CHARGE SEPARATION AND ENERGY TRANSFER IN THE MITOCHONDRIAL MEMBRANE

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It is clearly emerging from the work of various laboratories that membrane-bound electron transfer chains are anisotropically arranged along an axis perpendicular to the plane of the membrane (1–6). Anisotropic organization of redox chains, composed of hydrogen and electron carriers can lead, as proposed by Lundegardh (7), to generation of transmembrane electrical fields and proton gradients if the proton-electron separation and proton-electron recombination processes occur at opposite sides of the insulating layer of the membrane (see Mitchell, [8]).

The respiratory chain of mitochondria is organized in four polymeric units or "complexes." The purpose of this paper is to summarize recent work on the topographical arrangement in the membrane of cytochrome *c* oxidase (complex IV) and ubiquinol cytochrome *c* reductase (complex III) and to discuss its relevance with respect to the mechanism of the redox proton pump.

As regards complex IV cytochrome *c* is located at the external side of the inner mitochondrial membrane (see ref. 4 for review). The location of the site where cytochrome oxidase reacts with oxygen and protons and electrons recombine is controversial (4, 9–11).

This problem has been attacked in our laboratory by analyzing with flow potentiometric and spectrophotometric techniques the kinetic and stoichiometric relationship between aerobic oxidation of the terminal electron carriers and oxygen protonation (5, 6, 12). Fast aerobic oxidation of cytochrome oxidase and *c* cytochromes is accompanied, in intact beef-heart mitochondria, by synchronous proton release in the medium instead of the expected stoichiometric proton consumption (6). In the presence of carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) oxidation of electron carriers is accompanied by proton disappearance from the medium. This proton uptake, which roughly corresponds to the content of the oxygen-terminal electron carriers, evidently takes place at the inner side of the membrane. It does, in fact, manifest itself to the electrode in the medium only when a high proton conductance is induced in the membrane by FCCP and still detection of proton consumption is delayed with respect to the time of oxidation of electron carriers (5, 6).

This conclusion is reinforced by results of experiments in sonic submitochondrial particles. In sonic particles, due to inverted orientation of the mitochondrial mem-

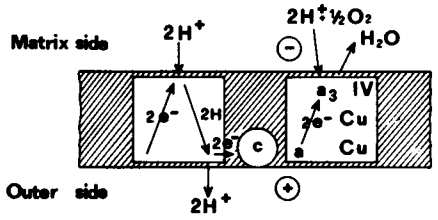

FIGURE 1

brane, the site of cytochrome oxidase reaction with oxygen should be directly exposed to the electrode in the medium. In fact the oxidation of oxygen-terminal electron carriers is accompanied in sonic particles by synchronous and stoichiometric consumption of protons (6).

In another set of experiments it has been found that valinomycin plus K^+ enhances the rate of aerobic oxidation of cytochrome *c* and of the accompanying proton consumption (5). This indicates that the aerobic oxidation of cytochrome *c* generates an electric field across the membrane, due to the fact that oxygen reduction, at the inner side of the membrane, utilizes electrons delivered from cytochrome *c* at the outer side and protons from water on the matrix side of the inner membrane (see Fig. 1). Complex IV thus appears to be oriented transversely across the mitochondrial membrane. The complex reacts with oxygen at the inner side and with cytochrome *c* at the outer side of the membrane and mediates a fast vectorial flow of electrons between the two sides of the membrane. This functional arrangement of the cytochrome-*c*-cytochrome oxidase region will lead to generation of a net transmembrane electrochemical proton gradient if other segments of the chain are topographically arranged so as to lead to proton electron separation at the outer side of the membrane and give rise to concomitant outward hydrogen pulses and inward electron flow.

Kinetics analysis shows that the aerobic oxidation of endogenous ubiquinol is accompanied by synchronous proton release from intact mitochondria and synchronous proton uptake by sonic particles. Thus electron flow along complex III is associated with proton translocation across the membrane. This proton pump appears to be electrogenic (13–15).

Flow analysis of the $H^+/2e^-$ stoichiometry for the aerobic oxidation of ubiquinol in sonic submitochondrial particles has revealed that, at least at pH 6.8, 4 gion H^+ are taken up *per* mole ubiquinol oxidized (5, 13).

$H^+/2e^-$ ratio of 4 has also been found by Lawford and Garland (16), by pulsing intact mitochondria with small amounts of exogenous quinols.

The finding that 4 H^+ are translocated per $2e^-$ flowing from ubiquinol to oxygen implies that one (or two) hydrogen carriers exist in complex III.

Additional evidence for the existence of a hydrogen carrier in complex III comes from studies on proton translocation in a reconstituted system consisting of phospholipid liposomes inlayed with purified complex III (14, 15).

