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Membrane catalysis: synchronous multielectron reactions at the interface between two liquid phases. Bioenergetic mechanisms

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Kinetics of multi-electron reactions at the interface between two immiscible liquids are considered. Calculations of the energy of solvent reorganization, of the work required to bring reactants and reaction products together, and of the electrostatic contributions to the Gibbs free energy of the reaction during electron transfer between reactants which are in different dielectric media are reported. Conditions under which the free energy of activation of the interfacial reaction of electron transfer decreases are established. The influence of the distance between reactants and of the dielectric permittivity of the non-aqueous phase on the solvent reorganization energy value is studied. Conditions under which multielectron reactions at the interface proceed are discussed. The biophysics and biochemistry of photosynthesis and respiration are considered as examples of multielectron processes.

Introduction

The quantum theory of chemical reactions in polar media [1–15] can be used as the basis for a theory of charge transfer at the interface between two dielectric media – oil/water and biomembrane/water; by this theory one can express the electron transfer rate in terms of the dielectric properties of the medium and the characteristics, describing the electronic properties of reactants.

Charge transfer at the interface between two dielectric media is an important stage in the chemical processes taking place at biological membranes [16,17]. The investigations of electron exchange reactions at the liquid/liquid interface began to develop 60 years ago [18]. In present communication the kinetics and thermodynamics of a liquid/liquid interface are discussed.

The theoretical and experimental investigation of synchronous multielectron reactions at biomembranes has lately drawn the attention of chemists and biologists. The biotechnological application of multielectron reactions makes it possible to realize redox reactions in relatively mild conditions under the action of weak oxidants and reductants. Synchronous multielectron reactions may proceed without formation of intermediate

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Abbreviations: Chl or Mg=Ch=O, chlorophyll; P-680, primary electron donor, special Chl *a* molecule; Pheo, pheophytin; PQ, plastoquinone electron acceptor; Q_a, bound plastoquinone electron acceptor; Q_b, second bound plastoquinone electron acceptor; RC, reaction center; S_n, state of oxygen-evolving complex; Z, plastoquinol electron donor; LHC, light-harvesting complex; A_i, bound acceptors of PS I which have the nature of Fe-S centers; Fd, ferredoxin; C, capacitance; R, resistance; E_s, solvent reorganization energy; E_a, free energy of activation; h_i, distance from interface.

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radicals, which are highly reactive and can readily enter into a side reaction of hydroxylation, oxidation and destruction of the catalytic complex or photosensitizer.

Since multielectron reactions do not poison the environment with toxic intermediates (and so are ecologically pure), they are used by nature for biochemical energy conversion in photosynthesis and respiration. Thus, for example, oxygen photoevolution from water due to solar energy may proceed through concerted 4- or 2:2-electron transfer mechanisms in the reaction center of the Photosystem II of chloroplasts or in model systems [19–27]. About 90% of the oxygen consumed on Earth is reduced in a four-electron transfer reaction by cytochrome-*c* oxidase (ferrocytochrome-*c*:oxygen oxidoreductase, EC 1.9.3.1) catalysis.

Thermodynamics of a heterogeneous reaction

Let us assume the following redox reaction takes place in a heterogeneous system:

$$\text{red} \rightleftarrows \text{ox} + ne \qquad (1)$$

The electrons which are the products of reaction (1) may be accepted by some other substances. The standard Gibbs energy of reaction (1) for each phase, I and II, is:

$$\Delta G^\circ_{\text{I}} = {}_{\text{I}}\mu^\circ_{\text{red}} - {}_{\text{I}}\mu^\circ_{\text{ox}} - n\mu^\circ_{\text{e}} \qquad (2)$$

$$\Delta G^\circ_{\text{II}} = {}_{\text{II}}\mu^\circ_{\text{red}} - {}_{\text{II}}\mu^\circ_{\text{ox}} - n\mu^\circ_{\text{e}} \qquad (3)$$

Subtraction of Eqn. 2 from Eqn. 3 gives the change in the standard Gibbs energy at the interface if the electron acceptor is in one phase only, or if it is localized at the phase boundary:

$$\Delta G^\circ_{\text{II}} - \Delta G^\circ_{\text{I}} = ({}_{\text{II}}\mu^\circ_{\text{red}} - {}_{\text{I}}\mu^\circ_{\text{red}}) - ({}_{\text{II}}\mu^\circ_{\text{ox}} - {}_{\text{I}}\mu^\circ_{\text{ox}}) \qquad (4)$$

or in the general case:

$$\Delta G^\circ = RT \ln \frac{B_{\text{red}}}{B_{\text{ox}}} \qquad (5)$$

where B_i is the distribution coefficient of the ith ion:

$$RT \ln B_i = {}_{\text{II}}\mu^\circ_i - {}_{\text{I}}\mu^\circ_i$$

In the case of a multielectron reaction, the standard redox potential at the interface is determined by:

$$\Delta E^\circ = \frac{RT}{nF} \ln \frac{B_{\text{red}}}{B_{\text{ox}}} \qquad (6)$$

where ΔE° is the change in the standard redox potential at the interface, and n is the number of electrons.

The change of the standard Gibbs energy at the liquid/liquid interface does not depend on the manner in which the reaction proceeds (multistage one-electron or synchronous multielectron charge transfer). It depends on the initial and final states of the system.

Thus, by selecting a corresponding solvent, we can shift the redox potential scale in the necessary direction and carry out the reactions that occur hardly at all in a homogeneous phase.

If the resolvation energies of substrates and products are very different, the phase boundary of two immiscible liquids may act as a catalyst.

Thus, for example, in Refs. 17–23 the four-electron reaction of water photooxidation sensitized by chlorophyll is described. At the octane/water interface, the ΔE° shift calculated from Eqn. 6 in the water photooxidation reaction amounts to 0.1 V, which makes the participation of chlorophyll cation in the water photooxidation reaction possible. At the nitrobenzene/water interface, this shift is equal to 0.03 V. This difference in ΔE° leads to a 10-fold increase of the equilibrium constant of the water oxidation reaction at the octane/water interface as compared to that at the nitrobenzene/water phase boundary.

For one-electron reactions the shift of ΔE° is n-times more, and results in a sharp increase of the reaction rate constant.

In the multielectron reaction which takes place in a series of consecutive one-electron stages, the Gibbs energy necessary per single electron transfer obviously cannot be completely uniformly distributed over the stages. The energy needs for various stages will be different and the excess energy in the easier stages will be converted into heat. In a synchronous multielectron reaction the energy will be utilized very economically [26].

