RECONSTRUCTION OF MITOCHONDRIAL H^+-TRANSPORTING SYSTEM IN PROTEOLIPOSOMES

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Summary. The proteolipid complex prepared from oligomycin sensitive ATPase had been included into phospholipid liposomes. The membranes of the proteoliposomes obtained thus are capable of transferring hydrogen ions down an electrochemical potential. Hydrogen ion transport is blocked by specific inhibitors of oxidative phosphorylation, namely dicyclohexylcarbodiimide or oligomycin.

INTRODUCTION

The mitochondrial ATPase is known to consist of, at least, two components. One of them, water-soluble protein (factor F_1) catalyses ATP hydrolysis. The second component, which may be designated as PLC*, is located in hydrophobic areas of membranes and lacks ATPase activity. A hydrophobic component (factor F_0) was first isolated and characterized by Kagawa and Racker (1) and by Tzagoloff et al. (2). It has been assumed that F_0 catalyses specific H^+ transport through the membranes (3), but direct evidence for such function of F_0 is absent.

In the present paper the incorporation of PLC into liposomes is described. PLC is shown to induce specific H^+ transport through the membranes. This transport in the reconstituted system is sensitive to DCCD and oligomycin.

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* Abbreviations: PLC, proteolipid complex; F_0, F_1, coupling factors of oxidative phosphorylation; DCCD, dicyclohexylcarbodiimide; DNP, 2,4-dinitrophenole.

MATERIALS AND METHODS

1. Isolation of PLC from oligomycin sensitive ATPase preparation of beef heart mitochondria was carried out by the method of Tzagoloff et al. (2) as modified by Chuchlova et al. (4).
2. Preparation of phospholipid vesicles. Soybean azolectin was purified according to method of Racker et al. (5). Azolectin (60 mg) was added to 4 ml of cold medium which consisted of 50 mM sucrose, 0.2 mM EDTA, 100 mM KCl and 10 mM Tris-HCl (pH 7.7) and sonicated for 5 min. with UZDN-1 (Moscow, USSR) sonicator (0.5 a, 22 kc). After sonication, the suspension was allowed to stand for 3-4 hours in ice bath. The electronemicroscopic analysis has shown the procedure described to result in formation of closed vesicles mainly having bilayer membranes. The lamellar structures and multilayer vesicles were also present. 1.5-2 ml aliquot of the suspension was applied to Sephadex G-75 column (1.6 x 18 cm) equilibrated with 0.3 M sucrose, 0.05 mM EDTA and 2 mM Tris-HCl, pH 7.7. Micelles were eluted by the same solution. The procedure of gel filtration was used for preparation of a homogeneous fraction of liposomes, and removal of external potassium and thus generation K^+ ion gradient on liposome membranes. The suspension obtained was used as soon as possible since its store for 1-2 hours resulted in releasing internal potassium.
3. Reconstitution of proteoliposomes. The suspension of PLC (about 0.5 ml) was added to azolectin before sonication. The mixture was then treated as described above. In the suspension of proteoliposomes obtained the final PLC concentration was 2-3 mg/ml. In some cases proteoliposomes were prepared in the presence of 0.2 mM DCCD which was added to suspension before sonication.
4. Determination of membrane ion conditivity. The K^+ and H^+ concentration changes in suspension were measured with K^+ and H^+ specific glass electrodes connected with LPU-01 (Gomel, USSR) amplifiers and EZ-2 (Kovo, Prague) recorders. The electrodes were calibrated with standard KCl and HCl solutions.

RESULTS AND DISCUSSION

The intact liposomes loaded with K^+ have low H^+ conductivity. As it seen in fig. 1A the addition of valinomycin does not affect H^+ concentration in the medium in spite of a H^+ electrochemical potential formation induced by K^+ efflux. DNP addition stimulates fast H^+ uptake and K^+ release by liposomes. DNP added before valinomycin induces very slow ion fluxes through the liposome membranes (fig. 1B). This fact demonstrates the low K^+ and SO_4^{2-} conductivity of membranes. In the presence of DNP valinomycin causes a fast H^+ uptake and K^+ release.

