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Ion permeability induced in artificial membranes by the ATP/ADP antiporter

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Abstract

The hypothesis on the additional function of the ATP/ADP antiporter (ANT) as uncoupling protein has been tested in proteoliposomes and planar bilayer phospholipid membranes (BLM). It is found that dissipation of the light-induced ΔpH in the dark is very much faster in ANT-bacteriorhodopsin proteoliposomes than in proteoliposomes containing bacteriorhodopsin as the only protein. Mersalyl treatment of ANT-bacteriorhodopsin proteoliposomes causes further increase in the ΔpH dissipation rate due to formation of a high conductance pore. The properties of this pore are studied on ANT incorporated to BLM. They proved to be similar to those of so-called multiple conductance channel or permeability transition pore of inner mitochondrial membrane. The conductance of the single channel is as high as 2.2 nS. The channel fails to discriminate between K^+, Na^+, H^+ and Cl^-. Thus the obtained data are consistent with the assumption that native and modified ANT might function as an H^+-specific conductor and as a permeability transition pore, respectively.

Key words: ATP/ADP antiporter; Bacteriorhodopsin; Proteoliposome; H^+-conductance; Permeability transition pore

1. Introduction

In recent years, the possibility that ANT can mediate the uncoupling of oxidative phosphorylation was widely discussed. There are at least two uncoupling mechanisms in which ANT seems to be involved: (1) fatty-acid induced, carboxyatractylate-inhibited selective increase of the proton leak [1–6] and (2) formation of a so-called permeability transition pore in the inner mitochondrial membrane, induced by Ca^{2+} in the presence of inorganic phosphate, which is stimulated by fatty acids as well as by carboxyatractylate [7–9]. Molecular mechanisms of these processes are still obscure.

In our previous papers [2,3], the hypothesis about fatty acid anion transport via ANT resulting in a H^+ permeability increase [5,6] was studied. The hypothesis in question assumes that in the presence of fatty acids, ANT functions like an 'uncoupling protein' (thermogenin) from brown adipose tissue [10]. Direct involvement of ANT in permeability transition pore formation was also assumed in the literature [9,11,12]. In the ANT-containing proteoliposomes Krämer and co-workers demonstrated the ability of ANT-mediated nucleotide transport to switch from counter-exchange to unidirectional flux of low selectivity after the mersalyl or Hg^{2+} treatment [13,14].

In the present paper model systems (proteoliposomes and BLM with ANT inlaid) were used to study permeability properties of ANT.

2. Materials and methods

ANT purification and incorporation into liposomes was carried out according to Krämer and Klingenberg [15,16] with some modifications [17]. Bovine heart mitochondria underwent osmotic shock in 10 mM Tricine-NaOH, followed by sedimentation of membranes (45,000 × *g*, 25 min) to remove matrix proteins. The next steps of the purification procedure [16] were carried out in the medium containing 150 mM Na_2SO_4, 40 mM Tricine-NaOH, pH 8.0, and 0.5 mM EDTA. Proteoliposomes were prepared from liposomes/protein/Triton X-100 mixture by the procedure of fast detergent removal on hydrophobic resin (Amberlite XAD-2) beads [16]. Liposomes were formed from egg yolk lecithin. Proteoliposomes were loaded with 20 mM ATP and 100 μM sodium azide which was added to prevent the ATP hydrolysis. External nucleotides and buffer were removed by gel filtration on Sephadex G-75 with the eluation medium containing 240 mM Na_2SO_4 or 600 mM sucrose, pH 7.0.

BR was isolated from purple membranes of *Halobacterium halobium* [18] and monomerized by preincubation with 2% Triton X-100 during 24 h before the proteoliposome formation. Generation and dissipation of H^+ gradient were monitored by non-penetrating optical pH probe pyranine [19]. pH response of proteoliposome suspension on white light flash (20 mJ, 0.4 ms) was calculated as difference in optical density traces at 450 nm in the presence and in the absence of the pH probe.

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Abbreviations: ANT, ATP/ADP antiporter (adenine nucleotide translocase); BR, bacteriorhodopsin; pyranine, 8-hydroxy-1,3,6-pyranetrisulfonate; BLM, bilayer (phospho)lipid membrane.

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The incubation medium contained 240 mM Na_2SO_4 (or 600 mM sucrose) and 100 μM pyranine, pH 7.0.

