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1-BUTANOL EXTRACTED PROTEOLIPID. PROTON CONDUCTING PROPERTIES

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Summary: The 1-butanol extracted proteolipid from mitochondria was incorporated to liposomes. This proteolipid mediates the H^+ transfer across the lipid bilayer in response to a negative charge produced by valinomycin and KCl. The process is sensitive to DCCD, but not to oligomycin. The flux of H^+ depends on the concentration of proteolipid and the inhibition of this flux depends on the concentration of DCCD.

INTRODUCTION

The function of the ATPase complex of mitochondria, chloroplast and bacteria is to link the H^+ gradient derived from oxido-reduction reactions to ATP synthesis (1, 2). In mitochondria the complex is formed by three main components: a water soluble protein (F_1) which possesses the catalytical site for ATP formation and hydrolysis; at least two peptides that attach F_1 to the membrane (OSCP and F_6) and which confer oligomycin sensitivity to F_1; and a membrane factor (F_0) whose function is to conduct H^+ across the membrane to F_1 (3, 4).

Recently some of the components of the membrane have been isolated (5-8), and the results of the experiments conducted with these fractions are consistent with the idea that they are H^+ conducting peptides. Nevertheless, it is not clear whether the proteolipids isolated by different extraction procedures are identical. In this work, The H^+ conducting properties of the 1-butanol extracted proteolipid have been examined. It has been observed that the proteolipid mediates the transfer of H^+ across the lipid bilayer of liposomes. The process is sensitive to DCCD but not to oligomycin, which contrasts with the H^+ transfer reaction carried out by the chloroform:methanol extracted proteolipid (9) which is sensitive both to oligomycin and DCCD. Moreover, the

Abbreviations: DCCD, N,N'Dicyclohexylcarbodiimide.

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results of this work also show that the 1-butanol extracted proteolipid induces the electrophoretic transfer of H^+ across the two sides of the bilayer on response to a negative electrical potential.

MATERIALS AND METHODS

Isolations of bovine heart mitochondria was carried out according to Low and Vallin (10); submitochondrial particles were prepared as described by Racker (11).

Either intact submitochondrial particles or particles which had been subject to extensive sonication (12) were employed. It was observed that in order to obtain maximal yields of the H^+ conducting peptide, either of the two preparations had to be stored for at least three days at -10°C. The proteolipid was extracted according to Sigrist et al (13). After solubilization of the proteolipid with butanol and precipitation with ethyl ether, the proteolipid was collected by centrifugation and suspended in a small volume of ether-butanol (5:1 v/v). For the preparation of liposomes, asolectin (Sigma Chemicals Co.) partially purified according to the procedure of Kagawa and Racker (14) was employed. The proteolipid (30-100 μg of protein) was mixed with 1 ml of 10 mM Tricine-NaOH pH 8.0, 0.25 M sucrose, 0.4 M KCl and 0.65 mM dithioerythritol that contained 22 mg of phospholipid. The mixture was sonicated for 30 min. in a water type sonicator under an atmosphere of nitrogen. Thereafter the liposomes were dialyzed overnight in the same buffer, except that KCl was omitted.

H^+ movements were monitored by following the self-quenching of fluorescence of 9-aminoacridine as described by Shuldiner et al (15) and Deamer et al (16). The wavelengths for excitation and emission were 365 and 451 nm respectively.

Protein was determined in the presence of 0.1% sodium dodecylsulphate (SDS) according to Lowry et al (17). SDS gel electrophoresis was carried out according to Weber and Osborn (18).

RESULTS

The valinomycin induced release of K^+ from K^+-loaded liposomes leaves a negative charge inside the vesicles. In the absence of H^+ conducting molecules no change in the fluorescence level of 9-aminoacridine takes place. On the other hand, if a pathway for H^+ exists, H^+ enter into the vesicles to compensate the valinomycin induced release of K^+. In this latter condition, quenching of 9-aminoacridine fluorescence occurs upon the addition of valinomycin. Nelson et al (8) reported that DCCD inhibits H^+ transfer, depending on the time of exposure of the liposomes to DCCD. These results were confirmed (Fig. 1, B and C); however, oligomycin which inhibits the H^+ conducting properties of the mitochondrial ATPase complex and the H^+ transfer mediated by the chloroform:methanol extracted proteolipid (9) failed to inhibit H^+ transfer mediated by the butanol extracted proteolipid (fig. 1-D).

