TRANSFER OF ELECTRONS AND PROTONS AT THE DECANE/WATER INTERFACE IN THE PRESENCE OF CHLOROPHYLL

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1. Introduction

Earlier we carried out experiments in which we observed the action of enzymes at the interface, charging the double layer at the expense of the energy of the chemical reaction at the interface [1–8], or at the expense of light energy [3,6,8].

In a chloroplast membrane the electron and proton transfers are coupled, which, possibly, means that at least one of the membrane pumps contains molecules in which redox reactions involve participation of protons. In this connection, of greatest interest is the Krasnovsky reaction, which is observed only in a homogeneous phase [9].

In the present study the authors have attempted to investigate the Krasnovsky reaction under heterogeneous conditions, when chlorophyll is adsorbed at the *n*-decane/water interface. As in previous studies, the charge transfer processes in the double layer at the interface were assessed from the potential jump at the decane/water interface in Chain 1:

Au | Air | Decane, charge acceptor, chlorophyll | Water, substrate | Electrolytic bridge | Reference electrode (1)

2. Methods and reagents

The changes of the potential jump between water and decane were measured by the vibrating electrode method (for details, see [2,3]).

The interface was illuminated by a light source with a 100 W incandescent lamp. The spectral characteristics were measured by means of interference filters with 20 nm band width. The dependence of $\Delta\varphi$ on the light intensity was measured by means of neutral filters. On the figures the change of the Volta potential in Chain 1 was referred to the potential of the supporting electrolyte solution, which did not contain the component plotted on the abscissa.

All solutions were prepared with twice-distilled water and Tris-buffer 'Sigma'. The solution pH was adjusted with twice-distilled hydrochloric acid. Chlorophylls 'a + b' had been isolated from nettle and purified on a saccharose column by the technique described in [10]. The system decane/water with electron or proton acceptors and aqueous phase of definite pH was maintained in equilibrium for 24 h after the introduction of components. As can be seen from Chain 1, the components necessary for the effect to take place include chlorophyll (previously dissolved in decane), the charge acceptor in decane and the redox system NADH/$K_3Fe(CN)_6$, which were added to the aqueous phase immediately before the experiment. Chlorophyll dissolved in decane was also added to the cell some minutes before starting the Volta potential measurements in Chain 1. If one of the components was absent, no change of Volta potential was observed in spite of the interface being illuminated

Results and discussion

When the interface was illuminated in the presence of all necessary components, a change of the Volta

Abbreviations: A, electron acceptor; Chl, chlorophyll; DNP, 2,4 dinitrophenol.

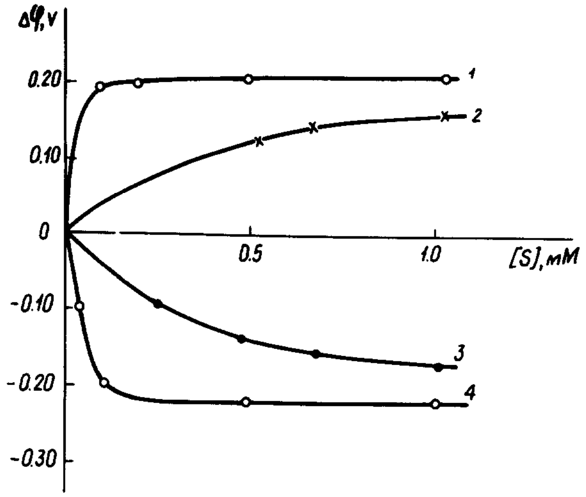

Fig.1. Dependence of the change of the Volta potential measured in Chain 1 on the concentration of substrate NADH (Curves 1,4) and ascorbate (2,3). The composition of the reaction medium: 10 μg/l chlorophyll, 2 × 10^{-2} M Tris-HCl, pH 7.4 and either (curves 1 and 2) 1 mM DNP, 1 mM $K_3Fe(CN)_6$, or (curves 3 and 4) 10^{-5} M 2-*N*-methylamino-1,4-naphthoquinone.

potential occurred in Chain 1, which depended on the concentrations of all reagents, the pH of the aqueous solution, the intensity and wavelength of incident light, but was independent of the buffer capacity of the solution (the concentration of the buffer Tris-HCl varying from 3 to 100 mM). This fact shows that the phenomena observed are not associated with the change of pH in the layer adjoining the interface. The optimum pH value of the aqueous fraction of the medium was 6.5–9.0.

Fig.1 gives the dependence of the potential jump at the decane/water interface in the presence of DNP as proton acceptor* under illumination (Curves 1,2) and, for comparison, without illumination (Curves 3,4) on the concentrations of the charge denor NADH (Curves 1,4) and potassium ascorbate (Curves 2,3). For the potential jump to take place in the absence of illumination, DNP was substituted by the electron acceptor 2-*N*-methylamino-1,4 naphthoquinone, or vitamin K_3 [4] **. Transfer of negative charges from water (w) to the part of the double layer adjoining the oil (o) phase is accompanied by an electrochemical reaction at the interface (s):

$$ {}^{w}(\mathrm{NADH}) + {}^{o}(\mathrm{A}) \xrightarrow{{}^{s}(\mathrm{Chl})} {}^{w}(\mathrm{NAD}) + {}^{o}(\mathrm{A}^-) + {}^{w}(\mathrm{H}^+) \quad (2) $$

The study of the dependence of the potential jump on the aqueous solution pH has shown that the potential jump has a maximum at pH = 6.5–9 and disappears in the acid and alkaline regions. As follows from eqn. (2), the reaction must be accompanied by proton ejection into the aqueous phase. Fig.2 gives the dependence of the Volta potential in Chain 1 on the incubation time of the reaction mixture at different buffer Tris-HCl concentrations. The aqueous phase pH was chosen so that acidification of the layer adjoining the interface should lead to inactivation of chlorophyll as the result of its pheophytinization. In a buffered solution this phenomenon is not observed.

