PHASE TRANSITIONS AND COUPLING IN ENERGY TRANSDUCING MEMBRANES

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1. Introduction

The mechanism of energy transduction in bacterial and organelle membranes is still a matter of considerable controversy [1]. Evidence has accumulated which confirms many of the postulates of Mitchell's chemiosmotic hypothesis [1–4]. It is generally accepted today that both the electron transport system and the ATPase are proton pumps, catalyzing a reaction in which spontaneous chemical reaction is coupled to the electrogenic transport of protons across the membrane. Both systems are capable of generating a large proton electrochemical gradient $\Delta\tilde{\mu}H$, between the bulk phases across the membrane [5]. It is also well established that these pumps are reversible and are capable of coupling the spontaneous flow of protons driven by $\Delta\tilde{\mu}H$ to a reversal of the chemical reactions such as ATP synthesis or electron transport from a low to a high potential. It is therefore clear that a membrane which contains both ATPase and electron transport chain is capable of utilizing a $\Delta\tilde{\mu}H$ generated by electron transport for the synthesis of ATP as postulated by Mitchell. However, it is still not entirely clear whether this is the only mechanism which provides the coupling in oxidative phosphorylation, or even whether this is the main mechanism. We have concluded [6] from the different effects of uncouplers and phosphorylation on $\Delta\tilde{\mu}H$ and on the rate of electron transport that there is a parallel, more direct, route of coupling which is not mediated by $\Delta\tilde{\mu}H$ between the bulk phases across the membrane. This conclusion supports a suggestion [6] of parallel coupling mechanism which is also favored by thermodynamic considerations of the coupling efficiency [7].

In this communication, I suggest that this direct coupling is a fast protonic current on the membrane surface which forms a direct connection between the two enzyme systems; one serving as proton source, the other as proton sink. This fast proton current on the membrane surface prevent equilibration during fast cycling of the domains of the ATPase and the electron transport chain with the bulk electrochemical proton potential. Thus, the surface proton current runs in parallel to the conventional chemiosmotic mechanism of proton flow through the bulk phases on both sides of the membrane. The fraction of the proton current carried directly on the surface would depend to a large extent on the distance between the ATPase and the electron transport chain.

2. Methods and results

Most biological membranes are known to be capable of existing in different structural phases. Generally at high temperatures the structure is liquid-crystalline, characterized by a loose packing, high fluidity and a high rate of in-plane diffusion of both proteins and phospholipids. At low temperatures the membrane exists as a gel phase, which is much more restrictive in motion particularly in the plane of the membrane [8]. Of particular interest is a transition state in which both liquid crystalline and gel phases co-exist. Because of the heterogeneity of natural membranes this transition state extends over a wide range of temperatures. From studies of various bacterial membranes, it appears that at the transition temperature range the proteins form clusters producing large areas of closely packed proteins and large areas of lipid membrane almost free of proteins [9–11].

The evidence for the existence of phase transition in mitochondria is not as substantial as in bacteria. Molecular motions have been studied [12,13] by spin labeling [12] and the energy of activation of respiratory enzymes [13] and it was concluded that a transition exists with T_m of about 23°C. I have extended these studies using several fluorescent probes and measuring activation energy for several membrane enzymes and other probes and conclude that a transition state exists over 20–30°C with $T_m \sim 25$°C (to be published).

It is therefore suggested that when the membrane is in this transition state, the ATPase and the electron transport chain come close together and the surface proton current is predominant. In this state a tighter coupling of oxidative phosphorylation and other energy-conserving processes is expected. Figure 1 is a scheme of an imagined view of the membrane surface at liquid-crystalline or gel phase (a), and the membrane surface at the transition state (b) which is based on this suggestion.

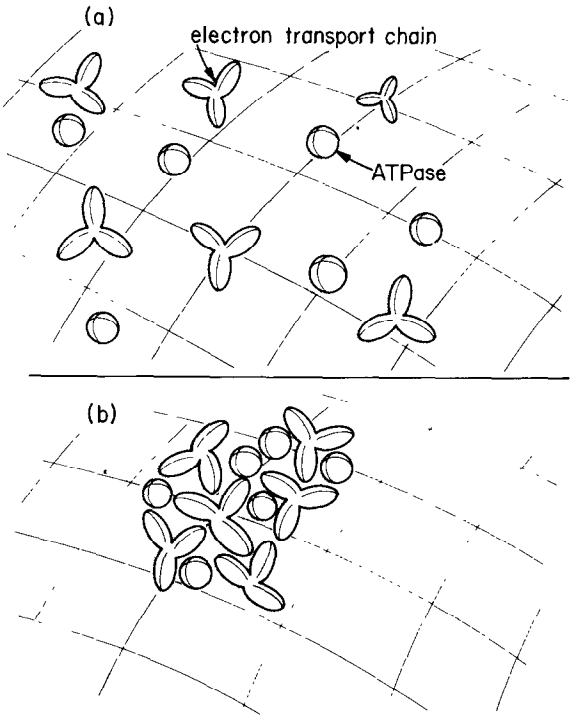

Fig.1. A schematic view of the mitochondrial membrane surface. (a) In liquid-crystalline or gel phase the enzymes are distributed at random on the membrane surface. (b) In the transition state, ATPase, the electron transport chain and other proteins form large protein clusters on the membrane surface.

