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A STUDY OF SPIN-PROBE SOLUBILITY IN MITOCHONDRIAL MEMBRANES CORRELATED WITH ATP-DEPENDENT CONFORMATIONAL CHANGES

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SUMMARY

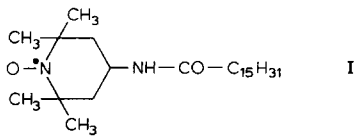
Addition of ATP to submitochondrial particles causes considerable changes in the ESR spectra of hydrophobic spin-probe bound to particles which indicate an improvement in probe solubilization in the submitochondrial particle membranes due to energization. This effect is abolished by 2,4-dinitrophenol (10^{-4} M) and oligomycin (1 μg/mg of protein). At lower concentrations (0.1–0.2 μg/mg), oligomycin, on the contrary, promotes the action of ATP on the submitochondrial particle-bound spin-probe ESR spectra as well as activates the ATP-dependent transhydrogenase reaction in submitochondrial particles.

Some years ago energy conservation processes in mitochondria were suggested to be accompanied by some kind of conformational transitions[1]. Using light-scattering and electron microscopy techniques one can observe changes in mitochondrial volume and shape at different functional stages[2,3]. But these phenomena cannot be considered as a direct proof of real conformational transitions in mitochondrial lipoprotein structures because they might occur as a result of water accumulation or effusion due to variations in ion transport during energization[4].

Using the spin-probe method we have observed conformational changes in mitochondrial membranes induced by redox transitions[5]. In the present work we succeeded in observing by the same method ATP-induced conformational changes in mitochondria and submitochondrial particles.

Beef-heart mitochondria were prepared by the method[6]. Submitochondrial particles were prepared from the light fraction of mitochondria according to SKULACHEV[7]. The light mitochondrial suspension in 0.25 M sucrose, 10 mM Tris–HCl (pH 7.5), 1 mM EDTA and 20–30 mg of protein per ml was sonicated twice under cooling in an MSE-500 w ultrasonic disintegrator at a frequency of 20 kcycles, 0.5 A and at maximal resonance for 1.5 min at intervals of 3–5 min. After sonication the pH of the mixture was again adjusted to 7.5 and the mixture was centrifuged at 1000 $\times$ g for 10 min. The sediment was removed and the supernatant was centrifuged at 105000 $\times$ g for 30 min. The sedimented submitochondrial particles were resuspended in a medium the composition of which is indicated in the legends to the figures.

A hydrophobic iminoxyle derivative prepared according to ROZANTSEV AND KRINITZKAYA[8] was used as a spin-probe. The spin-probe I is insoluble in water but can be readily solubilized in less polar solvents (ethanol, hexane). An ethanolic solution of probe I was added to the particle suspension, the final concentration not exceeding



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1–2 %. Usually we added potassium ferricyanide to the suspension to prevent the reduction of the radical by endogenic electron donors[5]. Component I was incubated with the mitochondrial suspension for 5 min at 25°. The suspension was mixed with an equal volume of ATP solution in the same medium at pH 7.5. The inhibitors were preincubated with mitochondrial preparations for 3–5 min before the addition of ATP. The electron spin resonance (ESR) measurements were carried out at 25° with an X-band ESR spectrometer of the type EPR-2 ICP[9].

Fig. 1a shows the ESR spectrum of the probe in submitochondrial particles without ATP. The spectrum represents a superposition of two signals: a singlet and a triplet. The singlet signal is similar to that of the hydrophobic probe suspension in aqueous medium (Fig. 1b). A large concentration of spins in probe particles results in broadening of the resonance line due to exchange and anisotropic interactions[10]. The triplet signal is typical for a probe molecular solution in organic solvents (Fig. 1d).

