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Energetics of multielectron reactions. Photosynthetic oxygen evolution

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In examining the energetics of the elementary act of reactions, involving a change in the number of particles, one has to consider not the standard free reaction energy, or the free energy at given reagent concentrations, but only the configurational component of the free energy. It is this quantity, independent of the reagents concentration, which affects directly the probability of the elementary act of the reaction. Using this approach it is shown that the oxygen-evolution reaction from water corresponds to the configurational redox potential about + 1.4 V, i.e., to a higher one than the redox potential of the primary acceptor of Photosystem II-oxidized P-680. The subdivision of the overall reaction into steps cannot eliminate this energy deficiency. It is shown that the potential in question can be considerably decreased if protons detached from water during the elementary act undergo immediate binding with sufficiently strong bases. An increase in the pH value of these bases upon reduction of manganese as well as the ionization of water molecules preceding their oxidation favours the reaction. The favourable energetics of the process in the form of a single four-electron or of two two-electron elementary acts are pointed out.

Introduction

In discussing the possibility of the occurrence of this or another redox reaction, it is customary to compare the redox potentials of the corresponding electron donor and acceptor. This approach is also widely used in considering multielectron processes. For example, for the oxygen evolution reaction from water

$$2H_2O - 4e = O_2 + 4H^+ \qquad (1)$$

the equilibrium potential at $pH = 7$ is 0.815 V. From this it is inferred that for the above reaction to occur an oxidant with the redox potential $E \geqslant 0.815$ V is necessary. Of course, this conclusion holds for the possibility of the process in principle, but it is doubtful that the value 0.815 V gives the real lower limit of the necessary redox potential value. The process which is quite possible from a purely thermodynamic point of view may have a very low probability to occur with a measurable rate.

The point here is not only that reaction (1) can occur in several steps, each of which is characterized by its proper redox potential (this we shall discuss below), but that there is a significant difference between the energetics of a single elementary act of the reaction, determining its activation energy and the energetics of the process as a whole that involves a large number of elementary acts, both in forward and backward directions and determines the equilibrium redox potential. The redox potential 0.815 V characterizes the free energy of formation of 1 mol of gaseous oxygen at the pressure of 100 kPa and of 4 mol of hydrogen

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Abbreviations: PS, Photosystem; Ph, pheophytin.

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ions at pH 7. This energy can be divided in two components: the energy necessary to perform the chemical transformation inside the reaction complex, and the energy change due to dilution of reaction products by the solvent up to their final concentrations *. The first contribution is independent of the concentration, and affects immediately the activation energy. The second part determines the concentration dependence of the free energy of the overall process.

The problem of the energetics of the elementary act of reactions involving many particles and especially involving a change in the number of particles is of a rather general significance. Here it will be considered as exemplified by the oxygen evolution reaction.

Discussion and General considerations

Let us assume that one electron acceptor (or more) A_{ox} removes simultaneously in one act four electrons from two water molecules, forming one O_2 molecule and detaching four protons. The activation energy of this process depends on the difference in the energy levels of initial and final states, i.e., on the energies of A_{ox}, A_{red}, H_2O, O_2 and H^+, but not on the concentrations of these reagents. Naturally, the probability of a given elementary act, taking place inside the reaction complex (nA + $2H_2O$), cannot depend on the presence and concentration of other reagents and reaction products. These concentrations define the number of reaction complexes for the forward and backward reactions, i.e., the overall process rate both in forward and backward directions and, hence, the equilibrium position. The probability of each elementary act, however, (and, accordingly, the activation energy reaction) does not depend on the concentration of reagents. In particular, the redox potential for reaction (1) depends significantly on the pH value but this dependence is defined by the dependence on pH of the rate of the back reaction, whereas the rate of the forward reaction does not depend on pH. Such phenomena are well known in the kinetics of electrode reactions where at constant potential the hydrogen evolution rate in alkaline solutions (H_2O molecules are the proton donors) or that of oxygen evolution in acid solutions (H_2O is the source of O atoms) are constant in a wide pH range (see, e.g., Refs. 1 and 2).