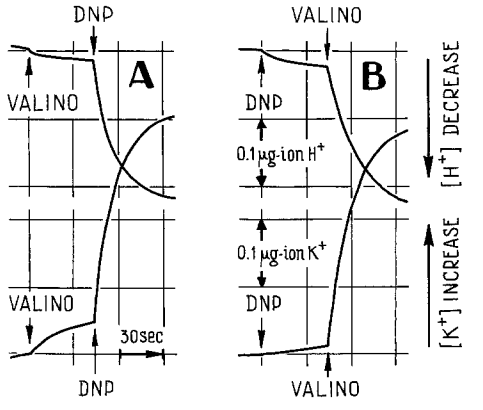

FIGURE 1

Effect of valinomycin and DNP on the K^+ and H^+ transport in liposomes. The fractions of 12-16 ml eluate from G-75 Sephadex column (see p.2 of "MATERIALS AND METHODS") were mixed and than used for measurements without dilution. The final azolectin concentration in liposome suspension was 10 mg/ml. The sample volume was 2 ml. Where indicated by the arrows, valinomycin (2 μg) and DNP (1 μmole) were added.

PLC incorporated into membranes changes the properties of proteoliposomes. Addition of valinomycin to these vesicles induces decreasing H^+ concentration in the external medium as it seen in fig. 2A. In this case valinomycin causes the appearance of a membrane potential as well as for liposomes (minus inside) while hydrogen ions are transferred into proteoliposomes through the selective channels formed by PLC. It should be stressed that H^+ conductivity is selective since H^+ translocation into proteoliposomes occurs when H^+ concentration in medium is as low as 2×10^{-8} M and the concentrations of anions and other cations are quite high.

The reconstituted system of H^+ transport seems to be similar to that of intact ATP-synthetase because of its sensitivity to specific inhibitors. When proteoliposomes are prepared in the presence of 0.2 mM DCCD inhibition of valino-

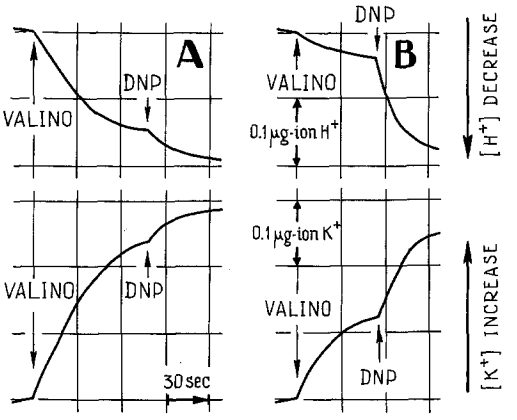

FIGURE 2

Effect of valinomycin and DNP on the K^+ and H^+ transport in proteoliposomes. The conditions were as in fig. 1. The final protein concentration in proteoliposomes was 2 mg/ml. A, proteoliposomes without inhibitors. B, proteoliposomes treated with DCCD or oligomycin.

mycin-induced H^+ conductivity results (fig. 2B). The same inhibition of H^+ transport may be achieved with oligomycin added before valinomycin. In all cases following addition of DNP to proteoliposomes restores valinomycin-induced hydrogen ion transport.

In separate experiments we have found H^+ transport rate to decrease 2-3 fold after incubation of proteoliposomes with the soluble factor F_1 (not shown here). At the same time, the ATPase acquires the oligomycin sensitivity. This fact indicates the specific interaction of F_1 and PLC incorporated in membranes as well as the coupling of H^+ transport and ATP hydrolysis in the reconstituted system. Including F_1 into liposome membranes induces H^+ transport per se but this transport is not sensitive to oligomycin or DCCD (6). It is likely PLC and F_1 (which consists of a few subunits) contain a common H^+-transporting component which is controlled by oligomycin or DCCD in the presence of PLC alone.

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