Charge transfer kinetics at the interface

The rate constant of charge transfer across the interface between two immiscible liquids was calculated in Refs. 11, 15, 28. Thus, allowing for interaction between the reactants, one can write the expression for the probability of electron transfer as

$$W = A \exp\left\{ -\frac{U_{\mathrm{i}}}{kT} - \frac{[E_{\mathrm{s}} + I_{\mathrm{f}}^{\circ} - I_{\mathrm{i}}^{\circ} + U_{\mathrm{f}} - U_{\mathrm{i}}]^2}{4E_{\mathrm{s}}kT} \right\} \tag{7}$$

where U_{i} is the work which must be performed upon the system in order to attain the reaction configuration where the reactants are at distances h_1 and h_2 from the interface, U_{f} is the corresponding work for the reaction products, I_{f}° and I_{i}° are the Gibbs energies of the reaction products and reactants when they are at a finite distance from the interface, E_{s} is the solvent reorganization energy, and A is the pre-exponential factor, which is proportional to the transmission coefficient.

The transmission coefficient, κ, of the reaction for a nonadiabatic process is proportional to the square of the electronic matrix element. Theoretical analysis shows [1,2,5] that the most effective electron transfer takes place at a close (practically contact) disposition of reaction centers. If κ is smaller than unity, the process may be considered as nonadiabatic. The transmission coefficient for n-electron transfer in this case also corresponds to a nonadiabatic process and may be substantially lower with respect to κ for a one-electron process. At the same time the activation factors for one- and multi-electron processes may differ essentially. The main attention of the present work will be centered on the investigation of the dependence of the expression for the activation factor for the charge transfer probability through the interface upon the system parameters.

For simplicity we shall limit the consideration of total reorganization energy by the exclusive consideration of the solvent reorganization energy, while the effects of molecular configurational changes and modifications of the first ionic coordination layer will be neglected.

The Gibbs free energy for substrates and products may be found using the Born relation

$$I_{\mathrm{i}}^{\circ} = \tilde{I}_{\mathrm{i}}^{\circ} + \frac{z_1^2 e^2}{2\epsilon_1 a} + \frac{z_2^2 e^2}{2\epsilon_2 b}, \tag{8}$$

$$I_{\mathrm{f}}^{\circ} = \tilde{I}_{\mathrm{f}}^{\circ} + \frac{(z_1 + n)^2 e^2}{2\epsilon_1 a} + \frac{(z_2 - n)^2 e^2}{2b\epsilon_2}, \tag{9}$$

where $\tilde{I}_{\mathrm{f}}^{\circ}$ and $\tilde{I}_{\mathrm{i}}^{\circ}$ are components which do not depend on the dielectric properties of media I and II.

Using Eqns. 8 and 9, the value for $I_{\mathrm{f}}^{\circ} - I_{\mathrm{i}}^{\circ}$ in Eqn. 7 may be written as:

$$I_{\mathrm{f}}^{\circ} - I_{\mathrm{i}}^{\circ} = \tilde{I}_{\mathrm{f}}^{\circ} - \tilde{I}_{\mathrm{i}}^{\circ} + e^2\left(\frac{n^2 + 2z_1 n}{2\epsilon_1 a} + \frac{n^2 - 2nz_2}{2\epsilon_2 b} \right) \tag{10}$$

The calculation of the electrostatic contribution to the solvation Gibbs free energy within the framework of the simple Born model has rather a qualitative than a quantitative character. More accurate calculation can be performed using non-local electrostatics; see, for example, Refs. 29, 30.

The solvent reorganization energy is an important parameter in the quantum theory of the elementary act of charge transfer in polar media,

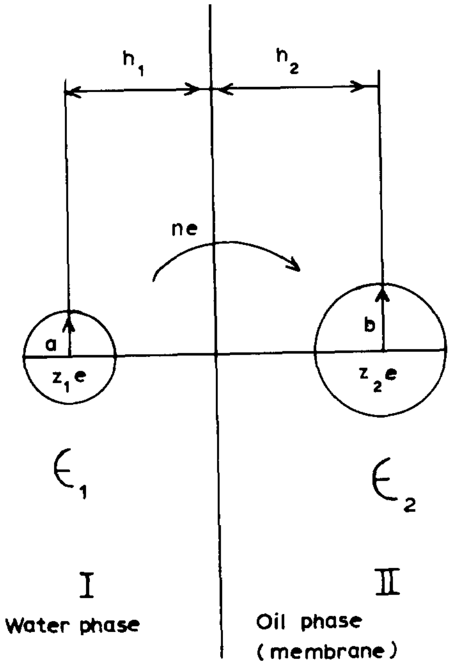


Fig. 1. Scheme of location of charge donors and acceptors at interface.

and in the case of homogeneous reactions which take place in one phase it can be described in the long-wave approximation by the relation:

$$E_s = \frac{1}{8\pi}\left(\frac{1}{\epsilon_{op}} - \frac{1}{\epsilon_s}\right) \int_{\infty - V_a - V_b} (\boldsymbol{D}_i - \boldsymbol{D}_f)^2 \, dV \qquad (11)$$

where ϵ_{op} and ϵ_s are the optical and the static dielectric permittivities of the medium, and D_i and D_f are the inductions of the electric field which are created in the solvent by the charge being transferred in the initial and final state. Integration in Eqn. 11 is carried out over the entire volume of the reactants.

In the case where the charge transfer occurs between reactants which are in two different dielectric media the following expression can serve as a generalization of Eqn. 11 [28]:

$$\begin{aligned} E_s &= \frac{1}{8\pi} \int_{\infty - V_a - V_b} dV \left(\frac{1}{\epsilon_{op}(r)} - \frac{1}{\epsilon_s(r)}\right)(\boldsymbol{D}_i - \boldsymbol{D}_f)^2 \\ &= \frac{1}{8\pi}\left(\frac{1}{\epsilon_{op1}} - \frac{1}{\epsilon_{s1}}\right)\int_{I} (\boldsymbol{D}_i - \boldsymbol{D}_f)^2 \, dV \\ &\quad + \frac{1}{8\pi}\left(\frac{1}{\epsilon_{op2}} - \frac{1}{\epsilon_{s2}}\right)\int_{II} (\boldsymbol{D}_i - \boldsymbol{D}_f)^2 \, dV \\ &= \frac{1}{8\pi}\left(\frac{1}{\epsilon_{op1}} - \frac{1}{\epsilon_{s1}}\right) I_1 + \frac{1}{8\pi}\left(\frac{1}{\epsilon_{op2}} - \frac{1}{\epsilon_{s2}}\right) I_2 \qquad (12) \end{aligned}$$

where subscripts 1 and 2 denote the dielectric permittivities in media 1 and 2, and the integration ranges I and II represent the two half-spaces of media 1 and 2 excluding the volumes of reactants; the latter we shall describe as spheres of radii a and b which lie at distances h_1 and h_2 from the interface with charges $z_1 e$ and $z_2 e$, respectively (Fig. 1).