Figure 2, A and B, show that the proteolipid mediated H^+ transfer across the lipid bilayer of liposomes depends on the concentration

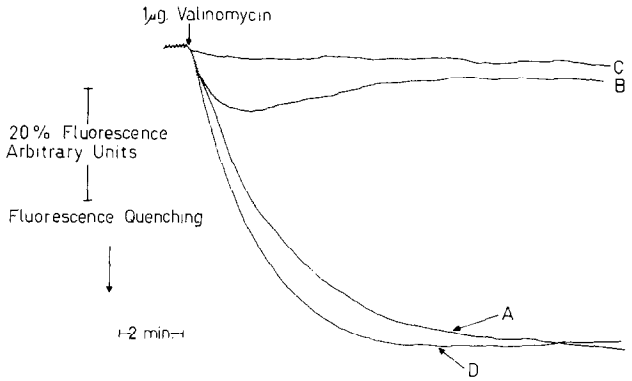

Fig. 1. Quenching of fluorescence produced by liposomes reconstituted with proteolipid extracted with 1-butanol. Liposomes containing 12 μg/ml proteolipid were suspended in a cuvette in 10 mM Tricine-NaOH pH 8.0, 0.3 M sucrose and 6 μM 9-aminoacridine, (final volume 1.0 ml); and the spectrofluorometer was then calibrated to 100 % fluorescence. After the base line was recorded, the reaction was started with 1 μg/ml valinomycin dissolved in dimethyl formamide (1 μl). The variables were as follow: A) Control, no inhibitor present; B) + 60 μM DCCD in 15 ul of 95 % ethanol and preincubated 15 min.; C) As B, but preincubated 30 min.; D) + 9 μg/ml of oligomycin in 15 μl of 96 % ethanol and preincubated for 10 min. Control experiment not shown: K-loaded liposomes but with no proteolipid show a trace as C.

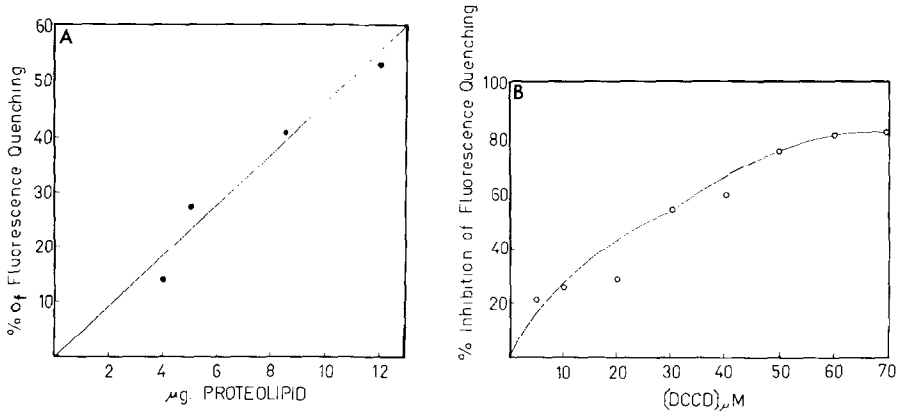

Fig. 2-A. Concentration curve of proteolipid in liposomes with constant phospholipid (PC) (22 mg/ml). Incubation medium, conditions and additions as in Fig. 1, trace A.

Fig. 2-B. Concentration curve of DCCD for inhibition of fluorescence quenching in proteoliposomes. The incubation media and proteolipid concentration are as in Fig. 1; the reaction was started with valinomycin (1 μg/ml). The concentration of DCCD was as shown; samples were preincubated 30 min. before the beginning of the measurement.