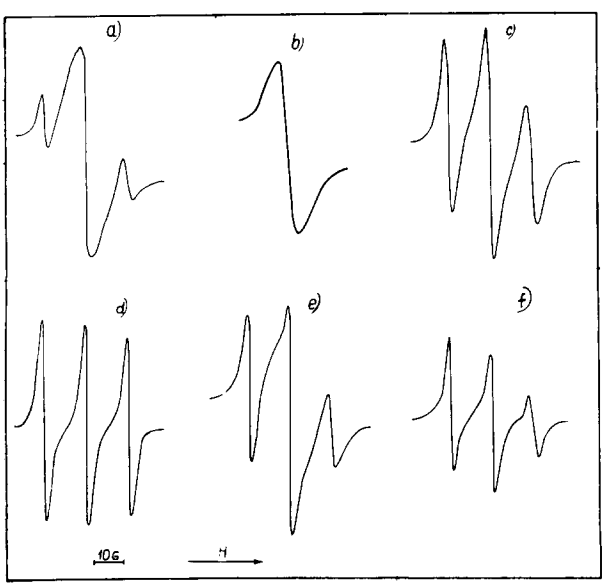


Fig. 1. ESR spectra of the probe. a, in submitochondrial particle suspension without ATP, pH 7.5; b, in water, 10^{-4} M of the probe; c, in submitochondrial particle suspension with 10 mM ATP, pH 7.5; d, in ethanolic solution, $1 \cdot 10^{-4}$ M of the probe; e, in submitochondrial particle suspension without ATP, pH 4.4; f, in mitochondrial suspension with 10 mM ATP, pH 7.5; In Exps. a, c, e and f the medium contains: 100 mM Tris–HCl (pH as pointed), 100 mM KCl, 10 mM $MgCl_2$, 0.25 M sucrose, 10 mM ferricyanide, 10 mg/ml mitochondrial protein, 10^{-4} M spin-probe, ATP as indicated.

One can suggest that the triplet part of the spectrum in Fig. 1a is exhibited by radicals dissolved in membrane lipoproteins.

In the presence of ATP the intensity of the triplet part of the spectrum increases while that of the singlet part decreases, thus indicating the improvement of the probe solubilization (Fig. 1c). The integral intensity of the spectrum does not change after energization.

Since the probe molecules have no electrical charge[11] the effect mentioned above can hardly be attributed to a redistribution of the probe between outside and inside of submitochondrial particles due to ATP-induced changes in ion transport. It seems much more probable that an enhancement of probe solubilization in membranes after ATP addition is due to a modification of the membrane structure resulting in increased acceptability of non-polar groups to the spin probe molecules.

From the Fig. 1e one can see that the acidification of the submitochondrial particles leads to similar changes as the exposition to ATP.

Since submitochondrial particles accumulate protons during energization the possibility arises that ATP acts on membrane conformation indirectly through the acidification of the internal space of submitochondrial particles. However, in intact mitochondria ATP causes a similar effect as in the case of submitochondrial particles (Fig. 1f) though mitochondria have an orientation opposite to that of internal membrane as compared with submitochondrial particles, and in this case protons are removed from the internal space[7]. The effect of ATP cannot be attributed to the acidification of the external space since there was no appreciable change in pH after the hydrolysis of ATP by mitochondria or submitochondrial particles. ADP does not cause any alteration in the ESR spectra of the probe bound to membranes. Preincubation of mitochondrial preparations with 2,4-dinitrophenol or oligomycin abolishes the ATP-induced conformational transitions.

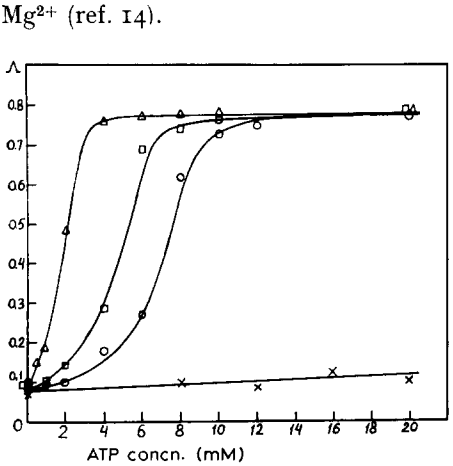
Thus the observed conformational transitions in membrane lipoproteins are directly caused by the energization.

It was found also that the ESR spectral changes induced by ATP are most pronounced at some optimal ratio of concentrations of the probe and the submitochondrial particle protein (10 nmoles of the radical per 1 mg of the submitochondrial particle protein). At a constant ratio of the probe and the submitochondrial particle protein concentrations the observed effect did not significantly change at varying the protein concentrations from 1 up to 20 mg/ml.

