner membrane fraction of French press vesicles was separated from the outer membrane fraction by sucrose density gradient centrifugation [23,27], and then washed with 1 mM MES-Tricine (pH 7.0) containing 0.5 mM EDTA, 5 mM monothioglycerol and 10% glycerol, suspended in 10 mM MES-Tricine (pH 7.0) containing 5 mM monothioglycerol and 10% glycerol at 10 mg of protein/ml, and stored at −80°C.

2.5. Solubilization and one-step purification of the TetA protein

Phospholipids (10 mg/ml, *E. coli* phospholipids or soybean L-α-phosphatidylcholine) were suspended in 10 mM MES-Tricine (pH 7.0) containing 1 mM dithiothreitol using a bath sonicator and stored at −80°C. The EDTA-washed inner membranes were solubilized with 1.25% octylglucoside in the presence of 5 mg/ml *E. coli* phospholipids [28] and then centrifuged at 210,000 × *g* for 30 min to remove insoluble materials.

Purification by DEAE-Sepharose CL-6B column chromatography was performed as follows: the octylglucoside extract (500 μl) was applied to a DEAE-Sepharose CL-6B anion exchange column (bed volume, 5 ml) equilibrated with 50 mM triethanolamine buffer (pH 8.0) containing 0.1% dodecylmaltoside, and eluted with 25 ml of a linear gradient of 0–0.5 M NaCl in 50 mM triethanolamine buffer (pH 8.0) containing 0.1% dodecylmaltoside at the flow rate of 1 ml/min. The absorbance at 280 nm of each fraction (800 μl) was monitored and the proteins were analyzed by SDS-polyacrylamide gel electrophoresis. Essentially pure TetA protein was obtained in fractions 16–18 corresponding to NaCl concentrations of 0.25–0.3 M.

2.6. Immunoblot analysis of the TetA protein

SDS-polyacrylamide gel electrophoresis of samples containing the TetA protein was followed by electroblotting of the protein. The TetA protein was detected by means of an enzyme-linked immunosorbent assay using an anti-carboxyl-terminal peptide antibody [11] and an Express blot assay kit (Bio-Rad), as described in the previous paper [12].

2.7. Reconstitution of the functional TetA protein in proteoliposomes

Proteoliposomes were reconstituted by two different methods: (1) freeze-thawing dilution, and (2) gel filtration chromatography. Reconstitution by freeze-thawing dilution was performed as follows: 100 μl (200 μg protein) of an octylglucoside extract or purified TetA protein solution was mixed with 100 μl (200 μg protein) of purified F_0F_1-ATPase and 60 μl (60 μg lipid) of briefly bath-sonicated phosphatidylcholine liposomes, followed by freezing and thawing for two times. Then the mixture was diluted 50-fold with 13 ml of 10 mM Tricine-choline (pH 8.0) containing 0.1 M KCl and 5 mM $MgCl_2$. The reconstituted proteoliposomes were collected by ultracentrifugation at 165,000 × *g* for 60 min using a HITACHI Himac CP 90α, and then resuspended in 150 μl of the same buffer and used for the tetracycline uptake assay.

Reconstitution by gel filtration chromatography was performed as follows: an octylglucoside-extract or purified TetA solution (500 μl, 1.2 mg of protein) was mixed with F_0F_1-ATPase (800 μl, 0.8 mg of protein) and sonicated liposomes (600 μl, 6 mg of phosphatidylcholine), followed by freeze-thawing twice, and then the mixture was applied to a Sephadex G-25 column (1 cm × 10 cm) equilibrated with 10 mM Tricine-choline (pH 8.0) containing 5 mM monothioglycerol. The turbid eluate was collected. After centrifugation at 200,000 × *g* for 30 min using a Beckman Optima TL Ultracentrifuge, the reconstituted proteoliposomes were suspended in 800 μl of the same buffer and immediately used for the tetracycline uptake assay.

