CONFORMATIONAL TRANSITION IN BIOLOGICAL MEMBRANE STUDYED BY THE SPIN LABEL METHOD

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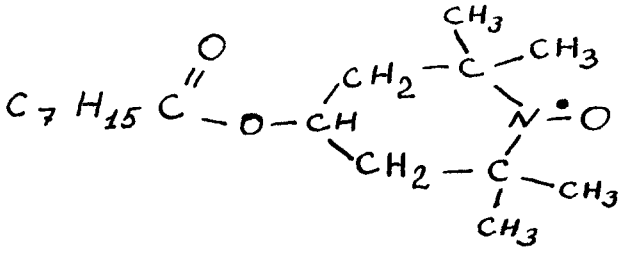
Abstract

Conformational transitions in electron transport particles (ETP) are recorded by variations in the electron spin resonance spectra of the spin label (2,2,6,6 - tetramethyl - 4 - piperidone-1-oxyl-4-caprilic ether) anisotropy. The spin is solubilized in ETP membrane and fixed by hydrophobic interactions. Conformational transition takes place after addition of oxidation substrates (succinate, NAD-H_2). Respiratory chain oxidation with ferricyanide restores the correlation time which is the ESR spectrum anisotropy quantitative measure, to its original value.

The weakly bound spin label method (Stone et al., 1965, Sukhorukov et all, 1967) has been used. for investigation of conformational transitions in electron transport particles (ETP) taken from bovin heart mitohondria by the well known technique (Crane et all, 1956). The free radical probe was 2,2,6,6-tetramethyl-4-piperidone-1-oxyl-4-caprilic ether (I)

I. 

I is water insoluble, but solubilises in ETP suspension. Conformational transitions, which take place by addition of oxidation substrate such as succinate, NAD-H_2, and back transition by oxidation of the respiratery chain with potassium ferrycianide were recorded by variations in the anisotropy of the electron spin resonance spectra iminoxyl radical, linked with membrane by hydrophobic interaction.

The correlation time, approximated by expression (Suchorukov, 1967):

$$\tau = -8{,}4\,\Delta H_{max(+1)}\left(1-\sqrt{\frac{I_{+1}}{I_{-1}}}\right)\cdot 10^{-10}\ \text{sec}$$

where $\Delta H_{max(+1)}$ - the distance between the maximal slope points of the ESR spectra component with $m_N = +1$ $I_{\pm 1}$ - intensities of components with $m_N = \pm 1$ respectively (Sukhorukov et al, 1967), is the ESR spectra anisotropy measure.

The applicability of this expression for investigation of systems of this kind is discussed elsewhere (Waggoner et al, 1967).

1 ml of the ETP suspension containing 30 mg of protein per ml was incubated with 0.01 ml of the radical 2.2×10^{-4} M ethanolic solution at 0°C. Then samples were taken and ESR spectra were recorded by an X-band ESR-spectrometer at room temperature.