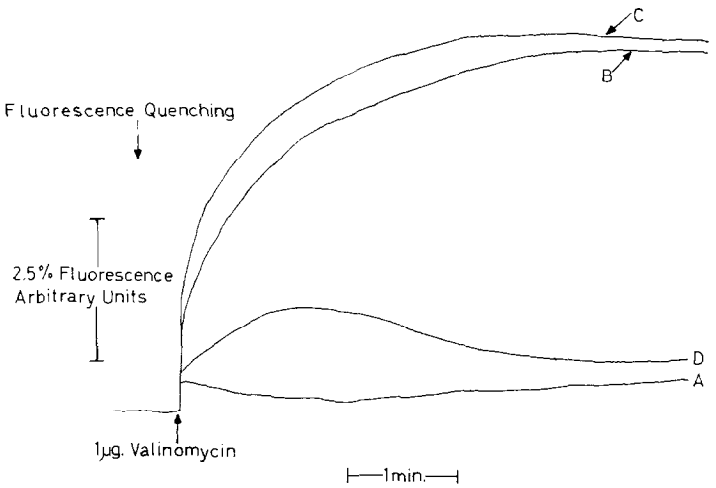

Fig. 3. Enhancement of fluorescence produced by liposomes reconstituted with proteolipid. Liposomes reconstituted with or without proteolipid. Sonicated without KCl, the media contained 10 mM Tricine pH 8.0 and 0.3 M sucrose. The assay medium contained 150 mM KCl, 10 mM Tricine pH 8.0 and 6 µM 9-aminoacridine. A) Control liposomes without proteolipid; B) Liposomes with 10 µg/ml of proteolipid; C) Liposomes as in B but with 60 µM DCCD, preincubated 30 min.

of proteolipid added (Fig. 2-A) and that the inhibition of H^+ fluxes depends on the concentration of DCCD (Fig. 2-B). It is worth mentioning that at all the proteolipid to phospholipid ratios that were studied (Fig. 2-A), the process was insensitive to oligomycin (data not shown). This latter finding suggests that the phospholipids are not modifiying the response to oligomycin.

In order to test whether the proteolipid mediates H^+ flow in both directions of the bilayer, the experiment detailed in Figure 3 was carried out. Liposomes were made with and without proteolipid in the absence of internal K^+.

In the presence of external K^+ valinomycin failed to induce H^+ translocation in the control liposomes (Fig. 3-A), but in liposomes made with proteolipid an important eflux was observed upon the addition of valinomycin (Fig. 3-B). The process was sensitive to DCCD, but not to oligomycin (Fig. 3-D, C), similarly to the experiments in which H^+ influx was observed.

It is important to note that the SDS electrophoretic analysis of the 1-butanol extracted proteolipid carried out according to Weber and Osborn (18) showed a single band with an approximate molecular weight of 8,000 daltons (not shown).

DISCUSSION

The present experimental data are consistent with the idea that the 1-butanol extracted proteolipid mediates H^+ translocation. On the basis of the sensitivity of the process to DCCD, the experimental results also support the idea that this proteolipid is involved in the transfer of H^+ carried out by the F_0 component of mitochondrial ATPase.

In this respect, it is interesting that the H^+ conducting properties of the chloroform:methanol extracted proteolipid from yeast is sensitive to both oligomycin and DCCD (9). I also have extracted a proteolipid from baker's yeast mitochondria by the butanol procedure and tested its ability to mediate H^+ translocation. Similarly to that extracted from heart mitochondria, this proteolipid mediates H^+ in a DCCD, but not oligomycin sensitive process (data not shown). The findings could indicate that extraction by organic solvents of membrane peptides may drastically alter their sensitivity to inhibitors, but it would be interesting to explore whether the chloroform:methanol proteolipid is composed by two peptides.

In mitochondria a protomotive force can be generated from electron transfer and from ATP hydrolysis. The latter indicates that the H^+ conducting peptides in mitochondria functions in a reversible form. The experiments shown in Figure 1 and 3 show that the 1-butanol extracted proteolipid mediates the flux of H^+ in either direction, but in response to a negative potential induced by valinomycin and K^+. These data further indicate that the DCCD binding component transfers H^+ electrophoretically across the lipid bilayer.

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