ANT incorporation into planar BLM was carried out by fusion of the proteoliposome membrane with BLM under an osmolarity gradient (800 mM urea) in the presence of 3 mM Mg^{2+} [20]. BLM was formed on a 0.3 mm partition from a decane solution of 2% azolectin and 1% cholesterol. The proteoliposome suspension was added in such an amount that showed the single ion-channel activity after 10–15 min mersalyl treatment. The incubation medium contained 180 mM Na_2SO_4, 20 mM MES-NaOH, pH 7.0. The sign of the applied potential was such that the side of BLM opposite to the proteoliposomes was virtual ground. Currents were amplified by a patch-clamp amplifier (OPUS, Moscow) with a cut-off frequency of 500 Hz and were stored on a videotape after digitizing with ADAREC (OPUS, Moscow). Computer analysis was carried out using a single-channel analysis program, PAT, kindly supplied by Dr. J. Dempster (Department of Physiology and Pharmacology, University of Strathclyde, Glasgow, UK), and a similar program developed by Dr. P. Ivanov (Department of Physics, Moscow State University).

The nucleotide transport activity of ANT was assayed by the luciferin–luciferase method as carboxyatractylate-sensitive ADP-induced efflux of ATP from (ANT + BR)-proteoliposomes pre-loaded with ATP. A 2500 W Xenon lamp with water solutions $CuSO_4$ and K_2CrO_4 as filters was used to actuate BR.

3. Results

In the experiments, three kinds of proteoliposomes have been studied, namely, with ANT, with bacteriorhodopsin and with ANT and bacteriorhodopsin co-incorporated.

Illumination of BR-liposomes for 0.4 ms resulted in fast increase in pH of the incubation medium, followed by a pH relaxation (Fig. 1A). This effect is due to H^+ pumping into liposomes by BR [21]. Relaxation of this response in the dark represents the passive dissipation of the H^+ gradient. An increase in the membrane conductance by gramicidin D led to a strong increase in the rate of pH response relaxation (data not shown). A similar effect was induced by ANT incorporation into the BR proteoliposomes (Fig. 1A). Such an activity of ANT appeared to be insensitive both to the palmitic acid and to the ANT inhibitor carboxyatractylate. However, some effects of palmitic acid were revealed in the nucleotide transport experiments. Nucleotide exchange ATP_{in}/ADP_{out} in the (ANT + BR)-liposomes was shown to be inhibited by continuous light. This inhibition was completely reversed by either artificial protonophorous uncoupler trifluoromethoxycarboxylcyanide phenylhydrazone or palmitic acid (data not shown).

The mersalyl treatment of (ANT + BR)-proteoliposomes led to the strong reduction of bacteriorhodopsin-generated ΔpH without any effect on the ΔpH dissipation kinetics (Fig. 1B). The possibility of a non-specific mersalyl action was excluded in the experiments with BR-liposomes. Mersalyl at the concentrations up to 0.4 mM did not cause any decrease in the amplitude of pH response of BR-liposomes (Fig. 1C). Apparently mersalyl modifies ANT in such a way that it starts operating as a pore or channel of such a high conductance that the H^+-pumping activity of bacteriorhodopsin appears to be too small to create measurable pH gradient. The residual pH response in mersalyl-treated samples can be due to the portion of proteoliposomes which still contain intact ANT.

A high conductance of proteoliposomal membrane could be induced by alameticin, hydrophylic pore-forming antibiotic. Experiments on BR-liposomes revealed that the alameticin effect was quite similar to that of mersalyl on (ANT + BR)-proteoliposomes. Alameticin (20–80 μg/ml) suppressed the amplitude of the pH response without alterations of kinetics (data not shown).