Expression (12) for the reorganization energy corresponds to neglect of the spatial dispersion of dielectric permeability. We use here the calculations, based on local electrostatics, since there are no calculations of the reorganization energy at present based on the nonlocal theory even for the simplest case of homogeneous charge transfer.

In terms of the Marcus approximation (see, for example, Ref. 8) we obtain:

$$\begin{aligned} E_s = (ne)^2 &\left(\frac{1}{\epsilon_{op1}} - \frac{1}{\epsilon_{s1}}\right)\left\{\frac{1}{2a} - \frac{1}{4h_1}\right. \\ &\left. + \left(\frac{2\epsilon_1}{\epsilon_1 + \epsilon_2}\right)^2 \left(\frac{1}{8h_1} + \frac{1}{8h_2} - \frac{1}{2(h_1 + h_2)}\right)\right\} \\ + (ne)^2 &\left(\frac{1}{\epsilon_{op2}} - \frac{1}{\epsilon_{s2}}\right)\left\{\frac{1}{2b} - \frac{1}{4h_2}\right. \\ &\left. + \left(\frac{2\epsilon_2}{\epsilon_1 + \epsilon_2}\right)^2 \left(\frac{1}{8h_1} + \frac{1}{8h_2} - \frac{1}{2(h_1 + h_2)}\right)\right\} \qquad (13) \end{aligned}$$

where ne is the charge transferred in the reaction. Expression (13) is obtained with an accuracy to the term of the order $(a/h)^3$ exclusive.

Similarly, U_i and U_f can be expressed in terms of integrals of inductions D_i and D_f:

$$U_i = \frac{1}{8\pi\epsilon_1}\int_{I} \boldsymbol{D}_i^2 \, dV + \frac{1}{8\pi\epsilon_2}\int_{II} \boldsymbol{D}_i^2 \, dV - \frac{z_i^2 e^2}{2a\epsilon_1} - \frac{z_2^2 e^2}{2b\epsilon_2} \qquad (14)$$

$$\begin{aligned} U_f = &\frac{1}{8\pi\epsilon_1}\int_{I} \boldsymbol{D}_f^2 \, dV + \frac{1}{8\pi\epsilon_2}\int_{II} \boldsymbol{D}_f^2 \, dV \\ &- \frac{(z_1 + n)^2 e^2}{2a\epsilon_1} - \frac{(z_2 - n)^2 e^2}{2b\epsilon_2} \qquad (15) \end{aligned}$$

Calculation of the integrals in Eqns. 5 and 6 is most conveniently carried out by changing to surface integrals. As a result we obtain for $\int_I \boldsymbol{D}_i^2 \, dV$ with the accuracy to $(a/h)^3$, $(b/h)^3$:

$$\begin{aligned} \int_{I} \boldsymbol{D}_i^2 \, dV = &\frac{4\pi e^2 z_1^2}{a} - \frac{2\pi e^2 z_1^2}{h_1} \\ &+ \pi e^2 \left(\frac{2\epsilon_1}{\epsilon_1 + \epsilon_2}\right)^2 \left(\frac{z_1^2}{h_1} + \frac{z_2^2}{h_2} + \frac{4 z_1 z_2}{h_1 + h_2}\right) \qquad (16) \end{aligned}$$

The expression for $\int_{II} \boldsymbol{D}_i^2 \, dV$ is obtained from Eqn. 16 by making the substitutions $a \rightarrow b$, $\epsilon_1 \rightarrow \epsilon_2$, $h_1 \rightarrow h_2$ and $z_1 \rightarrow z_2$. The corresponding expression for $\int_{I+II} \boldsymbol{D}_f^2$ is obtained by making the substitutions $z_1 \rightarrow (z_1 + n)$ and $z_2 \rightarrow (z_2 - n)$.

The simplest expressions for E_s, U_i and U_f are obtained in the case when the reactions take place at equal distances from the interface, $h_1 = h_2 = h$:

$$E_s = n^2e^2\left(\frac{1}{\epsilon_{\text{op1}}} - \frac{1}{\epsilon_1}\right)\left(\frac{1}{2a} - \frac{1}{4h}\right)$$

$$+ n^2e^2\left(\frac{1}{\epsilon_{\text{op2}}} - \frac{1}{\epsilon_2}\right)\left(\frac{1}{2b} - \frac{1}{4h}\right) \qquad (17)$$

$$U_i = \frac{z_1z_2e^2}{(\epsilon_1 + \epsilon_2)h} + \frac{z_1^2e^2(\epsilon_1 - \epsilon_2)}{4\epsilon_1(\epsilon_1 + \epsilon_2)h} + \frac{z_2^2e^2(\epsilon_2 - \epsilon_1)}{4\epsilon_2(\epsilon_1 + \epsilon_2)h} \qquad (18)$$

$$U_f = \frac{(z_1 + n)(z_2 - n)e^2}{(\epsilon_1 + \epsilon_2)h} + \frac{(z_1 + n)^2e^2(\epsilon_1 - \epsilon_2)}{4\epsilon_1(\epsilon_1 + \epsilon_2)h}$$

$$+ \frac{(z_2 - n)^2e^2(\epsilon_2 - \epsilon_1)}{4\epsilon_2(\epsilon_1 + \epsilon_2)h} \qquad (19)$$

In the case of homogeneous electron transfer in a dielectric medium of constant ϵ_{st}, the work required to bring the reactants or reaction products together became zero when one of the reactants or products was electrically neutral, whereas in the process discussed here, U_i values are always different from zero because of the interactions with the image charges.

The activation energy of electron transfer can change differently depending on the charges of the reactants and dielectric permittivity of the nonaqueous phase. This can be used when choosing a pair of immiscible liquids, in order to decrease the activation energy of the reaction in question or to inhibit an undesired process.