For the quantitative estimation of the ability of membrane preparations to solubilize the probe it is convenient to introduce a parameter of solubilization $A = I_s/I_c$. Here I_s is the integral intensity of a side component of the spectrum and I_c that of the central one. Both side components are equivalent because they are determined only by a triplet part of the spectrum[12]. The central component is the sum of the singlet from unsolubilized radicals and of the central component of the triplet from solubilized radical. Therefore A reflects a probe distribution between membrane and aqueous phases. It is clear that $A = 0$ for the probe in water and $A = 1$ for a molecular solution of the probe in organic solvents (Fig. 1).

Fig. 2 shows changes of A due to submitochondrial particle energization. The plot of A *versus* ATP concentration have a markedly sigmoidal shape (Fig. 2a). The Hill coefficient for the middle part of the curve is 6 (calculated according to CHANGEAUX AND PODLESKI[13]). A cooperative character of mitochondrial membrane tran-

sitions follows also from data obtained with dinitrophenol (Fig. 2b). Here the Hill coefficient is also 6. It is interesting that the same value of the Hill coefficient was calculated from the data on dinitrophenol-induced labilization of intramitochondrial Mg^{2+} (ref. 14).



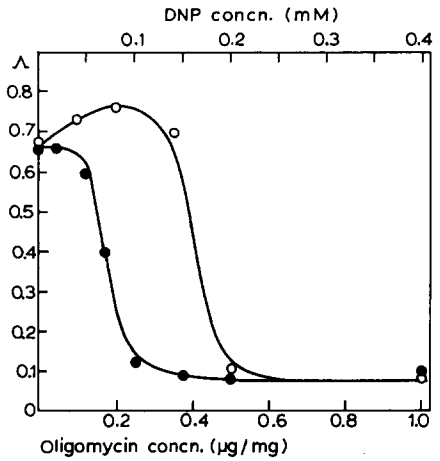


Fig. 2A. The parameter of solubilization of the probe in submitochondrial particle suspension as a function of ATP concentration. ○—○, without uncouplers; □—□, △—△ and ×—× in presence of different concentrations of oligomycin (0.1, 0.2 and 1.5 μg/mg of protein).

Fig. 2B. Plot of the parameter Λ *versus* dinitrophenol and oligomycin concentration in presence of 8 mM ATP. ○—○, oligomycin (μg/mg); ●—●, dinitrophenol (mM). The medium content as in Fig. 1, pH 7.5.

An interesting effect takes place in the case of oligomycin. At a concentration about 1 μg/mg of protein it inhibits the ATP-induced conformational transitions, while at lower concentrations (0.1–0.2 μg/mg) it promotes them. At activating concentrations oligomycin decreases the Hill coefficient for the action of ATP (as much as to 5 in the presence of 0.1 μg of oligomycin per mg of protein and to 4 in the presence of 0.2 μg of oligomycin per mg of protein). Such behaviour is typical for allosteric activators of biomembranes[13].

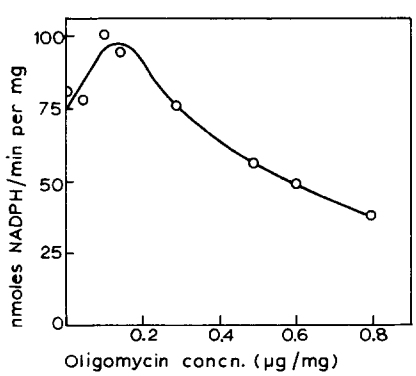


Fig. 3. Effect of oligomycin on the ATP-dependent transhydrogenase reaction. Medium contains: 0.25 M sucrose, 50 mM Tris–HCl (pH 7.5), 10 mM $MgSO_4$, 0.6% ethanol, 0.1 mg/ml alkohol-dehydrogenase, 5 mM NaCN, $1.7 \cdot 10^{-4}$ M $NADP^+$, $8 \cdot 10^{-5}$ M NAD^+, 2 mM ATP, 1 mg/ml submitochondrial particle protein. $NAD(P)^+$ reduction was followed fluorimetrically, excitation at 360 nm, recording at 460 nm.

At the same low concentrations oligomycin also activates the ATP-dependent transhydrogenase reaction in submitochondrial particles (Fig. 3; see also ref. 15).

The similarity between the curve representing the effect of oligomycin on ATP-dependent conformational transitions recorded by changing A, and the curve representing the effect of oligomycin on the transhydrogenase reaction indicates a close correlation between structural transitions in the membranes and changes in the activity of mitochondrial enzymes.

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