2.8. [^{3}H]Tetracycline uptake by proteoliposomes

[^{3}H]Tetracycline uptake by reconstituted proteoliposomes was performed using a Sephadex G-50 centrifuge column. 30 μl of proteoliposomes was mixed with 30 μl of 10 mM Tricine-choline (pH 8.0) containing 0.1 M KCl, 10 mM $MgCl_2$, 1 μg/ml valinomycin and 10 mM ATP. After 1 min incubation at 30°C, 90 μl of the same buffer containing 10 μM [^{3}H]tetracycline instead of ATP was added. At the indicated times, the assay mixture was applied to a Sephadex G-50 centrifuge column and centrifuged at 400 × *g* for 2 min. Radioactivity and protein in the effluent were determined. Assays were also carried out in the absence of ATP or in the presence of 2.5 mM NADH instead of ATP.

2.9. Proton translocation in proteoliposomes

Proton translocation in proteoliposomes was assayed by measuring the fluorescence quenching of acridine orange (excitation, 492 nm; emission, 540 nm). Proteoliposomes were reconstituted by the freeze-thawing dilution procedure [23]. An octylglucoside-extract or purified TetA solution (100 μl, 200 μg of protein) was mixed with F_0F_1 (100 μl, 100 μg of protein) and bath-sonicated liposomes (60 μl, 600 μg of phosphatidylcholine). After freeze-thawing twice, 50–100 μl of the mixture was diluted with 2 ml of 10 mM Tricine-choline (pH 8.0) containing 0.1 M KCl, 10 mM $MgCl_2$, 0.625 μg/ml valinomycin and 1.25 μM acridine orange. Ten μl of 0.1 M ATP, 10 μl of 2 mM tetracycline, and 5 μl of 1 M NH_4Cl were successively added at the indicated times.

3. Results and discussion

3.1. Overproduction of the TetA protein

The Tn10-*tetA* gene encoding the metal-tetracycline/H^+ an-

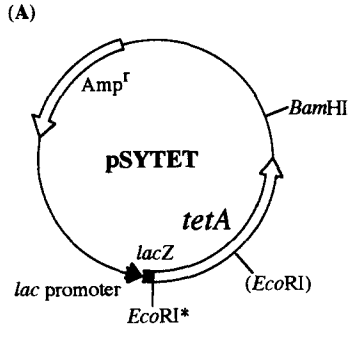

Fig. 1. Structure of the plasmid carrying the *tetA* gene downstream of the *lac* promoter. (A) Plasmid pSYTET was constructed as described in the previous paper [21]. *Eco*RI* and (*Eco*RI) indicate the newly introduced and removed restriction sites, respectively. (B) The nucleotide sequence around the *lacZ-tetA* fusion site and the deduced amino acid sequence of the fusion protein. The underlined amino acid residues are derived from β-galactosidase. The numbers of the amino acids are the same as in the native TetA protein.