The ion-conducting properties of the mersalyl-treated

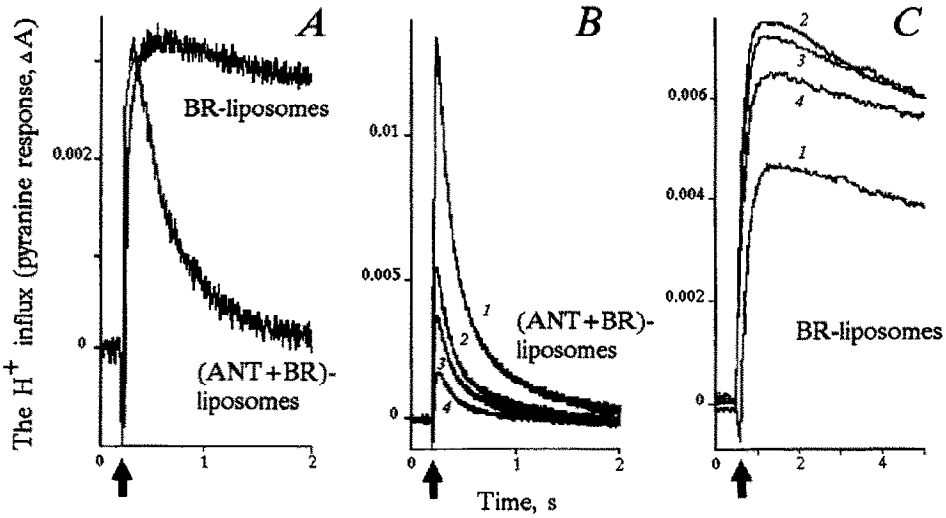

Fig. 1. Generation and dissipation of ΔpH in the BR and (BR + ANT)-proteoliposomes. Incubation medium contained 240 mM Na_2SO_4 (A) or 600 mM sucrose (B,C), 100 μM pyranine, pH 7.0. Mersalyl additions (B,C): curve 1, without mersalyl; curve 2, 100 μM; 3, 200 μM; 4, 400 μM. Arrow, 0.4 ms white light flash.

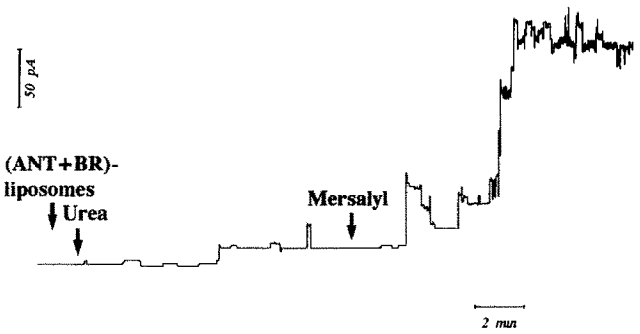

Fig. 2. The mersalyl-induced current transitions in the BLM fused with the ANT-liposomes. Voltage applied, +30 mV. Additions, 180 μl proteoliposome suspension, 800 mM urea (on the one side of BLM), 100 μM mersalyl (on the both sides of BLM).

ANT were studied in the planar BLM after its fusion with ANT-liposomes.

Fig. 2 demonstrates characteristic current trace recorded at constant voltage. It is seen that under the conditions used, neither proteoliposomes nor urea did cause significant alterations in the BLM conductance. Discrete jumps of the conductance were observed after mersalyl addition to BLM, which indicated the ion channel opening (Fig. 2). This mersalyl-induced conductance increase had complex pattern probably due to the opening of several channels.

Further experiments were carried out under the conditions of single channel insertion and much higher time resolution. Fig. 3 demonstrates single channel current traces at different voltages applied. Corresponding histograms (Fig. 4) present probability of different current states. Channels were shown to have multiple conductance sub-levels with current transitions between almost all pairs of the sub-levels occurred. Current-transition values varied within the range of 50–700 pS. A number of conductance sub-levels rose with the increase of applied potential (Fig. 4), channel conductance being decreased. Conductance levels altered from the range of 1.2–2.2 nS at 25 mV to 260–800 pS at 80 mV. Furthermore it should be noted that channels tended to transit to lower (relatively closed) sub-levels with time after voltage application, the probability of transitions being progressively decreased (data not shown).

The activation of higher conductance levels after the application and removal of rather high voltage (≃ 80 mV) was another characteristic feature of the channels under study. The channels were observed to transit from 200–500 pS sub-levels to 500 pS–1.5 nS sub-levels (at 50 mV) as a result of such activation procedure. The channels were shown to be non-selective. They failed to discriminate between K^+, Na^+, H^+ and Cl^- ions (data not shown).

4. Discussion

The above data indicate that in the model membranes ANT can dissipate the proton potential at least by two different mechanisms.