Let us consider some examples. Let an electron be transferred from the donor in aqueous phase 1 to the acceptor in organic phase 2 (Fig. 1). Let us discuss the character of the dependence of the free energy activation, E_a, upon the dielectric permittivity of nonaqueous phase, ϵ_2:

$$E_a = U_i + \frac{(E_s + I_f^\circ - I_i^\circ + U_f - U_i)^2}{4E_s} \qquad (20)$$

where U_i, U_f and E_s are determined by Eqns. 17, 18 and 19. The reorganization energy increases with ϵ_2, acquiring the maximum asymptotic value at $\epsilon_2 \gg \epsilon_{\text{op2}}$:

$$E_s^{\max} = n^2e^2\left(\frac{1}{2a} - \frac{1}{4h}\right)\left(\frac{1}{\epsilon_{\text{op1}}} - \frac{1}{\epsilon_1}\right) + n^2e^2\left(\frac{1}{2b} - \frac{1}{4h}\right)\frac{1}{\epsilon_{\text{op2}}} \qquad (21)$$

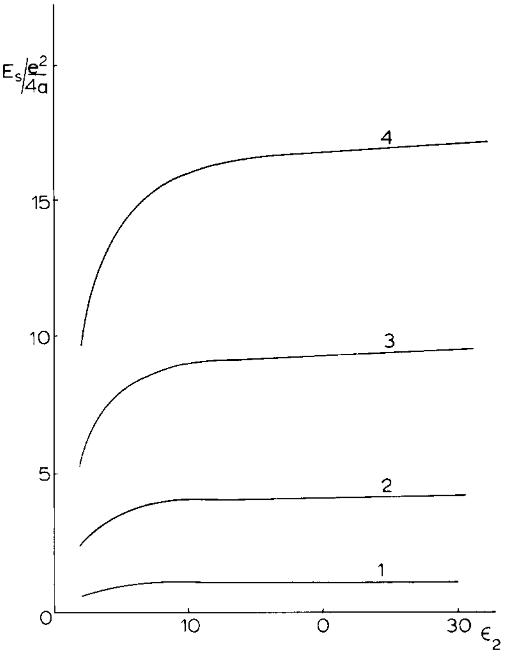


Fig. 2. Dependence of solvent reorganization energy on dielectric permittivity of nonaqueous phase ϵ_2 at parameters: $\epsilon_{\text{op1}} = \epsilon_{\text{op2}} = 1.8$, $\epsilon_1 = 80$, $a = b = h$, and $n = 1$ (1), $n = 2$ (2), $n = 3$ (3) and $n = 4$ (4).

and at $\epsilon_2 = \epsilon_{\text{op2}}$ becoming minimal and equal to:

$$E_s^{\min} = n^2e^2\left(\frac{1}{2a} - \frac{1}{4h}\right)\left(\frac{1}{\epsilon_{\text{op1}}} - \frac{1}{\epsilon_1}\right) \qquad (22)$$

Keeping in mind that the first two terms in Eqns. 18 and 19 are small, since $\epsilon_1 = 80 \gg 1$, the dependences of U_i and U_f on ϵ_2 are determined mainly by the third term and, in contrast to E_s, depend not only on the value of the charge being transferred but also on the values of the reactant changes proper. Examples of the dependences of E_s, E_a and U_i for different sets of parameters z_1, z_2 and h/a are presented in Figs. 2, 3 and 4.

Eqn. 17 shows that in synchronous multielectron reactions the solvent reorganization energy increases n^2 times as compared to E_s in one-electron processes, which leads to a significant rise in the free energy activation (Fig. 2). At the same time there are reactions for which the multielectron mechanism is thermodynamically preferable. For instance, the reaction of water photooxidation in the reaction center of Photosystem II [23–25],

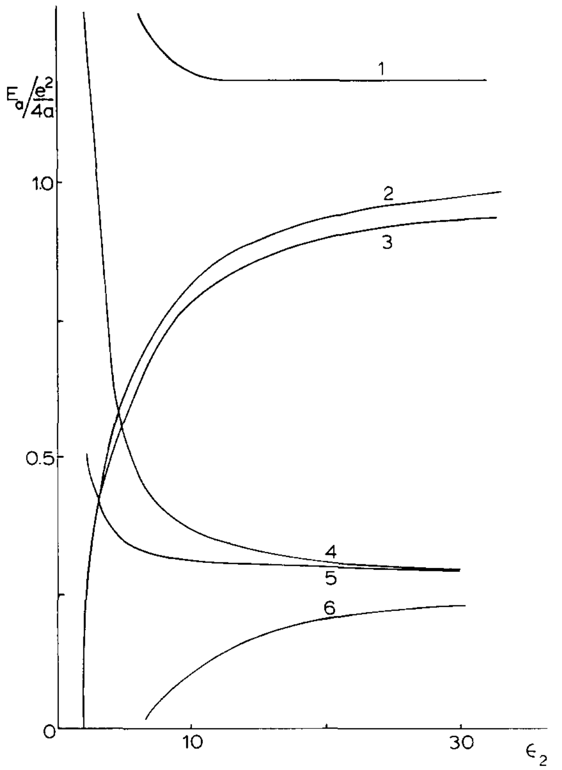


Fig. 3. Dependence of the activation energy, E_a, on dielectric permittivity of a nonaqueous phase, ϵ_2, calculated by Eqns. 10, 17–20) with parameters: $a=b=h$; $n=1$ (3–6), $n=2$ (1, 2) and (1, 5): $z_2=0$, $z_1=-1$, $\Delta\tilde{I}=0$; (2–4, 6): $z_1=0$, $z_2=1$, $\Delta\tilde{I}=(n^2e^2)/4a$ (3); $\Delta\tilde{I}=-2.2(n^2e^2)/4a$ (4); $\Delta\tilde{I}=0.7(n^2e^2)/4a$ (2); $\Delta\tilde{I}=0$.