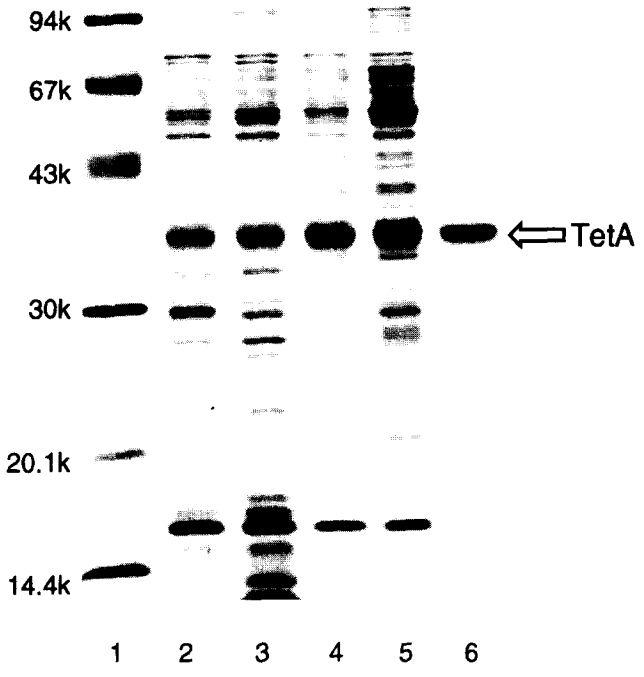

Fig. 2. SDS-polyacrylamide gel electrophoresis of various fractions from *E. coli* RB791 cells. Samples prepared from *E. coli* RB791/pSYTET cells were subjected to SDS polyacrylamide (12%) gel electrophoresis and stained with Coomassie brilliant Blue. Lane 1, molecular weight markers; lane 2, French press vesicles; lane 3, inner membranes; lane 4, EDTA-washed inner membranes; lane 5, 1.25% octylglucoside-extract; lane 6, TetA protein purified by DEAE-Sepharose CL-6B column chromatography.

tiporter (TetA) was placed downstream of the *lac* promoter in pUC118, and the resulting plasmid designated pSYTET (Fig. 1A, also see [21]). Since the *tetA* gene in this plasmid is fused inframe to the *lacZ* gene, the expression of the gene is controlled by the *lac* promoter/operator, and 4 amino acid residues, Thr-Met-Ile-Thr, derived from LacZ are inserted into the N-terminal of the TetA protein (Fig. 1B). The insertion of these residues did not affect the tetracycline/H^+ antiport activity [21]. An *E. coli* strain carrying the inducible (wild-type) *lacI* gene could not be transformed with this plasmid, suggesting that overproduction of the TetA protein is lethal for the host cells. The *E. coli* strain required the $lacI^q$ mutation for transformation with pSYTET.

Following 2 h induction of *E. coli* RB791 cells harboring pSYTET with 0.1 mM isopropyl β-D-thiogalactopyranoside, the cells were harvested and fractionated. A dense 36 kDa band was observed for the membrane fraction on SDS-polyacrylamide gel electrophoresis (Fig. 2, lane 2), which was identified as the TetA protein using anti-TetA-carboxyl-terminal-peptide antiserum (data not shown). The TetA protein accounted for nearly 20% of the total membrane protein, as judged using a densitometer. *E coli* RB791/pSYTET cells produced at least 10-fold more TetA protein than RB791/pLGT2 cells did (data not shown). In addition, cells carrying pSYTET expressed a 16 kDa protein, which is probably a degradation product of the TetA protein (Fig. 2, lane 2).

3.2. *Solubilization and reconstitution of the TetA protein*

The membrane fraction was separated into inner and outer membrane fractions on a sucrose density gradient. Then the inner membrane was washed with a low ionic strength buffer containing 0.5 mM EDTA to remove peripheral membrane proteins. The TetA protein occupied 21 and 38% of the protein in the inner membrane and EDTA-washed inner membrane fractions, respectively (Fig. 2, lanes 2 and 3). We used the EDTA-washed inner membrane fraction as a starting material for solubilization and reconstitution of the TetA protein.

Solubilization of the EDTA-washed inner membrane fraction with 1.25% octylglucoside was carried out in the presence of 5 mg/ml *E. coli* phospholipids, as in the case of lactose permease [28]. About 40% of the total inner membrane protein was recovered in the supernatant on centrifugation. The TetA protein comprised about 50% of the octylglucoside-extract proteins (Fig. 2, lane 4). The octylglucoside-extract was mixed with sonicated liposomes, followed by freezing and thawing. Then the mixture was applied to a Sephadex G-25 gel filtration column to remove detergents. About 30% of the octylglucoside-extract proteins was recovered in the proteoliposomes. Through this process, the TetA protein was further concentrated up to about 60% of the proteoliposomal protein (Fig. 3, lane 2). Most of the 16 kDa protein and two high molecular weight contaminants (about 60 and 70 kDa) were excluded from the proteoliposomes. The proteoliposomes were also re-