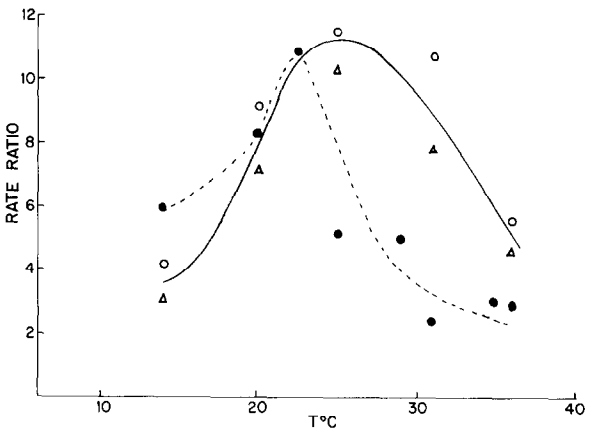

Fig.2. The respiratory control ratio in rat liver mitochondria with ADP (△), with FCCP (○), and the ATPase ratio (±) FCCP (●), as function of temperature. Rat liver mitochondria were prepared by differential centrifugation in a medium containing 0.25 M sucrose and 1 mM NaEDTA (pH 7.0). Respiratory control was measured by an oxygen electrode in a medium composed of: 0.2 M sucrose, 50 mM KCl, 10 mM Tris–Cl, (pH 7.0), 5 mM Na_2HPO_4–NaH_2PO_4, 2 mM $MgCl_2$. Additions were: mitochondria (0.5 mg protein/ml), rotenone 2 μM, Na_2-succinate 10 mM, ADP (0.125 μM X3), FCCP 1×10^{-7} M. ATPase rate was measured by following the pH change in the same basic medium except that Tris–Cl was omitted and Na_2HPO_4 (pH 7.0) was reduced to 2 mM. Additions were: mitochondria (1 mg protein/ml); rotenone 2 μM; ATP 1 mM; FCCP 2×10^{-7} M.

I have performed several experiments with rat liver mitochondria which lends substantial support to this model [14]. Here I show that respiratory control (both by ADP and uncouplers) is strongly temperature dependent. Very similar dependence was also observed for the control of the ATPase rate by uncouplers. Figure 2 shows these three types of measurements as a function of temperature. It is observed that the respiratory control by ADP (Δ) is maximal at 25°C dropping sharply at higher and lower temperatures. Similarly, respiratory control by FCCP (○) shows the same temperature profile at slightly higher values. The control of the ATPase rate by an uncoupler (●) is also temperature dependent. However while the maximal value is similar

to the respiratory control one at 24°C the drop is sharper at high temperature and significantly less pronounced at low temperatures. Note that each of these ratios shows the rate of the same reaction under two different conditions and its dependence on temperature cannot be explained by the general temperature dependence of an enzymic reaction. Moreover, one common feature of FCCP and phosphorylation, which release the control of these reactions, is the acceleration of proton cycling. It is therefore reasonable to assume that the temperature dependence of the control reflect both the reduction in proton cycling at the transition temperature in the controlled state and enhancement of proton cycling at this temperature either by the ATPase or by FCCP. However, I have found that $\Delta\tilde{\mu}H$ does not have the same dependence on temperature as shown here for the rate ratios [14] and that the general membrane proton permeability is not minimal at the transition temperature (to be published).

3. Conclusion and discussion

I therefore conclude that the highly controlled rate at the transition temperature is due to protein clustering which inhibits proton dissipation at the controlled state but allows relatively accelerated dissipation from the cluster either by the ATPase or FCCP. It is perhaps somewhat surprising that the maximal efficiency of oxidative phosphorylation in rat liver mitochondria would be found at 25°C, well below the physiological temperature (37°C). However, transition temperatures of charged membranes depend to a large extent on the ionic composition of the medium [8], and the conditions of our experiments are not identical to the cytoplasm. Moreover, the preparation of mitochondria in ion-free medium is probably responsible for a loss of both ions and proteins from the membrane which might greatly alter their temperature dependence.

Preliminary results indicate that indeed the ion composition of the medium has a large influence on the temperature dependence of the respiratory control. I would therefore like to believe that in situ the transition temperature is much closer to the physiological temperature. In fact it would be useful if the transition were close but not at the physiological temperature. Since the transition temperature of the membrane can be shifted by altering the charge density of the membrane surface, such a situation provides a convenient switch mechanism for controlling the efficiency of energy transduction.

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