In the absolute reaction rates theory [3] the rate constant of each reaction step is written as

$$K = \frac{kT}{h} \kappa \exp\left(- \frac{\Delta G^{\neq}}{RT} \right) \tag{2}$$

where κ is the transmission coefficient, $\Delta G^{\neq}$ the standard free activation energy and the other designations are conventional. For the given class of reactions $\Delta G^{\neq}$ is a single-valued function of ΔG^0 – the standard free energy of the given step (generalized Brønsted relation, see Ref. 2). The quantity ΔG^0, however, does not characterize the energetics of the elementary act itself, since it depends on the choice of standard states, i.e., on the choice of units of concentration measurements. A quantity specific for the elementary act of reaction is the change in configurational component of the free energy ΔG_c, i.e., the part of the free energy not depending on the transpositional contribution to entropy (it is this contribution that defines the direct dependence of ΔG on concentration) [4,5]. The transpositional component of entropy for an ideal solution is equal to $-\Sigma_1^n R \ln X_i$, where X_i is the mole fraction of each component. Hence

$$\Delta G_c = \Delta G^0 + \sum_1^n RT \ln X_i^0 - \sum_1^m RT \ln X_f^0 \tag{3}$$

Here X_i^0 and X_f^0 are the mole fractions of n initial and m final reagents in their respective standard states. If for all reagents the standard states were chosen identical, then

$$\Delta G_c = \Delta G^0 + (n - m) RT \ln X^0 \tag{3a}$$

It was mentioned above that the activation energy of the elementary act depends directly on the energy levels of all reagents, i.e., seemingly on potential energy rather than on free energy. In actual fact, however, an averaging takes place over

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* In the general case one more contribution is to be taken in account, namely the free energy of the reaction complex formation at the given initial concentration of reactants. In the case of reaction (1) taking place in rather dilute aqueous solutions (at H_2O mole fraction $X_{H_2O} \approx 1$), this energy change is negligibly small.

a large number of system configurations that have similar energies corresponding to potential barriers of similar height. As a result, the final expression for the mean probability of the elementary act contains the configurational free activation energy $\Delta G_c^{\neq}$, depending directly on the configurational free reaction energy ΔG_c [6,7].

The quantity $-\Delta G_c$ is no other than the average work of one elementary act of reaction. It differs from the work of the reaction as a whole by the work of mixing reagents depending on the concentration. Therefore, ΔG_c can also be called the free energy of the elementary act, and the quantity $\Delta G_c^{\neq}$ the true free activation energy.

Thus, to analyse the elementary act of the reactions of interest we need not the redox potential value at the given reagent concentrations E_r and not that of the standard potential E_0, but the value of the potential corresponding only to the change of the configurational component of free energy. Let us illustrate the computation of this quantity by the example of reaction (1).

The standard potential of this reaction $E_0 = 1.229$ V (against normal hydrogen electrode). It refers to usual standard states: $X_{H_2O} = 1$, $C_{H^+} = 1$ M (i.e., $X_{H^+} = 1/55.5$) and the gaseous O_2 pressure of 100 kPa, which corresponds to the solubility $C_{O_2} = 1.26 \cdot 10^{-3}$ M, i.e., $X_{O_2} = 2.27 \cdot 10^{-5}$. Introducing corrections in accordance with Eqn. 3, we have $E_c = E_0 - (\frac{1}{4} \ln X_{O_2} + \ln X_{H^+})(RT/F) = 1.401$ V. In a similar manner we calculated the values of E_c for different versions of the stoichiometry both of the overall four-electro reaction and for its possible components, the one- and two-electron steps. The results of the computations for the most important reactions are listed in Table I *. The difference between E_c and E_0 is as a rule quite substantial.

It is expedient to mention here again that the configurational electrode potential is independent of concentration. The same value of E_c we obtain starting with the value of E_r at any pH. For instance, the potential at pH 7 (E_0') is 0.41 V lower than the standard potential E_0, but when calculating E_c from E_0' we have to add with the same 0.41 V as when calculating from E_0.

