CHANGE IN SURFACE CHARGE DENSITY AND MEMBRANE POTENTIAL OF INTACT MITOCHONDRIA DURING ENERGIZATION

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1. Introduction

Application of respiratory chain substrates or ATP to mitochondria leads to the so-called 'energized state'. Various phenomena such as change in morphology [1,2], light scattering [3], redox states of respiratory chain components [4–6], or fluorescence of hydrophobic probe such as 1-anilino-8-naphthalene sulfonate (ANS) [7–10] have been observed during the energization. Here the surface charge densities of intact mitochondria determined by electrophoretic mobility, in energized or de-energized state, are reported. The negative charges appear when mitochondria are energized by substrates or ATP, and disappear when mitochondria are treated with respiratory inhibitors or uncouplers. It is shown, moreover, that the change in surface charge correlates with uptake of dibenzyldimethyl ammonium cation (DDA^+), which is regarded as an indicator of membrane potential [11].

2. Experimental

Mitochondria from rat liver were isolated according to the standard method by differential centrifugation in 0.25 M sucrose and 0.2 mM EDTA.

The surface charge density of mitochondria, σ, was calculated from the electrophoretic mobility, u, with use of the following equation [12]:

$$\zeta = \chi\pi\gamma u/D$$

$$\sigma = \sqrt{\frac{DRTT}{500\pi}}\ \frac{F\gamma}{2RT} \qquad (1)$$

Here, D, R, T, F, I, γ, and ζ stand for the dielectric constant of the dispersion medium, gas constant, absolute temperature, Faraday constant, ionic strength, the viscosity of the dispersion medium, and zeta-potential, respectively. The dielectric constant was assumed to be the same as that of pure water. Ionic strength was calculated with use of the pK_a value for the respective substance, taken from the appropriate table [13,14]. The electrophoretic mobilities were measured by a micro-electrophoretic apparatus (Cytopherometer, Karl Zeiss, West Germany). The velocity of electrophoresis varied from one mitochondrion to the other. The histogram shows under various conditions the frequency within the range of the surface charge density ± 0.025 $\mu C/cm^2$ (fig.1).

The DDA^+-uptake was measured with a DDA^+-selective electrode, developed in our laboratory. The construction and properties of the DDA^+-selective electrode were described elsewhere [15].

3. Results and discussion

Histograms showing the distribution of the surface charge density measured under de-energized and energized conditions are illustrated in fig.1. Figure 1(A) represents the electrophoretic character of mitochondria in the buffer solution (10 mM potassium phosphate, 250 mM sucrose, 0.1 mM EDTA, pH = 7.4). De-energization by addition of rotenone brought about a decrease in negative surface charge. The surface charge density was normally distributed with a maximum frequency of approx. $-0.7\mu C/cm^2$. Energization by addition of succinate to mitochondria which were previously

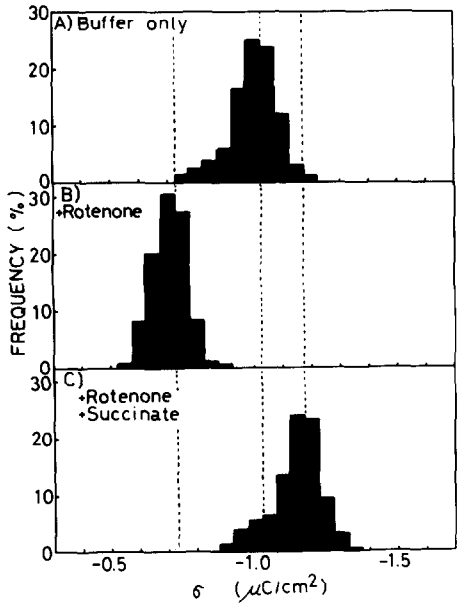

Fig.1. The effect of rotenone and succinate on surface charge density. Measurements were made by direct observation of the electrophoretic velocity of mitochondria at the stationary layer in a flat quartz cell regulated at 25°C. The sedimented mitochondria were suspended in the following media: (A) buffer solution containing 10 mM potassium phosphate (pH 7.4), 0.1 mM EDTA and 0.25 M sucrose; (B) buffer plus rotenone (0.1 μg/mg of protein); (C) buffer plus 3 mM succinate in the presence of rotenone. The mitochondrial concentration was 0.03–0.1 mg protein/ml. The measurements were carried out within 1 h after the addition of the various chemicals. Under a given condition, 25–30 min mitochondria were timed in each direction of the electric field (approx. 6 V/cm) to eliminate the polarization of the electrodes from which the current was delivered.