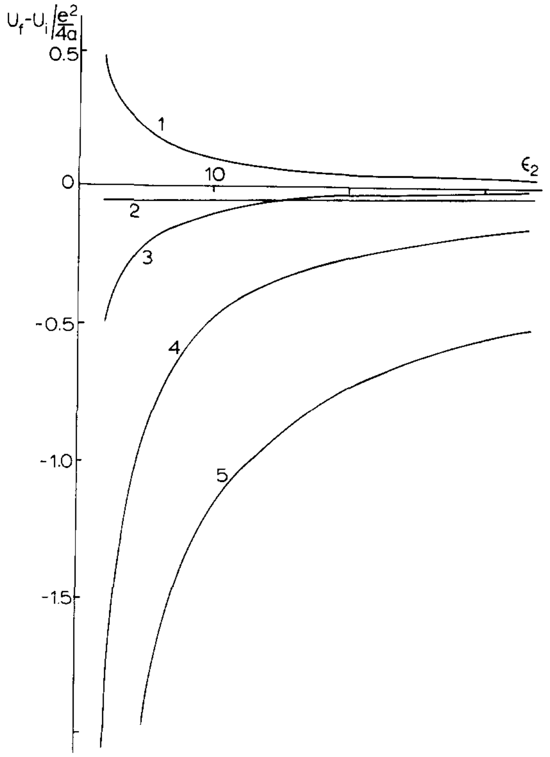


Fig. 4. Dependence of U_f-U_i on dielectric permittivity of nonaqueous phase ϵ_2 at parameters: (1) $z_2=0$, $z_1=-1$, $n=1$; (2) $z_2=0$, $z_1=1$, $n=2$; (3) $z_2=1$, $z_1=0$, $n=1$; (4) $z_2=1$, $z_1=0$, $n=2$; (5) $z_2=1$, $z_1=0$, $n=4$.

water photooxidation at the octane/water interface, sensitized by chlorophyll [19–22,31], reduction of NAD$^+$ and NADP$^+$ [32–34], reduction of nitrogen and oxygen, catalysed by nitrogenase [11–14,26] and cytochrome-*c* oxidase, are multielectron processes. The conditions for decreasing the activation energy are examined in Refs. 12–14, 25, 35–37. In multielectron processes the charge being transferred can be redistributed among a number of reaction centers. Calculation of the solvent reorganization energy in reactions with complex charge redistribution in reactants was discussed in Refs. 35–37.

Eqn. 19 shows that, in contrast to homogeneous reactions, U_f can contribute greatly to the activation energy of redox reactions at the oil/water interface at small ϵ_2. This contribution is comparable with E_s at $z_2=0$ and even exceeds E_s at $z_2 \leqslant -1$. At $z_2>0$ the U_f contribution to the activation energy is much less than that of the solvent reorganization energy.

It is clear from Fig. 3 that the activation energy of the process decreases (increases) greatly at small ϵ_2 and, accordingly, the rate constant of charge transfer across the interface increases (decreases) sharply at relatively small ϵ_2.

The influence of distance h between the reactants on the value of the activation energy is shown in Fig. 5, where the case $a=b$, $a \leqslant h < \infty$ is considered. When the relation h/a decreases, the activation energy diminishes and at $a=h$ it becomes minimal. This leads to significant intensification of the process when the ions are in contact with the interface.

Fig. 6 shows the influence of the relation of the reagents' radii on the value of the activation energy.

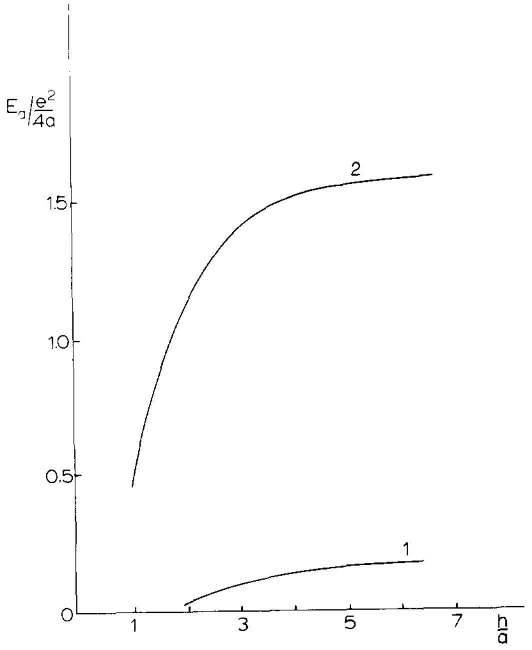


Fig. 5. Dependence of activation energy on distance h at $a = b$, $\epsilon_{\text{op1}} = \epsilon_{\text{op2}} = 1.8$, $\epsilon_2 = 4$, $\epsilon_1 = 80$, $z_1 = 0$, $z_2 = 1$ and $n = 1$ (1), $n = 2$ (2).

Thus, the analysis given here shows that the kinetics of interfacial charge transfer depends to a great extent upon the value of the charge being transferred, the charges of reactants, their location in relation to the interface, as well as upon the dielectric properties of the media in contact.

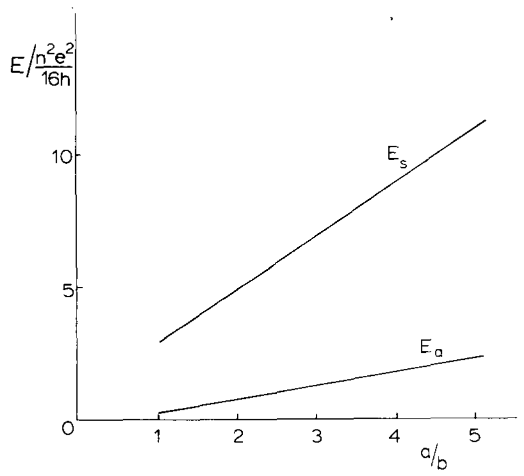


Fig. 6. Dependence of solvent reorganization energy, E_s, and activation energy, E_a, on the ratio a/b at parameters: $\epsilon_{\text{op1}} = \epsilon_{\text{op2}} = 1.8$, $\epsilon_1 = 80$, $\epsilon_2 = 4$, $z_2 = 1$, $z_1 = 0$.

The equations given below describe the charge transfer process at the oil/water interface within the framework of the simplest model and can be used as a basis for the description of more complicated processes in membrane, micellar and emulsion catalysis as well as bioenergetic processes taking place on the surface of the membranes of mitochondria, bacteria and chloroplasts.

Let us consider some examples of the most important biochemical processes transforming energy in living systems through multielectron mechanisms.

Cytochrome oxidase is a multielectron H$^+$ pump

Cytochrome oxidase (EC 1.9.3.1) is the terminal member of the respiratory chain catalyzing the reduction of dioxygen to water by ferrocytochrome *c*. This function has been demonstrated to be linked to proton translocation across the inner mitochondrial membrane [38].

