CHARGE TRANSFER BETWEEN WATER AND OCTANE PHASES BY SOLUBLE MITOCHONDRIAL ATPase (F_1), BACTERIORHODOPSIN AND RESPIRATORY CHAIN ENZYMES

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Received 18 November 1974
Revised version received 2 December 1974

1. Introduction

According to Mitchell's chemiosmotic theory of energy coupling, there are special enzymic systems carrying out translocation of electrons or protons across biological membranes [1]. Such a translocation should include the stages of electron (proton) transfer through a water-lipid interface, the processes being catalyzed (a) by certain enzymes operating in the energy coupling sites of the respiratory and photosynthetic redox chains, and (b) by H^+–ATPase.

In this paper, we shall report a method of investigation of the enzyme-mediated charge transfer across a water-lipid interface. The method consists in the measurement of the Volta potential difference between an octane and water solution, containing the enzyme system studied. Positive charging of the octane phase was found to be induced by soluble mitochondrial ATPase (F_1) in an ATP-dependent fashion, or by bacteriorhodopsin from *Halobacterium halobium* in a light-dependent fashion. In both cases, a lipid-soluble proton acceptor proved to be necessary. Cytochrome oxidase and succinate-cytochrome *c*-reductase were shown to carry out negative charging of octane if a lipid-soluble electron acceptor and water soluble electron donor were added. Succinate-cytochrome *c*-reductase catalyzed the positive charging of octane if a lipid-soluble electron donor and water-soluble electron acceptor were used.

Abbreviations:
DNP, 2,4-dinitrophenol
FCCP, trifluoromethoxycarbonylcyanidephenylhydrazone
MANQ, 2-*N*-methylamino-1,4-naphthoquinone
F_1, coupling factor F_1 (soluble mitochondrial ATPase)

2. Methods

Coupling factor F_1 was isolated from beef heart mitochondria according to Horstman and Racker [2], bacteriorhodopsin sheets after Oesterhelt and Stoeckenius [3], the cytochrome oxidase after Yonetani [4], and the succinate-cytochrome *c*-reductase after Erecinska [5].

The system for Volta potential measurements included the following components: Au/air/octane/water/sat.KCl/Hg_2Cl_2/Hg. The vibrating gold electrode was connected to a mechanical vibration generator (the electrode vibrates at a distance of 0.2–1 mm from the surface of the octane). The vibration of the gold electrode resulted in periodical changes in the capacitance of the part of the system which was situated between the gold electrode and the water surface. The displacement current, generated in this circuit due to capacitance changes, was counterbalanced by an external battery. The potential of the latter, which was equal to the Volta potential in the above system, was measured by a voltmeter (for details see [6]). Any redistribution of the charges between water and octane changed the Volta potential. It should be mentioned that chemical ingredients presented in high concen-

trations in the experimental system (e.g., buffers) must be extremely pure. The best results were obtained with Tris from 'Sigma'.

3. Results and discussion

In the first series of experiments, soluble ATPase from beef heart mitochondria (coupling factor F_1) was studied. F_1 was added to the water phase supplemented with Mg^{2+} and DNP. Then the ATPase reaction was initiated by addition of ATP. As the measurements showed, ATP induced a strong Volta potential change indicating the positive charging of the octane phase. Further experiments showed that the value of the potential change did not depend on whether ATP, Mg^{2+} or F_1 was the reaction-initiating component. A lipid-soluble proton acceptor was found to be absolutely necessary for the above effect to take place. Its amplitude was shown to vary depending on the type of the proton acceptor (table 1). The Volta potential change as a function of DNP concentration is shown in fig.1.

It is noteworthy that the protonated form of some of the proton acceptors used is neutral (DNP and FCCP), whereas that of the others is positively charged (anizidin and methyl ester of phenylalanine). In special experiments it was shown that 1 mM anizidin does not increase the proton conductance of the artificial phospholipid membranes.

The effect observed was of a non-equilibrium nature. It paralleled the rate of the ATPase reaction. Inhibition of the ATPase activity of F_1 by ADP caused a decrease in the Volta potential change. Oligomycin

Table 1
The ATPase-linked Volta potential change in the octane/water system containing different proton acceptors

Proton acceptor	Concentration (M)	Volta potential changes (V)
DNP	10^{-3}	1.00
FCCP	10^{-6}	0.15
Anizidin	10^{-3}	0.20
Methyl ester of phenylalanine	2×10^{-3}	0.35