Fig.3 shows the absorption spectra of chlorophyll in decane (Curve 3), the spectrum of the 'red' form of chlorophyll (borrowed from [11]) (Curve 1) and the spectral dependence of the Volta potential in Chain 1 (Curve 2). The spectral dependence of on the wavelength of incident light coincides with the absorption

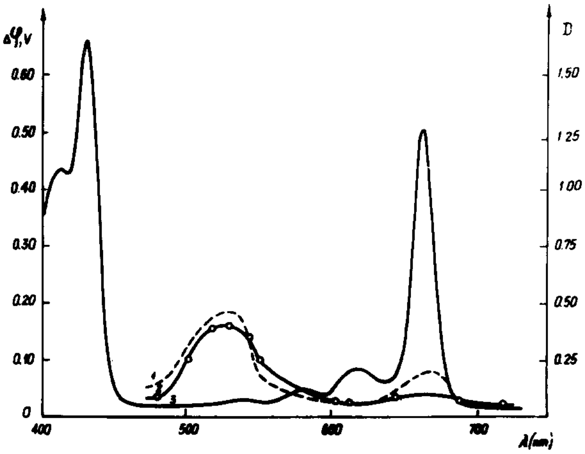

Fig.2. Dependence of the Volta potential change in Chain 1 on the incubation time of reaction mixture (Reaction 2). The medium contained 2 × 10^{-4} M NADH, 10^{-6} M chlorophyll, 10^{-4} M vitamin K_3, the following concentrations of Tris-HCl: (1) 10 mM; (2) 1 mM; (3) 0 mM. pH = 6.5.

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* There is no effect in the presence of electron acceptors alone.

** There is no effect in the presence of proton acceptors alone.

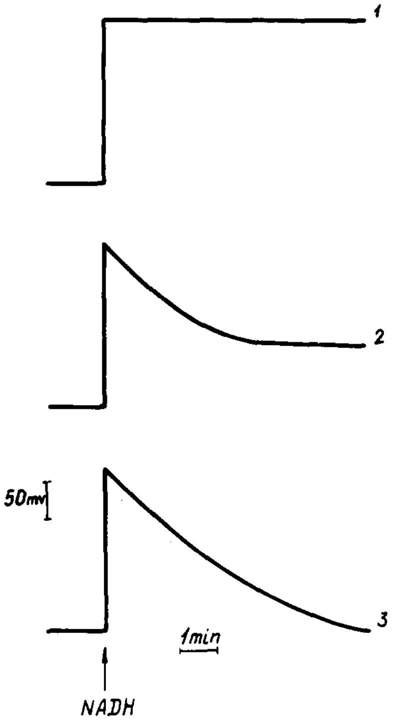

Fig.3. Dependence of the change of the potential jump at the interface and the absorption coefficient of chlorophyll solution in octane on the wavelength of incident light: (1) the absorption spectrum of the 'red' form of chlorophyll [11], (2) the spectral dependence of the Volta potential measured in Chain 1 (medium as in fig.1, Curve 4), (3) the absorption spectrum of initial chlorophyll dissolved in octane.

spectrum of the 'red' form of chlorophyll, the maximum value of $\Delta\varphi$ being observed at the incident light wavelength in the region of 530 nm. This coincidence points to the occurrence at the interface of a reaction of the type:

$$^{w}(DH_2) + {}^{s}(Chl) \xrightarrow{h\upsilon} {}^{s}(Chl.H_2) + {}^{w}(D) \qquad (3)$$

Since $Chl.H_2$ exists only in non-aqueous phases [11], we can conclude that Reaction (3) takes place in the part of the double layer between octane and water where the dielectric constant is lower than in the bulk of the water phase.

If the potential-forming stage of the reversible chlorophyll photoreduction reaction at the decane/water interface proceeds according to the equation:

$$(Chl.H_2)^{s} + (Acceptor)^{w} + (RO^-)^{o} \longrightarrow$$
$$(Chl)^{s} + (Acceptor^-)^{w} + (ROH)^{o} \qquad (4),$$

where RO^- and ROH are the ionized and protonated forms of proton acceptor, this means that this reaction takes place precisely at the interface because some of components are present in the water phase and others in the oil phase. It should be emphasized that the RO^- molecule should be located in the double layer in the part adjoining the nonaqueous phase since at the water–air interface no effect is observed.

In the heterogeneous system studied, adsorbed chlorophyll transforms light energy into electrical energy. This process is accompanied by a redox reaction, which results in proton transfer to the part of the double layer adjoining the non-aqueous phase. The data obtained show that at the interface between two immiscible liquids, chlorophyll can act either as a proton pump (under illumination), or as a system coupled with a redox reaction at the interface and causing a purely electron transfer (in the absence of illumination).

Chlorophyll photoreduction at the interface between two immiscible liquids is a reversible reaction, the time of the setting up of the potential jump (or its disappearance) not exceeding 2 sec.

Acknowledgements

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