It was shown in Refs. 12, 14, 38 and 39 that in native conditions cytochrome oxidase may reduce oxygen by the multielectron pathways. Fig. 7 shows schematically the energetics of possible reaction pathways:

$$4e + 4\,H^+ + O_2 \rightleftarrows 2\,H_2O \qquad (23)$$

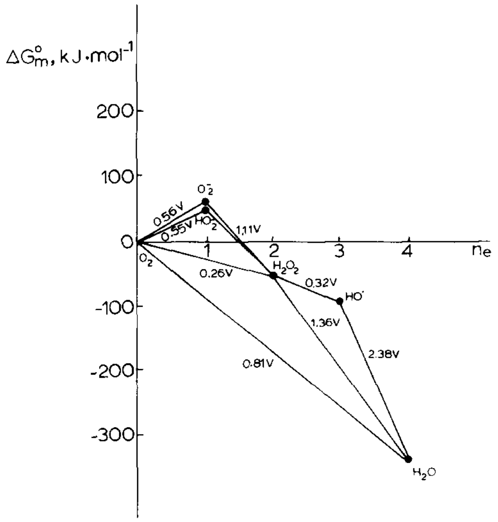
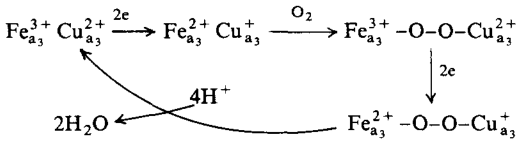
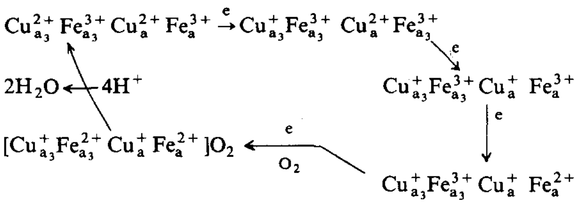


Fig. 7. Energy diagram of possible routes of the reaction $O_2 + 4H^+ + 4e \rightleftarrows 2\ H_2O$. Abbreviations: G°_m, midpoint free Gibbs energy; n_e, number of electrons.

Fig. 7 shows that only two mechanisms – 2 : 2 electron



and 4-electron



can take place without production of highly reactive intermediates which can destroy the biomembrane and the enzyme itself. In 2 : 1 : 1 electron transfer mechanism, the reduction of O_2^{2-} can proceed through a radical intermediate. Neither is hydrogen peroxide a desirable substance for the biomembrane – it may initiate peroxide oxidation of the membrane. Evolution could preserve the 2 : 2- and 4-electron mechanisms in which the intermediate is strongly bound in the catalytic center.

The dependence of the rate of oxygen reduction by cytochrome oxidase on temperature is investigated in Ref. 40. The activation energy of dioxygen reduction by fully reduced oxidase at temperatures between 23 and 35 °C is 15.9 kJ/mol. The low value of E_a shows that, in vivo, one-electron mechanisms of O_2 reduction, 1 : 1 : 1 : 1, 1 : 2 : 1, 1 : 3 and 1 : 1 : 2, are impossible, since the heat of the endothermal reaction of the addition of the first electron to dioxygen is several times greater than the measured activation energy (even with account of the bonding energy of dioxygen with enzymes, which is equal to 21 kJ/mol [41]). Therefore, in the first step of the reaction two or four electrons must be synchronously transferred from enzyme to dioxygen. So, in vivo, 2 : 2- and 4-electron pathways of dioxygen reduction by cytochrome oxidase are possible. The energy released may be dissipated into heat or used for proton transfer through the mitochondrial membrane. The endothermicity of the step of the addition of the first electron to O_2 is the reason for the relative chemical inertia of dioxygen in nature. This is the reason for the existence of life on Earth.

Oxygen evolution in photosynthesis

The redox map of photosynthesis can be described by the well-known Z-scheme of photosynthesis (Fig. 8). The molecular organization of the thylakoid membrane and its equivalent electrical circuit are shown in Fig. 9. The molecular mechanism of the photoevolution of oxygen from water is the key problem of the bioelectrochemistry of photosynthesis.

Oxidation of water to molecular dioxygen is, seemingly, a multielectron process provided that the reaction proceeds with high quantum efficiency [21]. This process can be realized in a variety of different reactions involving a Mn-cluster $Mn_4^{k+}(H_2O)_2$ [23–25,41], a chlorophyll cluster [21,22] or one chlorophyll dimer and two manganese ions bound into a multicentered complex [23]. In the present communication we consider only the first system.

Fig. 7 shows schematically the energetics of reaction [23]. O_2 evolution in photosynthesis takes place under illumination at the wavelength 680 nm, which excludes the thermodynamic possibility for one-electron reactions to proceed; a stronger oxidant than the cation radical P-680$^+$ is needed. Thus, the only possible route is the process of four-electron oxidation of water to molecular oxygen. That is why we do not discuss consecutive one-electron mechanisms. It should be mentioned that one-, two- and three-electron reactions are thermodynamically possible if the intermediate particles formed are in the adsorbed state as cryptoradicals, but such particles have not been discovered in vivo.

The possibility of four-electron [19–26,42] and two-electron [27] mechanisms of oxygen evolution in photosynthesis has already been discussed in literature.

A possible molecular mechanism of water photooxidation in photosynthesis by an Mn-cluster is shown in Fig. 10. The cation radical P-680$^+$ oxidizes the Mn-ions in a series of consecutive one-electron stages. Each single-turnover flash induces separation of charges, transforming the re-