For conditions see fig.1

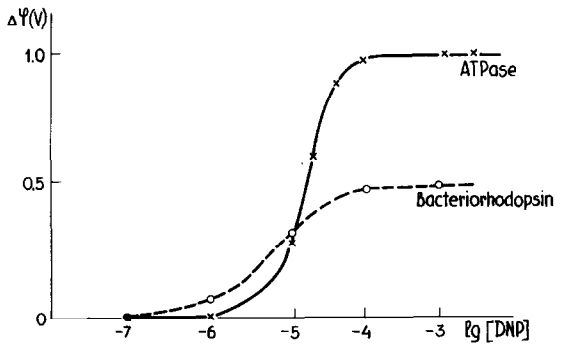

Fig.1. ATPase- and bacteriorhodopsin-mediated Volta potential changes at the octane/water interface ($\Delta\lambda$) as a function of the DNP concentration. The composition of the water phase: in the ATPase experiment, 10 mμM F_1, 1 mM $MgSO_4$, 10 mM Tris-HCl, pH 7.4; in the bacteriorhodopsin experiment, bacteriorhodopsin sheets (0.02 mg protein/ml), 10 mM Tris-HCl, pH 7.4. The process was initiated by addition of 1 mM ATP (ATPase exp.) or switching on the light (bacteriorhodopsin exp.).

was without effect. The dependence of the potential change upon the F_1 concentration showed saturation at 5×10^{-9} M F_1.

The above phenomenon was shown not to be a result of the ATP hydrolysis-induced pH change in the water phase. The addition of 1 mM HCl to the complete or ATP-deficient system did not cause any potential change. Besides, lowering of pH from 7.3 to 6.2, i.e. down to the level at which pH changes accompanying ATP hydrolysis are negligible, affected the above electric response only slightly. The increase in the buffer capacity from 3 mM to 100 mM Tris-HCl was shown not to influence the value of the ATP-dependent Volta potential. Soluble inorganic triphosphatase from *Neurospora crassa* hydrolysing inorganic triphosphate to phosphate and causing the same pH changes in the solution as ATP hydrolysis did not affect the Volta potential.

The data obtained are indicative of the positive charge transfer from water to octane in conditions of ATP hydrolysis by F_1. It seems to be most probable that F_1-translocated positive charges are H^+ ions, since F_1 is a part of the H^+–ATPase complex whose function consists in H^+ ion transport across the mitochondrial membrane at the expense of ATP energy [1,7–11].

In other experiments bacteriorhodopsin complexes ('violet sheets') functioning as the light-dependent electrogenic proton pump in the *Halobacterium halobium* membranes [12–14] were studied. The bacteriorhodopsin sheets were added to the water phase, and the light source was switched on. It was found that illumination induced a Volta potential change of the same direction as did ATP addition in the F_1-containing system. Switching off the light reversed the effect. Again, a lipid soluble proton acceptor, like DNP, was found to be necessary. Dependences of the Volta potential changes upon DNP concentration were very similar in samples with F_1 and bacteriorhodopsin (fig.1).

Experiments on planar phospholipid membranes, carried out by this group, showed an important difference in the behaviour of F_1 and bacteriorhodopsin sheets. The latter were found to be competent in transmembrane hydrogen ion translocation (see [14, 15]), whereas F_1 was ineffective, even in the presence of a lipid-soluble proton acceptor (anizidin). To translocate H^+ across the phospholipid membrane in an ATP-dependent fashion, reconstitution of F_1 with hydrophobic proteins of the oligomycin-sensitive ATP-ase complex was found to be required [15]. So, F_1 seems to mediate an H^+ transfer through the water/membrane interface on one of the two membrane surfaces (injection of H^+ from water into the membrane). As to hydrophobic proteins, they may be necessary for the H^+ ions to cross the membrane/water interface on the other surface of the hydrophobic barrier.

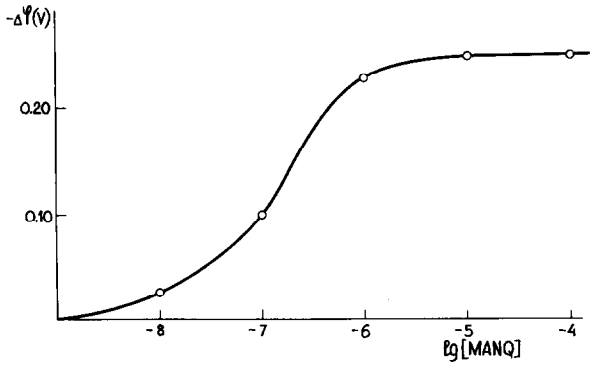

Fig.2. The cytochrome oxidase-linked changes in the Volta potential (Δλ) at the octane/water interface at different concentrations of a lipid soluble electron acceptor, 2-*N*-methylamino-1,4-naphthoquinone (MANQ). The water phase composition: 50 μM cytochrome *c*, 4 mM ascorbate, 50 μg/ml cytochrome oxidase, 10 mM Tris-HCl, pH 7.4.