TABLE I

Reaction	Electrode potential (V) (n.h.e.)	
	standard E_0	'configurational' E_c
Overall four-electron reactions		
$2H_2O = O_{2(gas)} + 4H^+ + 4e$	1.229	1.401
$OH^- + H_2O = O_{2(gas)} + 3H^+ + 4e$	1.022	1.142
$2OH^- = O_{2(gas)} + 2H^+ + 4e$	0.815	0.884
$3OH^- = O_{2(gas)} + H_2O + H^+ + 4e$	0.608	0.626
$4OH^- = O_{2(gas)} + 2H_2O + 4e$	0.401	0.367
Two-electron steps		
$2H_2O = H_2O_2 + 2H^+ + 2e$	1.776	1.931
$2OH^- = H_2O_2 + 2e$	0.948	0.896
$H_2O_2 = O_{2(gas)} + 2H^+ + 2e$	0.682	0.870
$H_2O_2 + 2OH^- = O_{2(gas)} + 2H_2O + 2e$	−0.146	−0.163
One-electron steps		
$H_2O = OH_{(gas)} + H^+ + e$	2.848	3.225
$OH^- = OH_{(gas)} + e$	2.020	2.191
$OH_{(gas)} = O_{(gas)} + H^+ + e$	1.996	2.099
$OH_{(gas)} + H_2O = H_2O_2 + H^+ + e$	0.710	0.642
$OH_{(gas)} + OH^- = H_2O_2 + e$	−0.118	−0.392
$H_2O_2 = HO_2 + H^+ + e$	1.495	1.598
$H_2O_2 + OH^- = HO_2 + H_2O + e$	0.667	0.564
$HO_2 = O_{2(gas)} + H^+ + e$	−0.130	0.144
$HO_2 + OH^- = O_{2(gas)} + H_2O + e$	−0.958	−0.890

Discussion of the photosynthetic oxygen evolution

Let us now discuss on the basis of these considerations some aspects of the photosynthetic oxygen evolution mechanism (for a review, see Refs. 8 and 9). Water decomposition occurs due to the action of the Mn-containing protein component linked through an electron carrier (or carriers) with the reaction centre of the Photosystem II (P-680). We know neither the redox potential of Mn ions, nor their immediate surroundings. It is clear, however, that the E_r value of manganese cannot be more positive than the E_r value of the primary oxidant pigment P-680. The value of the latter was obtained by Klimov et al. [10] from the experimen-

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* In calculating the solubility, the value for gaseous OH was assumed to be equal to that for O_2. Considering that the solubilities of O_2 and H_2 differ only by the factor of one and a half, the error involved in hardly larger than ≈ 5 mV. The standard potentials for different steps are known to different degrees of accuracy. Because of this the sums over the steps can disagree with the potential of the multielectron reaction by several millivolts. For our further analysis this error is immaterial.

tally measured redox potential of pheophytin (Ph) in the reaction centre of PS II (-0.61 ± 0.03 V), the activation energy for electron transfer from Ph^- to $P\text{-}680^+$ (0.08 eV) and the excitation energy of P-680 (1.81 ± 0.01 eV). The resulting magnitude of E is close to $+1.1$ V (referred to an aqueous normal hydrogen electrode). In what follows we shall use this value, bearing in mind, however, that the true value of E_r (Mn) is less due to some potential drop in the chain of electron transfer.

Comparison of $E_r = +1.1$ V with E_c for reaction (1), i.e., with $+1.4$ V shows that the redox potential of oxidant is lower than the configurational potential of reaction (1). An important point must be stressed here. Generally speaking, for the elementary act to occur there is no need for the oxidant potential to be higher than that of reductant (or for them to be equal). Indeed, it is well known that endoergonic reactions are possible. But it should be borne in mind, however, that the activation energy cannot be less than the energy of the elementary act $\Delta G_c^{\neq} \geqslant \Delta G_c$. For the four-electron reaction under consideration $\Delta G_c = 4\ (E_c - E_r)e = +1.2$ eV (here e is the electron charge). Taking this value as the lower limit of activation energy and supposing the transmission coefficient $\kappa = 1$ we can calculate by means of Eqn. 2 the upper limit of the reaction frequency, or, correspondingly, the lower limit of the reaction characteristic time. The latter equals to 10^7 s, i.e., it is quite unreal.