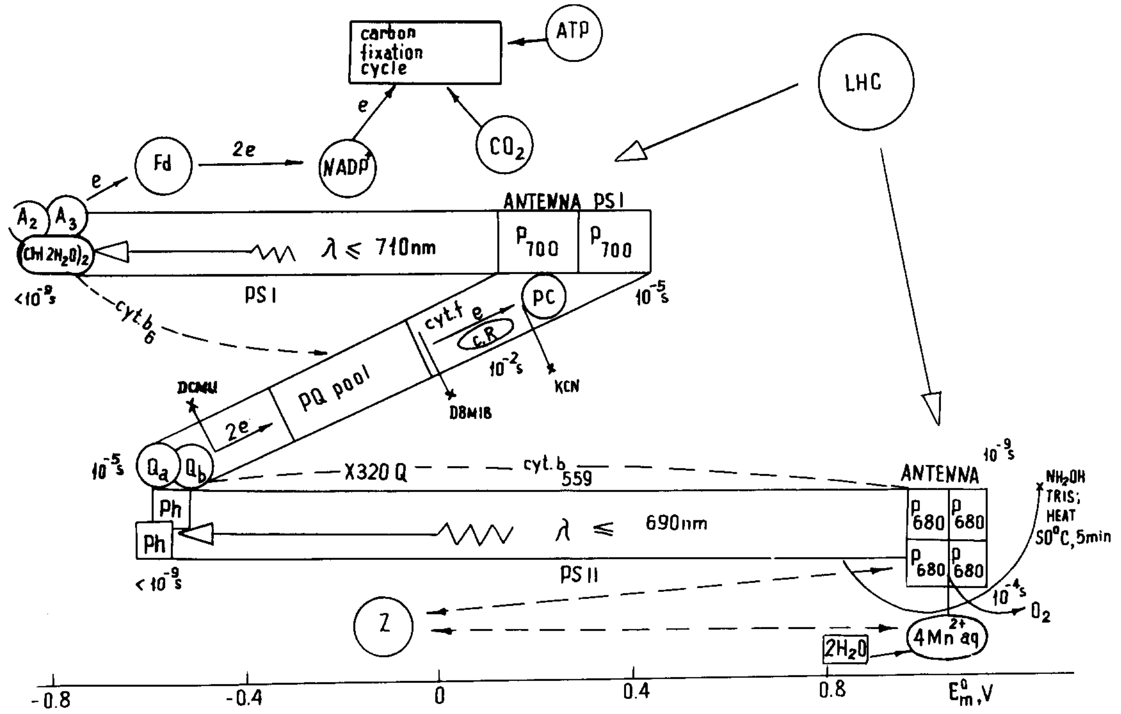


Fig. 8. Scheme of electron transfer in photosynthesis of higher plants. Times of pulsed kinetic variations are given. Scale of redox potentials is plotted at the bottom.

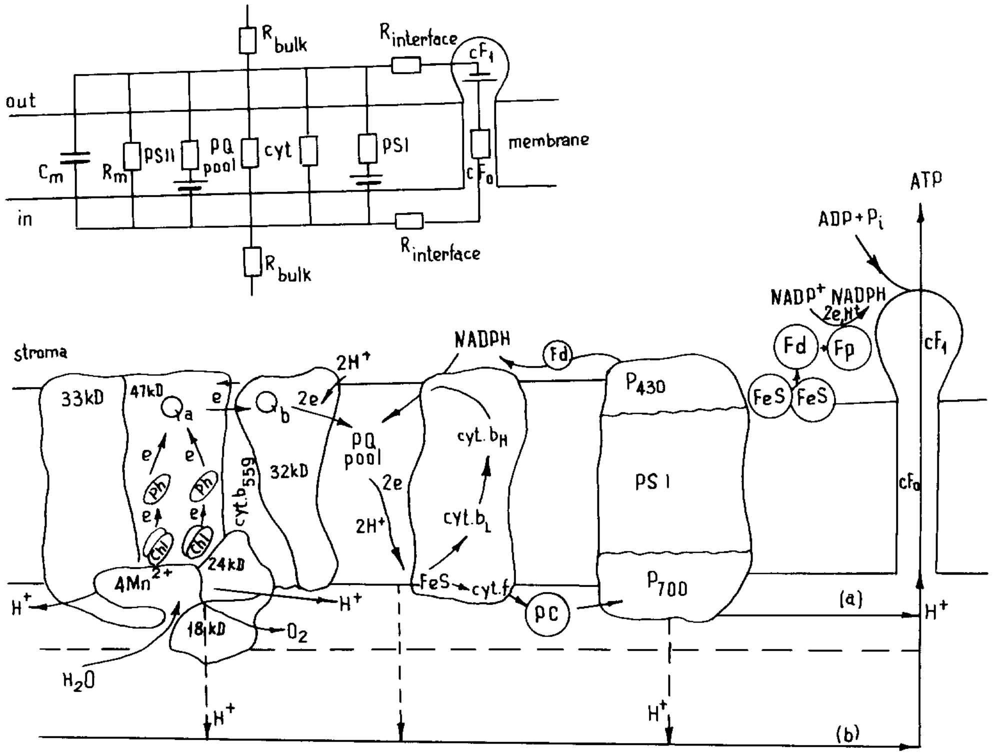


Fig. 9. Molecular organization of the thylakoid membrane: (a) equivalent electrical circuit; (b) scheme of charge transfer in thylakoid membrane.

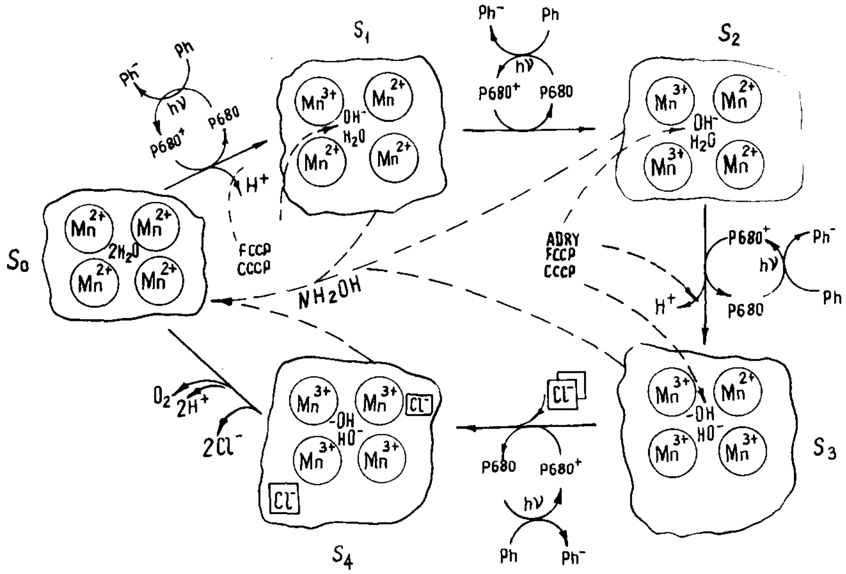


Fig. 10. Tentative model for water photooxidation in the Mn-cluster of Photosystem II in chloroplasts.

action center in redox state $[Mn_4^{k+}(H_2O)_2]Pheo$ (S_n) to redox state $[Mn_4^{k+}(H_2O)_2]^+Pheo^-$ (S_{n+1}). The positive charges generated are stored by the dark relaxation $S_n^* \rightarrow S_{n+1}$ which corresponds to the oxidation of S_n by the electron chain Pheo → $Q_a \rightarrow Q_b \rightarrow$ PS I via univalent electron transfer reactions until four redox equivalents are accumulated, S_4 decomposes to S_0, water decomposition by four-electron abstraction occurs and oxygen is evolved (Fig. 10). This mechanism is correlated with the four oxidation states of the water-splitting enzyme systems. The electron release pattern 1 : 1 : 1 : 1 is accompanied by the pattern of charge formation 0 : + : + : 0 and the intrinsic proton release stoichiometry 1 : 0 : 1 : 2. The protons released are mainly bound to a membrane and can be used for ATP synthesis via ATP-synthetase of chloroplasts.