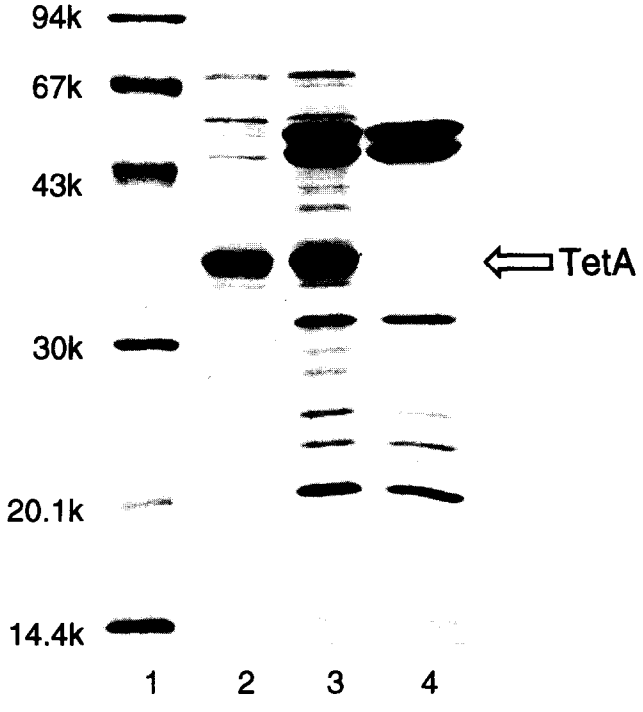

Fig. 3. SDS-polyacrylamide gel electrophoresis of reconstituted proteoliposomes. Proteoliposomes were reconstituted with only the octylglucoside-extract (lane 2), the octylglucoside-extract and F_0F_1-ATPase (lane 3), or F_0F_1-ATPase alone (lane 4) by gel filtration chromatography. Lane 1, molecular weight markers. SDS-polyacrylamide gel electrophoresis was performed in the same way as in Fig. 2.

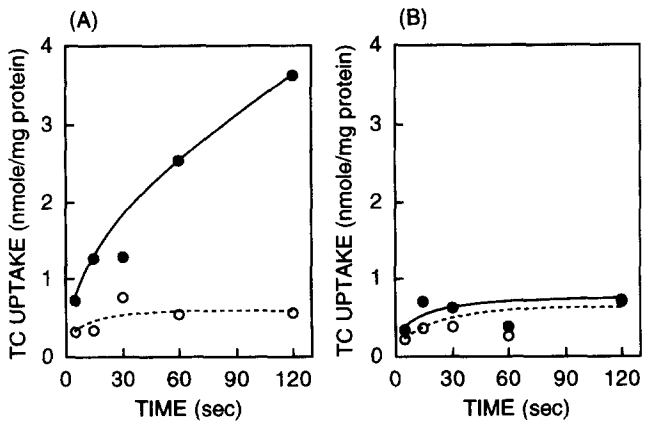

Fig. 4. [^{3}H]Tetracycline (TC) uptake by reconstituted proteoliposomes. (A) Proteoliposomes reconstituted with the TetA protein and F_0F_1-ATPase. (B) Proteoliposomes reconstituted with F_0F_1-ATPase alone. Reconstitution was performed by the gel filtration chromatography method. Assays were performed as described in section 2. Closed circles with a solid line and open circles with a dashed line indicate the uptake in the presence and absence of ATP, respectively.

constituted in the presence of purified F_0F_1-ATPase in the same way (Fig. 3, lane 3).

3.3. [^{3}H]Tetracycline uptake by reconstituted proteoliposomes

[^{3}H]Tetracycline uptake was assayed using a Sephadex G-50 centrifuge column. Reconstituted proteoliposomes containing the TetA protein and F_0F_1-ATPase exhibited substantial [^{3}H]tetracycline uptake in an ATP-dependent manner (Fig. 4A). The initial rate of uptake was 2 nmol/mg protein/min, which is comparable to the specific activity in inverted membrane vesicles. The reason why the specific activity of the reconstituted proteoliposomes was not higher than that in the inverted vesicles may be because a difference in the efficiency of energy supply and/or partial inactivation of TetA during the solubilization/reconstitution process. NADH-dependent [^{3}H]-tetracycline uptake was not observed in the proteoliposomes (data not shown), indicating that there was no contamination by insoluble inverted membrane vesicles. Proteoliposomes containing only F_0F_1-ATPase (Fig. 4B) or TetA (data not shown) showed no [^{3}H]tetracycline uptake, regardless of the presence or absence of ATP. These observations clearly indicated that the solubilized TetA and F_0F_1-ATPase were integrated in the same vesicles and acted in the chemiosmotic coupling.