Let us now consider the possibilities of this reaction occurring in four successive one-electron steps. A diagram of the levels for each step is given in Fig. 1. As can be seen from the figure, the one-electron steps involving formation of HO_2, and particularly OH, radicals are characterized by too high E_c values, making these steps extremely disadvantageous ($\Delta G_c \approx 0.5$ and 2.1 eV). Formation of H_2O_2 in the course of one two-electron reaction is also unfavourable ($\Delta G_c \approx +1.6$ eV). In spite of higher statistical probability of two-particle reaction (e.g., OH formation) in comparison with four-particle process (oxidation of $2H_2O$ to O_2) the first one needs too high activation energy, and hence is strongly retarded.

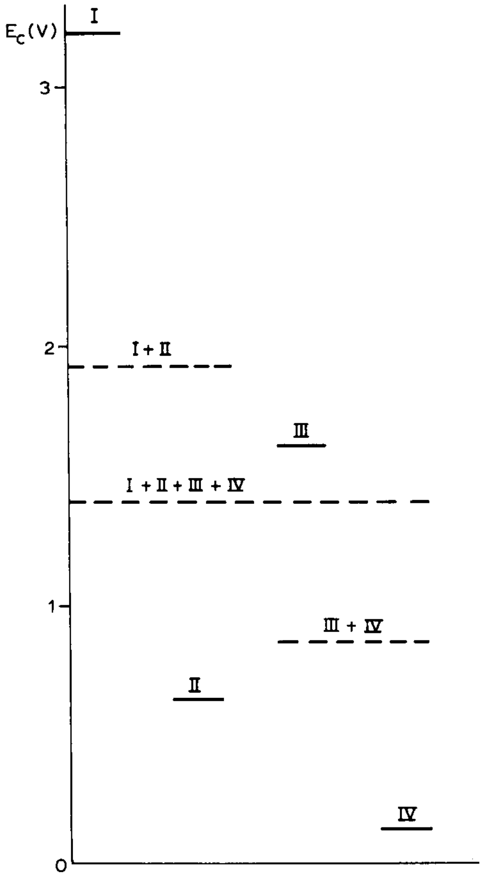

Fig. 1. Levels of configurational potential for one- and two-electron steps and overall reaction. Steps: (I) $H_2O = OH + H + e$; (II) $OH + H_2O = H_2O_2 + H^+ + e$; (III) $H_2O_2 = HO_2 + H^+ + e$; (IV) $HO_2 = O_2 + H^+ + e$.

As is clear from Fig. 1, the subdivision of the overall reaction into one- and two-electron steps is energetically of less advantage than a four-electron process. Some years ago this conclusion was drawn from the data on standard potentials by Semenov et al. [11]. Another argument against such subdivision is intermediate formation of free radicals capable of entering into side reactions with the active site, destroying it. It is sometimes suggested that these difficulties can e avoided by assuming, e.g., the OH radical to be strongly bound ('cryptohydroxyl'), which greatly decreases its oxidation potential and prevents side reactions (Refs. 12 and 13; for reviews, see also Refs. 8 and 9). A more detailed examination shows, however, that strong binding of radicals is insufficient for changing essentially the situation.

Let us assume, for instance the binding energy of the OH radical with the reaction site to be

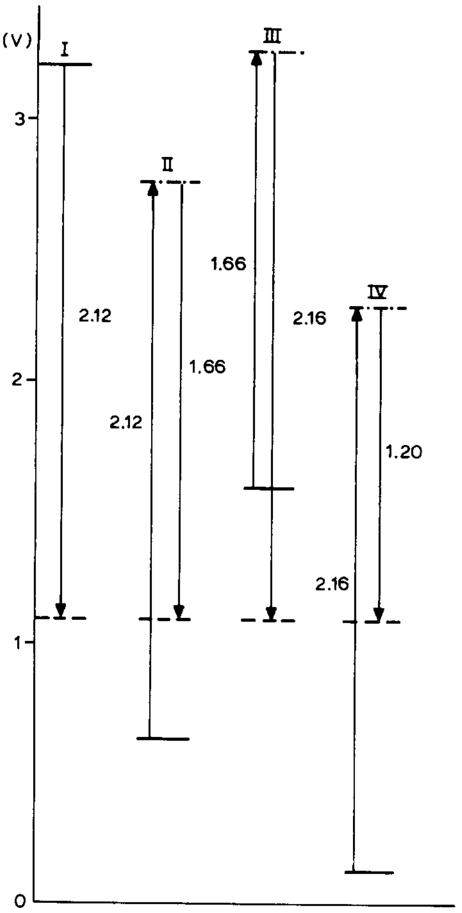

Fig. 2. Levels of configurational potential for one-electron reaction steps. Designation of steps as in Fig. 1. Designation of levels: solid line, without taking account of binding energies; dash-and-dot line, taking into account the binding energies of initial reagents; dashed line, taking into account the binding energies of initial and final reagents. Vertical arrows mark the binding energies, the figures indicate their values. The resulting level $E_c = +1.1$ V is common to all steps.