One of them, inherent to the native ANT was revealed

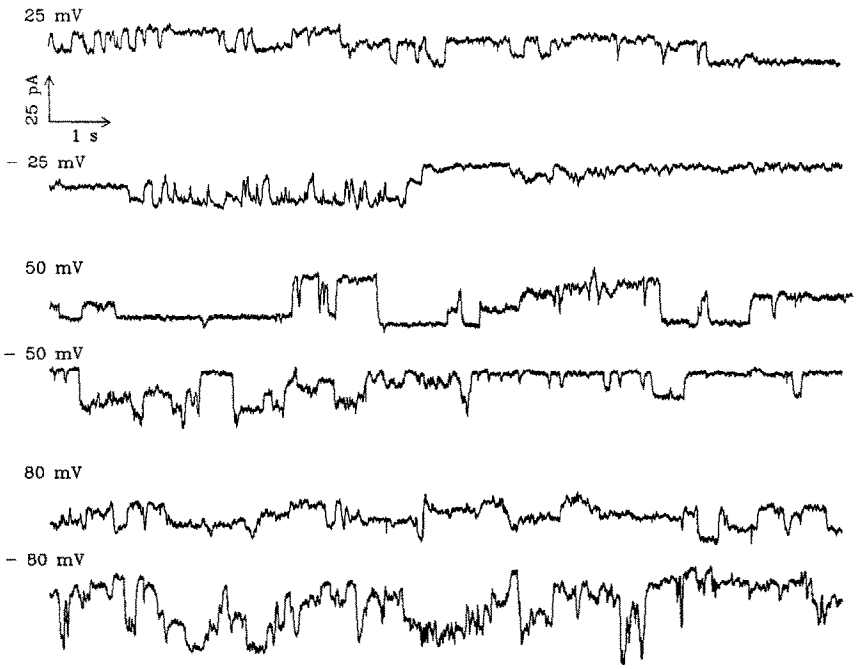

Fig. 3. Single channel current traces for the mersalyl-treated BLM fused with the ANT-liposomes. Mersalyl addition (100 μM) were made to the side of BLM opposite to proteoliposomes.

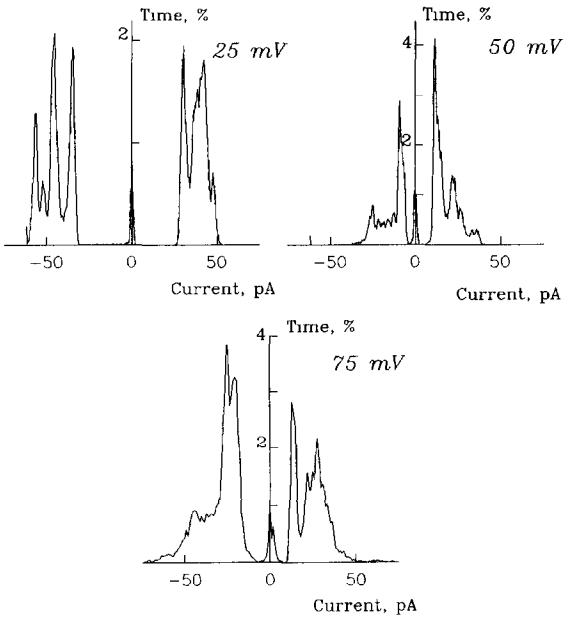

Fig. 4. Current amplitude distribution. Current bin width, 1 pA; duration of the experiment, 60 s. Other conditions, see Fig. 3.

in intact (ANT + BR)-proteoliposomes. Here stimulation by ANT of the dark dissipation of the light-induced ΔpH was described. In principle, this effect may be related to the ANT-mediated Ca^{2+}-independent uncoupling in mitochondria.

The other mechanism requires the ANT proteoliposomes to be treated with mersalyl. It seems to model formation of the Ca^{2+}-dependent mitochondrial permeability transition pore of high conductance and low selectivity. This effect could also be demonstrated on the ANT-inlaid BLM when mersalyl was added on the side of the planar membrane opposite to proteoliposomes. The phenomenon in question is clearly related to induction of non-specific permeability by SH-reagents discovered by Krämer and others in the ANT proteoliposomes [13]. Moreover, characteristics of the non-specific pore revealed in our experiments are similar to those of so-called multi-conductance channel observed in patch-clamp experiments with mitoplasts [22–24]. Among them there are existence of multiple conductance sub-levels, activation of channel activity by high voltage, slow transition to more closed sub-levels after the high voltage applied. According to Zoratti and Szabo [25], this channel could be attributed to the permeability transition pore in the intact mitochondria. On the other hand, there are many indications that treatment of mitochondria by the SH-modifying reagents results in ion fluxes which can be due to the formation of a permeability transition pore (for review see [26]). Our results suggest that ANT can function as a permeability transition pore-forming protein in mitochondria.

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