No classical hydrogen carrier is known to exist in complex III. However metal electron carriers might act as effective hydrogen carriers and might be responsible for transmembrane proton translocation if oxidoreduction of the metal were linked to proton equilibria of acidic groups in the apoprotein (17). In this case the redox potential of the electron carrier has to be pH dependent. It can be noted that such a mechanism emphasizes the role of chemical and conformational events at the level of the apoproteins of the respiratory carriers and differs in this respect from that proposed by Mitchell (8) which is based upon the alternation of currents of hydrogen and electron carriers from one side to the other of the membrane and the coupling is envisaged as a purely physical consequence of the supramolecular arrangement of the respiratory chain in the membrane.

The electron carriers of complex III are two *b* cytochromes b_{566} and b_{562}, cytochrome c_1 and two Fe-S-protein (18). There are however other factors not better identified (19). The midpoint potential of c_1 is pH independent at least in the physiological range. Prince and Dutton (20) have reported at this meeting that the midpoint potential of the Rieske's Fe-S-protein (g 1.90) is pH independent in the range pH 6.3 to pH 8.3.

The midpoint potential of the two *b* cytochromes is however pH dependent (21). Utilizing the pK values of the oxidized and reduced *b* cytochromes it is possible to construct the curves for the ionization state of the oxidized and reduced *b* cytochromes as a function of the pH, and from these curves it is possible to calculate to what extent at a given pH the *b* cytochromes function as electron or hydrogen carriers. These properties of the *b* cytochromes have been utilized to design experiments to examine the topographical arrangement in the membrane of *b* cytochromes and their role in the redox proton pump of complex III (22, 23).

Work from various laboratories has revealed that energization of the mitochondrial membrane slows down the rate of aerobic oxidation of cyt b_{566} but has no appreciable effect on cyt b_{562} (see ref. 24 for review). It has been found that this effect on b_{566} is abolished by agents which collapse the membrane potential (22). On the basis of these and related observations Wikstrom has proposed that cytochrome b_{566} is predominantly located at the outer and cytochrome b_{562} at the inner side of the membrane. With this arrangement the *b* cytochromes will constitute an electron carrying arm. Electrons move vectorially from the outer to the inner side of the membrane and a membrane potential positive outside will specifically depress the delivery of electrons from cytochrome b_{566} to redox components at the inner side of the membrane.

If indeed cytochrome b_{566} is located at the outer side of the membrane the depression of its oxidation by the membrane potential should decrease as the *b* cytochromes change, upon increasing the pH from 5 to 8.0, from an electron to an hydrogen carrier.

In fact it has been shown (22) that oligomycin which inhibits passive proton diffusion in EDTA submitochondrial particles, and thus allows the establishment of aerobic transmembrane electrochemical proton gradient, causes at pH 7 dramatic inhibition of the oxidation of cyt b_{566}. This inhibition is completely released by valino-

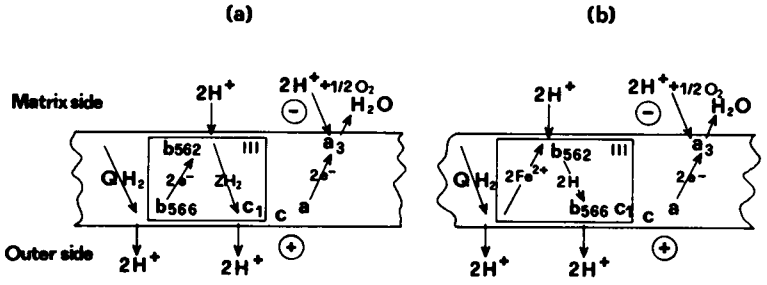

FIGURE 2

mycin plus K^+. The inhibitory effect of oligomycin and the releasing effect of valinomycin are much less marked at pH 7.7 and disappear completely at pH 8.5. Also the oxidation of cyt b_{562} is inhibited by oligomycin and stimulated by the subsequent addition of valinomycin. However in this case the effects are much less pronounced and practically pH independent. These results are consistent with the proposed location of cyt b_{566} at the outer side and cyt b_{562} at the inner side of the membrane.

Two models based on this arrangement of *b* cytochromes are shown in Fig. 2. According to model *a* (favored by Wikstrom (24) as well as by Mitchell (25)) cytochrome b_{566} would be closer to the substrate than cytochrome b_{562}. The *b* cytochromes will, in this case, give rise to a vectorial flow of electrons or hydrogens—this depends upon the actual pH of the system—from the outer to the inner sides of the membrane.

According to model *b* cytochrome b_{562} would be closer to the substrate than b_{566}. In this case the *b* cytochromes will give rise to a vectorial flow of electrons (or hydrogens) from the inner to the outer side of the membrane.

It is expected that further studies along these lines might contribute to clarify the spatial arrangement of the components of complex III and general aspects of structure-function relationships in membrane-bound redox systems.

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