Reconstitution was also performed by the freeze-thawing dilution procedure [23], and the [^{3}H]tetracycline uptake was measured by a rapid filtration method similar to the assay for inverted vesicles [2]. ATP-dependent [^{3}H]tetracycline uptake was certainly observed (data not shown), but the amount of tetracycline actively taken up was significantly less than in the case of the column method.

3.4. Tetracycline-coupled H^+ translocation in reconstituted proteoliposomes

Proton translocation coupled with tetracycline transport was measured by monitoring the fluorescence change of acridine orange. Proteoliposomes reconstituted with F_0F_1-ATPase alone showed ATP-dependent ΔpH formation, as judged from the fluorescence quenching, but no change in fluorescence intensity on the addition of tetracycline was observed (Fig. 5A). Proteoliposomes reconstituted with the TetA protein and F_0F_1-ATPase also showed the formation of ΔpH through ATP hydrolysis (Fig. 5B). The addition of tetracycline caused a significant increase in the fluorescence intensity (Fig. 5B), indicating efflux of H^+ out of the proteoliposomes coupled with the influx of tetracycline into them.

3.5. Effect of sulfhydryl reagent on tetracycline/H^+ antiport by reconstituted proteoliposomes

The wild-type TetA protein has a cysteine residue at position 377. However, *N*-ethylmaleimide (NEM) did not inhibit tetracycline transport in inverted membrane vesicles containing the wild-type TetA protein [10,14], because NEM did not bind to Cys377 [14]. In contrast, the activity in vesicles containing the Cys65 mutant TetA protein was completely abolished by NEM [10,14]. Thus proteoliposomes containing the Cys65 mutant TetA protein were prepared in the same way. Tetracycline-coupled H^+ translocation by the wild-type proteoliposomes was not affected by NEM (Fig. 5C). In contrast, tetracycline-dependent H^+ efflux in the proteoliposomes containing the Cys65 mutant TetA protein was completely inhibited (Fig. 5E). These results confirm that the tetracycline/H^+ antiport observed in this reconstitution system is certainly mediated by the TetA protein.

3.6. One-step purification of the TetA protein and reconstitution of tetracycline-coupled proton translocation from the purified TetA protein

Essentially pure TetA protein was obtained on DEAE-Sepharose Cl-6B anion exchange column chromatography of the solubilized TetA protein (Fig. 2, lane 6). Proteoliposomes reconstituted from the purified TetA protein also showed tetracycline-coupled proton translocation (data not shown). These experiments confirmed that no cytoplasmic, periplasmic or peripheral membrane proteins are required for the tetracycline/H^+ antiport function.

Acknowledgements: This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education of Japan.

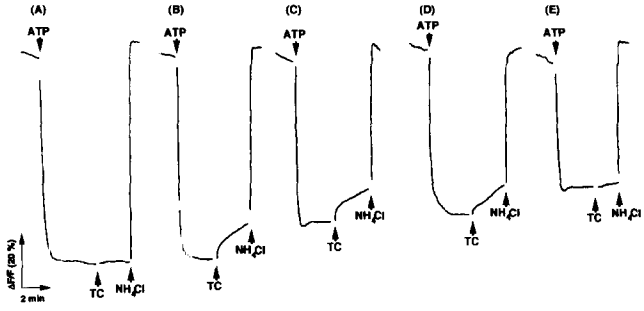

Fig. 5. Proton translocation in reconstituted proteoliposomes. Proton translocation across proteoliposomes was measured by monitoring the fluorescence quenching of acridine orange. Proteoliposomes were reconstituted with (A) F_0F_1-ATPase alone. (B and C) the wild-type TetA protein and F_0F_1-ATPase. (D and E) the Cys65 mutant TetA protein and F_0F_1-ATPase. Reconstitution was performed by the freeze-thawing dilution method. ATP (0.5 mM), tetracycline (TC, 1 mM), and NH_4Cl (2.5 mM) were successively added at the indicated times. In (C) and (D), proteoliposomes were preincubated with 1 mM NEM for 1 min before ATP was added.