exactly sufficient for E_c for the first step to be equal to 1.1 V, i.e., $\Delta G_c = 0$. This binding energy is equal to -2.12 eV (see Fig. 2). The OH radical, however, being a product of the first step is at the same time an initial substance for the second step. Therefore, the whole energy gain in the first step due to strong binding of OH proves to be a loss in the second step; the value of E_c for the second step increases to 2.76 V. To reduce it to the level 1.1 V, the binding energy of H_2O_2 must be assumed to be equal to 1.66 eV, but this shifts E_c of the third step, etc. As a result, an excess energy $\Delta G_c = 1.2$ eV arises in the last step, i.e., the value found above for the four-electron reaction. Strictly speaking, this is just what would be expected, since the total energy deficiency – the difference in the energies of final and initial reactants – does not depend on the shift of the levels of intermediate substances. E_c could be decreased to 1.1 V in the last step as well if the binding energy of O_2 were assumed to be 1.2 eV but thus we only would shift the main-energy barrier from the electron-transfer process to that of liberation of the dioxygen molecule already formed, without accelerating thereby the reaction as a whole.

We have considered the kinetics of the sequence one-electron steps proposed as a probable mechanism by Renger [12,13]. Other versions are also possible. We can at once reject the mechanism according to which OH radicals are formed in two redox steps, undergoing later recombination to form hydrogen peroxide. Indeed, ΔG_c of the recombination reaction of two free OH radicals computed from known thermodynamic data is equal to -2.23 eV [5], i.e., for adsorbed radicals with allowance for the above estimate of OH binding energy $\Delta G_{cB}(\mathrm{OH}) \approx -2.12$ eV, we have $\Delta G_c = +2.01$ eV. At no reasonable adsorption energies of H_2O_2 can this value be lowered to an acceptable level.

Another path is oxidation of OH_{ads} to O_{ads}, followed by recombination of two O atoms or further oxidation $O_{ads} \rightarrow O_2H_{ads} \rightarrow O_2$. For the oxidation reaction of free OH radical to O atom $E_c = 2.1$ V. It is possible to reduce it to the level 1.1 V at $\Delta G_{cB}(\mathrm{OH}) = -2.1$ eV by taking the O binding energy $\Delta G_{cB}(\mathrm{O}) = -3.1$ V. Further recombination of two O_{ads} is characterized by too large a value of $\Delta G_c \approx 1$ eV (for free O atoms $\Delta G_{c_{\mathrm{recomb}}} = -5.22$ eV [5]). Oxidation of O_{ads} to the adsorbed superoxide radical HO_2 at $E = 1.1$ V requires $\Delta G_{cB}(HO_2) \approx -2.2$ eV. At this point the path in question merges with the one considered above.

The picture presented in Fig. 2 corresponds to such a distribution of the overall ΔG_c where it is all concentrated in the last step; and in the preceding steps $\Delta G_c = 0$. In principle, other distributions are also possible. For example, if 1.2 eV is divided equally between all steps, then in each of them $\Delta G_c = 0.3$ eV, which, generally speaking, does not present too serious an obstacle both on the path