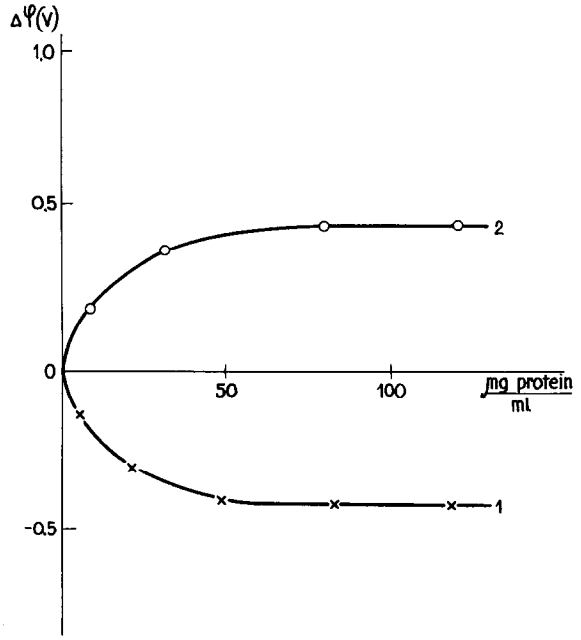

Fig.3. The succinate-cytochrome *c*-reductase-mediated Volta potential changes at the octane/water interface as a function of the concentration of this enzyme. The water phase composition; curve 1: 4 mM succinate, 0.1 mM 2-*N*-methylamino-1,4-naphthoquinone, 50 μM cytochrome *c*, 10 mM Tris-HCl, pH 7.4; curve 2: 50 μM cytochrome *c*, 10 mM ferrocene, 10 mM Tris-HCl, pH 7.4.

In the concluding series of experiments, an attempt was made to use the same method for studying the respiratory chains enzyme complexes which carry out the charge transfer across the mitochondrial membrane. It turned out that cytochrome oxidase and succinate-cytochrome *c*-reductase are capable of catalysing the charge transfer between water and octane if lipid-soluble electron donors or acceptors are present in the system. Cytochrome oxidase negatively charged the octane phase when cytochrome *c*, ascorbate and 2-*N*-methylaminoaphthoquinone were added (fig.2). Cytochrome *c*-reductase catalyzed the negative charging of octane in the presence of succinate, cytochrome *c* and 2-*N*-methylaminoapthoquinone and the positive charging – in the presence of cytochrome *c* and ferrocene (fig.3). The cytochrome oxidase-mediated effect was abolished by cyanide, those of the cytochrome *c* reductase by antimycin A.

Acknowledgements

The authors would like to thank Academician A. N. Frumkin, Drs L. A. Drachev, E. A. Liberman, L. S. Yaguzhinsky, and A. A. Jasaitis for discussing the results, Drs L. P. Kayushin, L. N. Chekulaeva and S. A. Ostroumov for preparation of bacteriorhodopsin, and Dr I. S. Kulayev for the preparation of phosphatase of inorganic triphosphate.

References

[1] Mitchell, P. (1966) Chemiosmotic Coupling in Oxidative and Photosynthetic Phosphorylation (Glynn Research, Bodmin).
[2] Horstman, L. L. and Racker, E. (1970) J. Biol. Chem. 245, 1336–1343.
[3] Oesterhelt, D. and Stoeckenius, W. (1971) Nature New Biol. 233, 149.
[4] Yonetani, T. (1967) Methods in Enzymol. 10, 332.
[5] Erecinska, M., Oshino, R., Oshino, N. and Chance, B. (1973) Arch. Biochem. Biophys. 157, 431–445.
[6] Frumkin, A. N., Gugeshasvily, M. I. and Boguslavsky, L. I. (1971) Dokl. Akad. Nauk SSSR, 198, 1452–1457.
[7] Mitchell, P. (1973) FEBS Letters, 33, 267–274.
[8] Skulachev, V. P. (1974) Ann. N. Y. Acad. Sci. U.S. 227, 188–202.
[9] Skulachev, V. P. (1974) Usp. Sovrem. Biol. 77, 211–225.
[10] Mitchell, P. (1972) J. Bioenerg. 3, 5–24.
[11] Glagolev, A. N. and Skulachev, V. P. (1974) Biokhimya, 39, 615–619
[12] Oesterhelt, D. and Stoeckenius, W. (1973) Proc. Natl. Acad. Sci. U.S. 220, 2853–2860.
[13] Kayushin, L. P. and Skulachev, V. P. (1974) FEBS Letters, 39, 39–42.
[14] Drachev, L. A., Kaulen, A. D., Ostroumov, S. A. and Skulachev, V. P. (1974) FEBS Letters, 29, 43–45.
[15] Drachev, L. A., Jasaitis, A. A., Kaulen, A. D., Kondrashin, A. A., Liberman, E. A., Nemeček, I. B., Ostroumov, S. A., Semenov, A. Yu. and Skulachev, V. P. (1974) Nature, 249, 321–324.