References

[1] McMurry, L., Petrucci, R.E., Jr. and Levy, S.B. (1980) Proc. Natl. Acad. Sci. USA 77, 3974–3977.
[2] Yamaguchi, A., Udagawa, T. and Sawai, T. (1990) J. Biol. Chem. 265, 4809–4813.
[3] Levy, S.B. (1992) Antimicrob. Agents Chemother. 36, 695–703.
[4] Chopra, I., Hawkey, P.M. and Hinton, M. (1992) J. Antimicrob. Chemother. 29, 245–277.
[5] Nguyen, T.T., Postle, K. and Bertrand, K.P. (1983) Gene (Amst.) 25, 83–92.
[6] Hillen, W. and Schollmeier, K. (1983) Nucleic Acid Res. 11, 525–539.
[7] Eckert, B. and Beck, C.F. (1989) J. Biol. Chem. 264, 11663–11670.
[8] Yamaguchi, A., Adachi, K. and Sawai, T. (1990) FEBS Lett. 265, 17–19.
[9] Allard, J.D. and Bertrand, K.P. (1992) J. Biol. Chem. 267, 17809–17819.
[10] Yamaguchi, A., Ono, N., Akasaka, T., Noumi, T. and Sawai, T. (1990) J. Biol. Chem. 265, 15525–15530.
[11] Yamaguchi, A., Adachi, K., Akasaka, T., Ono, N. and Sawai, T. (1991) J. Biol. Chem. 266, 6045–6051.
[12] Yamaguchi, A., Akasaka, T., Ono, N., Someya, Y., Nakatani, M. and Sawai, T. (1992) J. Biol. Chem. 267, 7490–7498.
[13] Yamaguchi, A., Ono, N., Akasaka, T. and Sawai, T. (1992) FEBS Lett. 307, 229–232.
[14] Yamaguchi, A., Someya, Y. and Sawai, T. (1992) J. Biol. Chem. 267, 19155–19162.
[15] Yamaguchi, A., Nakatani, M. and Sawai, T. (1992) Biochemistry 31, 8344–8348.
[16] Yamaguchi, A., Kimura, T., Someya, Y. and Sawai, T. (1993) J. Biol. Chem. 268, 6496–6504.
[17] Yamaguchi, A., Akasaka, T., Kimura, T., Sakai, T., Adachi, Y. and Sawai, T. (1993) Biochemistry 32, 5698–5704.
[18] Eckert, B. and Beck, C.F. (1989) J. Bacteriol. 171, 3557–3559.
[19] Hickman, R.K., McMurry, L.M. and Levy, S.B. (1990) Mol. Microbiol. 4, 1241–1251.
[20] Guay, G.G. and Rothstein, D.M. (1993) Antimicrob. Agents Chemother. 37, 191–198.
[21] Yamaguchi, A., Someya, Y. and Sawai, T. (1993) FEBS Lett. 324, 131–135.
[22] Kaneko, M., Yamaguchi, A. and Sawai, T. (1985) FEBS Lett. 193, 194–198.
[23] Moriyama, Y., Iwamoto, A., Hanada, H., Maeda, M. and Futai, M. (1991) J. Biol. Chem. 266, 22141–22146.
[24] Brent, R. and Ptashne, M. (1981) Proc. Natl. Acad. Sci. USA 78, 4204–4208.
[25] Klionsky, D.J., Brusilow, W.S.A. and Simoni, R.D. (1984) J. Bacteriol. 160, 1055–1060.
[26] Bligh, E.G. and Dyer, W.J. (1959) Can. J. Biochem. Physiol. 37, 911–917.
[27] Osborn, M.J., Gander, J.E., Paris, E. and Carson, J. (1972) J. Biol. Chem. 247, 3962–3972.
[28] Newman, M.J. and Wilson, T.H. (1980) J. Biol. Chem. 255, 10583–10586.