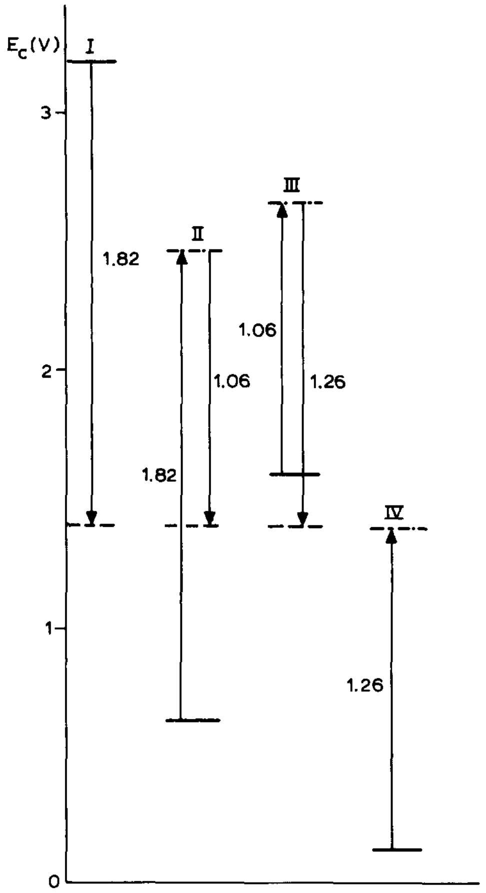

Fig. 3. Levels of configurational potential for one-electron reaction steps. Designations as in Fig. 2. The resulting level of E_c for the first three steps is equal to +1.4 V.

via H_2O_2 (Fig. 3) and on the path via O_{ads}. Such division seems, however, unlikely for the following reasons. In this case, all intermediates and final product would be at the level of E_c higher than E_r of oxidant. In the case of such a situation a back reaction would be very likely, i.e., reduction of molecular oxygen. To avoid that reaction one should reduce ΔG_c at least for the last step (and also better for formation of H_2O_2) to a certain negative value. But in that case significant positive values of ΔG_c in the intermediate steps would as well be inevitable.

As was shown above, the hypothesis of strong binding of intermediates can hardly remove large energetic obstacles on the path of reaction (1). It should be emphasized that we assumed the binding energies necessary for optimization of the path without discussing the reality of such large energies both for OH, O and HO_2 radicals and for the H_2O_2 molecule. It is surprising that it should be necessary to assume the binding energy of H_2O_2 close to that for radicals. One would rather expect here values similar in their order of magnitude to that of the binding energy of water. We do not know the value of this energy, but for aquacomplexes of Mn^{2+} it can be estimated as ≈ 0.3 eV (from the data on the exchange rate of ligands; see, e.g. Ref. 14). If we take into account the binding of the initial reagent, i.e. H_2O, the corresponding correction will increase by 0.3 V E_c of the first two steps (both in the one- and two-electron versions) and by 0.15 V E_c for the four-electron reaction.

The total process can be made more advantageous by increasing the energy of initial substances or decreasing that of final products. As was clear from the foregoing, strong binding of one of the products – molecular oxygen – is not only unlikely, but also disadvantageous because it will slow down O_2 evolution too much. This is due to the fact that the product of the four-electron reaction is only one O_2 molecule. In the case of binding of H^+, the total energy deficiency compensated for the binding energy will be distributed among the four particles, so that each of them will prove to be bound not strongly enough to render its liberation too difficult.

Binding of protons can be effectively carried out in two ways. First, substitution of the initial reagent H_2O by OH^-, i.e., increase of the energy of the initial state and decrease of the number of liberated protons. Second, participation in the reaction of some bases accepting protons immediately during the elementary act (for reaction (1) these bases are water molecules forming H_3O^+).

Substitution of one of the two water molecules by OH^- reduces E_c for the four-electron reaction to +1.14 V, i.e., almost to the required level, while substitution of two water molecules by OH^- reduces already to +0.88 V (see Table I). It should be taken into account, however, that in neutral solutions the OH^- concentration is very low, so that if the pK value of water bound in the active site does not differ from that in the bulk, the fraction of active sites with one OH ion is $10^{-7}/$