The oxygen atoms of the two water molecules bound in the reaction center should be sufficiently close to one another, as, for example, is depicted in Fig. 10. Otherwise the multielectron process proves to be impossible on account of the large value of the activation energy.

Water photooxidation evidently is not the only multielectron process in photosynthesis.

Plastoquinone in non-cyclic electron transfer between the reaction centers of the two photosystems acts as a transmembrane electron and proton carrier:

$$Q_aQ_bH_2 \xrightarrow[PQ \qquad PQH_2]{} Q_aQ_b$$

Q_b is a two-electron 'gate' [42] of photosynthesis. Q_bH_2 transfers $2H^+$ and 2e to plastoquinone on the inner side of the thylakoid membrane. Thus, Q_b always carries electrons and protons across the thylakoid membrane in pairs.

Further examples of the multielectron reaction are the redox reactions of NADH and NADPH:

$$NADH \rightarrow NAD^+ + 2e + H^+$$

and

$$NADPH \rightarrow NADP^+ + 2e + H^+$$

which take place on the surface of biomembranes in photosynthesis and in mitochondria during respiration. An energy diagram has the following form:

$$\underbrace{NAD^+ \xrightarrow[-850\text{ mV}]{e} NAD^{\cdot} \xrightarrow[210\text{ mV}]{e,H^+} NADH}_{320\text{mV}}$$

This diagram shows that the activation energy of

NAD$^+$ reduction by the two-step one-electron mechanism must be $E_a \geqslant 83$ kJ/mol. For a synchronous two-electron process the activation energy can be smaller: $E_a \geqslant 60$ kJ/mol.

In photosynthesis, as is shown on Fig. 8, one-electron reduction of NADP$^+$ is energetically improbable because the redox potential of NADPH-reductase is less than -0.4 V.

An example of a multielectron reaction is nitrogen reduction:

$$N_2 + 8\,H^+ + 8e \rightarrow 2\,NH_3 + H_2$$

The enzyme nitrogenase, which catalyses the reduction of nitrogen in some bacteria and algae, contains several iron and molybdenium atoms, bound in clusters. The multielectron mechanism of this reaction is proposed in Ref. 26.

Oxygen evolution during water photooxidation at the oil/water interface

The interface between two immiscible liquids on which photosynthetic pigments are immobilized is the simplest, convenient model of a biological membrane for studying photoprocesses accompanied by a spatial charge separation reaction. Illumination of an octane/water interface in the presence of chlorophyll, a proton acceptor, dinitrophenol (DNP) or pentachlorophenol (PCP), in octane and of an electron acceptor in water (NAD$^+$, FeCN$_6^{3-}$ or NADP$^+$) results in evolution of molecular oxygen from water with a quantum yield of about 10–20% [31].

A possible four-electron mechanism of dioxygen evolution at the octane/water interface is shown in Fig. 11.

The hydrated oligomer Chl$_4$(H$_2$O)$_2$, adsorbed on the interface and close-packed, so that overlapping of the electron clouds of the porphyrin rings is possible, is excited under the action of light; an oxidized form of the pigment and a reduced form of the acceptor are produced. Water in the reaction complex is coordination-bound to the magnesium of one of the chlorophyll molecules and hydrogen-bonded with the carbonyl group of another chlorophyll molecule and a phenol anion, also adsorbed at the interface and forming part of the catalytic complex. The hydrophobic proton acceptor, PCP, is essential for adsorption binding of water in the reaction center and for the protection of chlorophyll from pheophytinization, since it activates part of the protons liberated in the course of the reaction. The artificial proton acceptor PCP does not participate in the action of Photosystem II. The role of the surface regulator of proton equilibrium on the membrane can be played by plastoquinone, lipids and polar groups of proteins.

Since the redox potential of hydrated chlorophyll oligomer is less than 1 V, the concerted four-electron oxidation of water by chlorophyll remains the only possible one of the pathways proposed in Fig. 7. It should be noted that the occurrence of one- and two-electron reactions (Fig. 7) is thermodynamically possible if the intermediate particles formed are in the adsorbed state. However, the radicals HO$^\bullet$ and HO$_2^\bullet$ thus formed

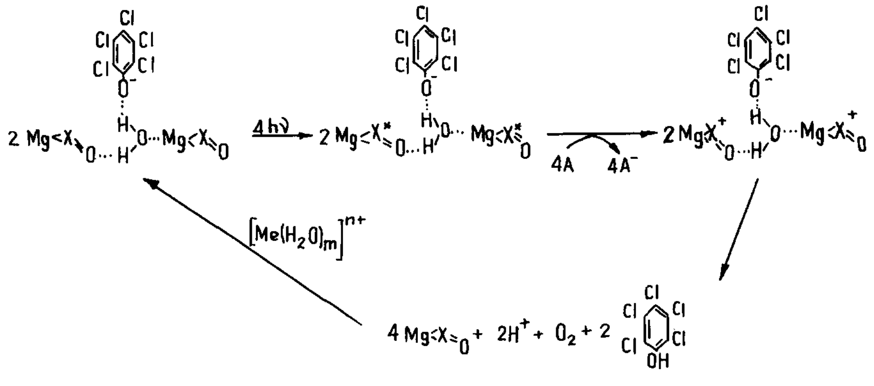


Fig. 11. Tentative model for water photooxidation at the oil/water interface.

are highly reactive and can readily enter into the side reactions of hydroxylation, oxidation and destruction of chlorophyll and other reagents in the catalytic complex. In fact, octane/water systems with chlorophyll can produce O_2 under illumination for approximately 4–5 h, which is evidence in favor of the multielectron mechanism.

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