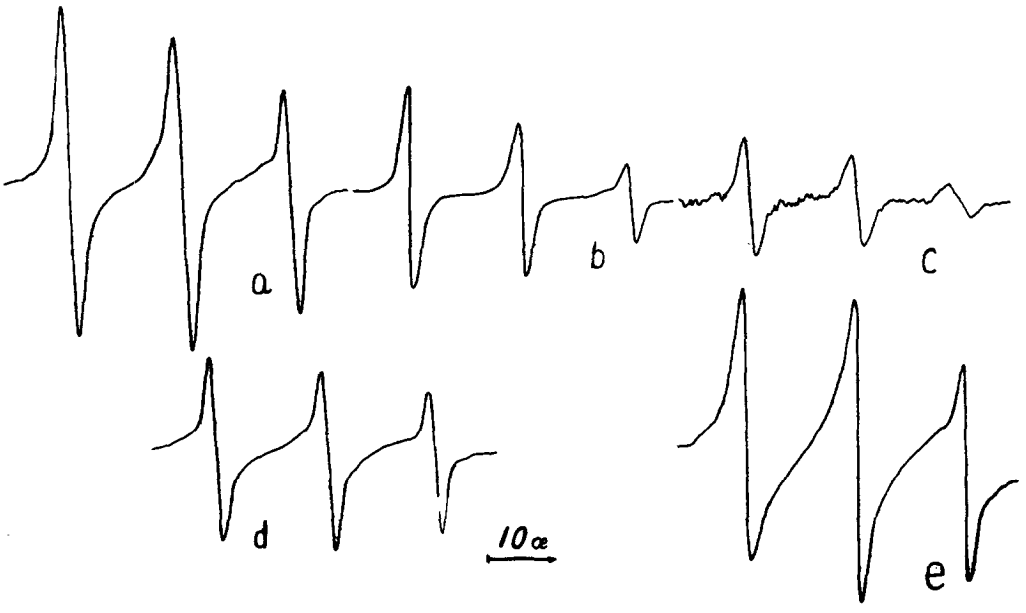


Fig. I. ESR spectra of I in ETP: a) oxidized ETP, after 5 min incubation with label; b) 3 min. after succinate addition; c) 12 min. after succinate addition: d) control sample spectrum - oxidized ETP after 20 min. incubation with label at 0°C. e) spectrum 3 min. after addition of $[Fe(CN)_6]$ to reduced ETP.

Typical results are shown in Fig.1. The iminoxyl ESR spectrum in ethanolic solution (10^{-4}M) is given for comparison in Fig.2.

Comparison of the signal shapes and the correlation times seem to show that ESR spectrum in oxidized ETP represents superposition of two signals, differing in anisotropy. The weak anisotropic signal is supposed to be due to radicals localised in the part of membrane of a rather large effective free volume. The strong anisotropic signal is exhibited by

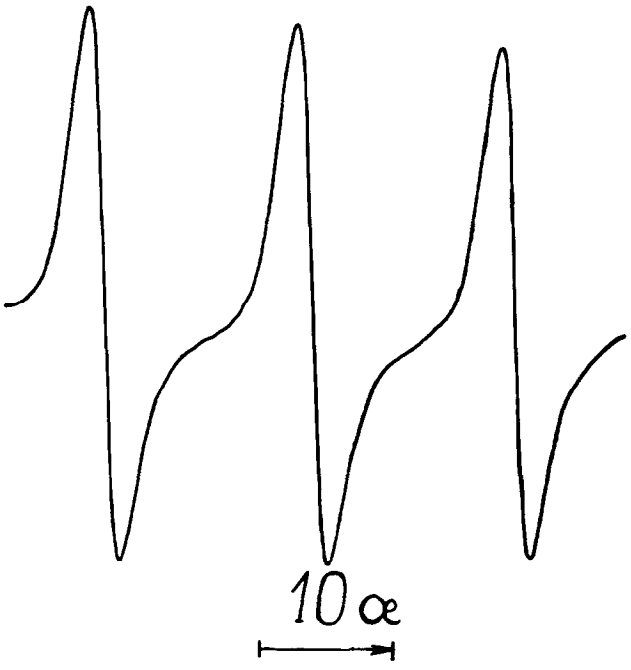


Fig. 2.
ESR spectrum of the radical (I) in ethanolic solution 10^{-4} M.

radicals localised in other membrane regions of a smaller free volume.

Cooperative conformational transitions accompany the reduction of respiratory chains with substrates. This results in anisotropy increase, due to increasing the fraction of small free volume regions ($\tau_{red} = 20 \times 10^{-10}$ sec against $\tau_{ox} = 4 \times 10^{-10}$ sec).

Oxidation with ferricyanide brings the system to its original state.

Besides decrease in the ESR spectra intensities, in consequence of label reduction to hydroxylamine, accompanies the respiratory chain reduction process. Addition of ferricyanide restores the signal.

Thus, there is an experimental confirmation of the idea of conformational transitions (Baum et al., 1967) accompanying the respiratory chain action.

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