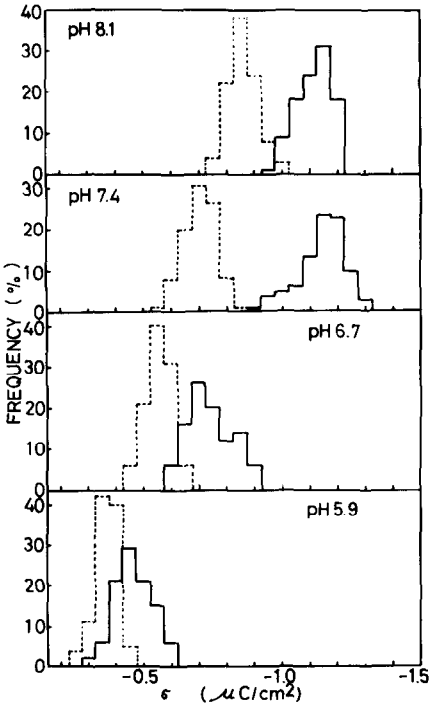

Fig.2. Effect of pH value on the surface charge density. The broken and solid lines represent the frequency in de-energized and energized states. Mitochondria were de-energized by rotenone and energized by 3 mM succinate. The pH value used is indicated in each histograms.

treated with rotenone led to negative surface charge. The peak value −1.1 to −1.2 $\mu C/cm^2$ was larger than that in buffer only. Then, the surface charge of state 4 mitochondria was large than that of state 1 mitochondria. The distribution became somewhat broader, especially in the region of low charge densities. In the following discussion, we consider the surface charge density for the maximum of mitochondrial populations (broken lines, fig.1). We discuss the average value instead of the maximum population value.

Under various medium pH values (5.2–8.1), electrophoreses of mitochondria were performed, the results shown in fig.2. The negative surface charge density decreases as the pH of the medium is decreased. This is due to the association of the acidic groups on the mitochondrial membrane. The difference in surface charge density between de-energized and energized states was largest at pH 7.4. Phosphate buffer (pH 7.4) was used therefore in subsequent experiments.

The membrane potential in small organelles or bacteria can be estimated from the uptake of lipophilic ions such as dibenzyl-dimethyl ammonium cation (DDA^+), according to Skulachev, Libermann et al. [16], Harold and Papineau [11]. The change in DDA^+ concentration in the medium during de-energization or energization was monitored with a DDA^+-electrode [15]. Results are shown in fig.3. The upward shift of an electrode potential in the figure indicates a decrease of the DDA^+ concentration in the medium, i.e., the uptake of DDA^+. Addition of mitochondria to the buffer solution containing 10^{-4} M DDA^+ and 10^{-6} M TPB^- (tetraphenyl boron anion) led to a fairly large

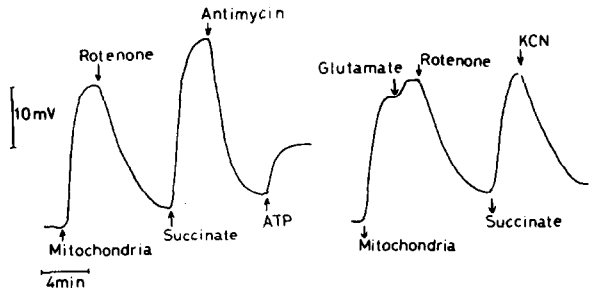

Fig.3. DDA^+ accumulation due to energization and release due to the addition of inhibitors. The vertical bar represents the 10 mV shift of the read-out in DDA^+ electrode. Incubation medium: 10 mM potassium phosphate pH 7.4), 0.1 mM EDTA, 0.25 M sucrose, 10^{-4} M DDA^+ and 10^{-6} M TPB^-. Additions: rotenone (0.1 μg/mg protein), antimycin A (0.1 μg/mg protein), succinate (3 mM), ATP (adenosine triphosphate, 0.4 mM) glutamate (3 mM), KCN (1 mM). Mitochondrial concentration: 2.4 mg protein/ml (right) and 3.0 mg protein/ml (left).