$55.5 \approx 10^{-8.7}$, and with two OH^- the fraction is $10^{-17.4}$, i.e., this way proves to be extremely ineffective. It is logical to suppose that H_2O in the coordination sphere of Mn^{3+} is a much stronger acid than in solution bulk, since OH^- must be more strongly bound than the water molecule. If, for example, the difference in the binding energies of OH^- andH_2O is approx. 0.45 eV, the fraction of once or twice ionized sties increases to $10^{-8.7}$ $e^{0.45/0.025} \approx 10^{-1}$ or 10^{-2}, respectively. Hence it can ensure reasonable process rates, though the utilization of active sites especially in the second case is not very effective. Increase of the binding energy of OH^-, i.e., decrease of the initial reagent energy, leads, however, to increase of E_c. If we take into account the estimate of the binding energy of water (≈ 0.3 eV) given above and the additional energy of binding of OH^-, we obtain higher E_c values: for the overall reaction ($OH^- + H_2O$) $E_c = 1.14 + \frac{1}{4}(0.3 + 0.75) = +1.4$ V, and for the reaction ($OH^- + OH^-$) $E_c = +1.26$ V. It should be noted that further increase of the degree of ionization of active sites θ would entail a rather rapid growth of the binding energy. Thus, for $\theta = 0.9$ an excess energy of binding of OH^- equal to 0.57 eV would be required and for $\theta = 0.99$ – 0.63 eV. In the latter case E_c for the reaction of two OH ions will be $+1.35$ V. Thus the gain is not large as compared to the reaction of two neutral molecules, and it may be attended by decrease in efficiency of utilization of active sites. The gain rises with increasing number of OH^- participating in reaction. Thus, for the reaction of $4OH^-$ ($E_c = 0.37$ V without allowance for the binding energies) at the maximum binding energy of OH^- assumed above, $E_c = +1.15$ V.

Another possible reaction path is incorporation into the system of some other base-binding protons

$$2H_2O + 4X^- - 4e = O_2 + 4HX \qquad (4)$$

These bases can be coordinated with Mn^{3+}, which provides the required reaction configuration. At pK of the bound HX equal to 7.5, E_c for reaction (4) drops (even allowing for the binding energy of water 0.3 eV) to the level $+1.0$ V, an even greater decrease of E_c being possible at larger pK. (It should be remembered that the change of E_c differs from that of the standard potential due to the change of the particle number). As a possible candidate for the role of the base X^- the anion radicals of plastosemiquinones can be supposed. They are believed to enter into the coordination sphere of manganese ions [15,16]. Other bases are also possible, basic groups of the protein, in particular.

Assuming pK_{HX} equal to 7.5 or greater, we, meet, however, with a significant difficulty. If HX readily exchanges protons with the aqueous phase, i.e., is in equilibrium with it, then at physiological pH the base is to a considerable degree (and, at larger pK, predominantly) in the protonated form, i.e., the fraction of efficient active sites proves to be low. To avoid this we are forced to take $pK \lesssim 6$. But in that case $E_c \gtrsim +1.1$ V. Thus, basically, here we encounter though perhaps in not so large a degree, the same difficulties as for reactions involving OH^-. In both cases the situation can be improved if we take into account the binding energy of O_2. If it is approx. 0.4 eV, which is reasonable for a binuclear complex, the value of E_c decreases by $0.4/4 = 0.1$ V, i.e., it reaches an admissible level.

Thus, the mechanisms described above seem possible though not very advantageous. Indeed, the estimates made above lead to E_c of the reaction as a whole and E_r of the primary oxidant, having approximately the same values. But in this way it is difficult to achieve a significant potential drop between oxidant and reductant, desirable from the viewpoint of the unidirectional process course. It should be recalled that our treatment was based on the data on the potential of oxidized P-680, whereas the potential of the manganese system is likely to be lower.

Unlike reactions with OH^-, in which the base is at the same time a source of oxygen atoms, another effect is possible for a reaction with coordinated bases X^-, capable of making the process much more advantageous. If the base, the semiquinone anion in particular, is coordinated with the manganese ion, its acidity should depend significantly on the oxidation state of the central ion. In the initial state of the reaction the base is coordinated with Mn^{3+}, and in the final state with Mn^{2+}. Naturally, its initial acidity is much higher, i.e., pK is much lower. Owing to this, initial pK can be less than 6, which provides practically complete ionization of all HX, whereas the final pK that alone affects the energetics of reaction (4) may be by several units higher *, which ensures a low enough value of E_c. This effect, allowing in addition for the binding energy of O_2, can cause an appreciable drop of the oxidation potential.

Combined versions are of course possible, e.g., the overall reaction

$$2OH^- + 2X^- - 4e = O_2 + 2HX \tag{5}$$

At the above estimates of the binding energies of OH^-, H_2O and O_2 and at $pK_{HX} = 10$, for reaction (5) $E_c = +0.8$–0.9 V, i.e., there is a significant drop of the oxidation potential.