uptake of DDA^+. Inhibition of the energy supply from endogenous substrates by rotenone brought about an efflux of DDA^+. Subsequent addition of succinate and antimycin A brought about a repeat of the uptake and release of DDA^+. It is noted that the mitochondria supplemented with succinate or glutamate exhibit large uptake than that observed with endogeneous substrates only. The DDA^+-concentration in the medium was approximately equal when mitochondria were de-energized by rotenone, antimycin A, or CN^-. The membrane potential of mitochondria with respect to the medium, $\Delta\varphi$, can be estimated from the shift of the electrode potential provided that DDA^+ is passively distributed in accordance with the Nernst equation [11]. When we denote the readout of a selective electrode by E, $\Delta\varphi$ is given by the following equation:

$$\Delta\varphi = 59 \log (v/V) - 59 \log [10^{(E-E_0)/59} - 1] \quad (2).$$

Table 1

THE SURFACE CHARGE DENSITY AND THE MEMBRANE POTENTIAL OF MITOCHONDRIA UNDER VARIOUS CONDITIONS

CONDITIONS	SURFACE CHARGE DENSITY (μ COULOMB/CM^2)				MEMBRANE POTENTIAL (MV)				
	-0.6	-0.8	-1.0	-1.2	-100	-120	-140	-160	-180
A) BUFFER ONLY			—					—	
B) +ROT.		—			—				
C) +ROT. +SUC.				—					—
D) +GLU.				—				—	
E) +GLU. +ROT.		—			—				
F) +GLU. +ANT.		—			—				
G) +ROT. +SUC. +ANT.		—			—				
H) +ROT. +SUC. +KCN		—				—			
I) +ADP			—				—		
J) +ADP +ROT.	—				—				
K) +SUC. +ANT. +ATP				—			—		
L) +ROT.+SUC.+FCCP		—			—				

ROT., rotenone (0.1 μg/mg protein). SUC., succinate (3 mM). GLU., glutamate (3 mM). ANT., antimycin A (0.1 μg/mg of protein). KCN, potassium cyanide (1 mM). ADP, adenosine diphosphate (0.4 mM). ATP, adenosine triphosphate (0.4 mM). FCCP, carbonyl cyanide p-trifluoromethoxyl phenylhydrazone (1 μM).

In the above equation, ν, V and E_o stand for the volume of mitochondria, that of the medium, and the electrode potential observed before the mitochondria are injected, i.e. the value of the base line in fig.3.

The effect of substrates, respiratory inhibitors, ADP or ATP on the surface charge density and the membrane potentials estimated from the DDA^+-uptake are summarized in table 1. In calculating the membrane potential, the volume of the matrix in mitochondria, ν is taken as 1 μl/mg protein according to Harris and van Dam [17]. Comparing the second and third column, we may conclude that the negative surface charge is formed concomitant with the membrane potential. Interior negative charge is generated when mitochondria are energized by the addition of substrates or ATP. The change in the membrane potential due to addition of ATP is smaller compared with the change in the surface charge. The reason is not clear to us at present. Mitochondria de-energized by the presence of FCCP (carbonyl cyanide *p*-trifluoromethoxyl phenylhydrazone) exhibit neither the negative charge nor change in membrane potential when substrates are applied.

The surface charge density calculated from electrophoresis is considered to be that of the outermost surface of the particle, i.e., the outer-membrane of mitochondria in the present experiments. But, the good correlation between the change in surface charge calculated from electrophoresis and that in the membrane potential generated across the inner-membrane, suggests that the electrophoretic character is affected significantly by the change in surface charge on the inner-membrane of the mitochondria, and that the negative charge appear on the surface of the inner-membrane when mitochondria are energized.

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