Let us consider for illustration the scheme of the four-electron reaction (5) similar to the one described in Ref. 15 and involving four manganese ions. Oxidation of the first manganese ion leads to detachment of a proton from the water bound with it and formation of an OH ion. Oxidation of the second Mn ion, if initially it was bound with the same water molecule, does not lead to detachment from OH^- of the second proton. There only appears near OH^- a proton acceptor X^-, or Cl anion enters into the coordination sphere. The third oxidized ion promotes the detachment of a proton from the second H_2O molecule; the fourth oxidation equivalent creates a situation that can give rise to a four-electron reaction (5) involving detachment from two OH^- of two protons, added to the primary acceptors X^-. This elementary act corresponds to $\Delta G_c < 0$ (at $E_r = 1.1$ V, the estimates of ΔG_c vary from -0.8 eV to -1.2 eV). The neutral molecules of semiquinone HX leave the coordination sphere of manganese and dissociate, donating two protons to the medium. Thus protons are detached in a stepwise manner in the ratio 1 : 0 : 1 : 2. Allowing for the possibility of parallel paths, this evolution of H^+ corresponds to Fowler's results [17] (cf. also Ref. 18, for a review see Refs. 9 and 19). Though there are several steps between transfer of the fourth electron and transfer into the aqueous medium of the last two protons, at sufficiently high rates of these steps they will be observed as being one process.

There exist some data indicating that of the four Mn ions present in the active site only two participate in redox reactions [20–23]. In such case the same scheme could include consecutive oxidation of Mn^{2+} to Mn^{3+} and Mn^{4+}. In the case of participation of only two Mn^{2+} a version of subdivision of reaction (5) into two two-electron steps is possible

$$2OH^- - 2e = H_2O_2 \tag{6}$$

$$H_2O_2 + 2X^- - 2e = O_2 + 2HX \tag{7}$$

For reaction (6) $E_c = 0.9$ V; if the binding energies of OH^- (and H_2O) estimated above are taken into account its value rises to 1.7–1.8 V. Assuming the binding energy of H_2O_2 to be equal to 0.6–0.8 eV (this is a reasonable order of magnitude because this value is approximately twice as large as the binding energy of H_2O with one site), one can reduce E_c to approx. 1.4 V, i.e., decrease ΔG_c of reaction (6) to an admissible level $+0.6$ eV. At such binding energy value of H_2O_2 and also at $\Delta G_{cB}(O_2) \approx -0.4$ eV and $pK_{HX} = 10$, the value of E_c for reaction (7) proves to be at the level $+0.2$–0.4 V. Thus, if reaction (6) is markedly endoergonic, reaction (7) is significantly exoergonic, and this causes a pronounced drop of the redox potential in the overall O_2 evolution process.

A scheme similar in a sense to the sequence (6)–(7) was proposed by Wydrzynski and Sauer [21]. It can be interpreted as follows: oxidation of the first Mn^{2+} is accompanied by detachment of one H^+, oxidation of the second ion does not lead to electrolytic dissociation. When the third oxidation equivalent is transferred to the active site of enzyme, the bound OH^- and H_2O undergo oxidation with participation of one of the Mn^{3+} and quinone X to form hydrogen peroxide and HX (ultimately with detachment of one proton):

$$OH^- + H_2O + X + Mn^{3+} = H_2O_2 + HX + Mn^{2+} \tag{8}$$

This can be treated as the reaction

$$OH^- + H_2O + X^- - 2e = H_2O_2 + HX \tag{8a}$$

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* The same reasoning is applicable, of course, to dissociation of water coordinated with Mn, but it should be remembered that in the given case oxidation of Mn^{2+} to Mn^{3+} results not only in increase of pK_{H_2O}, but also in stronger binding of OH ion that ultimately must become detached from manganese to form O_2.

in which X and Mn^{3+} are electron acceptors. These can be assumed, as was done above, to have the mean oxidation potential +1.1 V. At the same estimates of ΔG_{cB} of OH^- and H_2O and of pK_{HX}, it proves possible to decrease E_c of reaction (8a) to the level of 1.1 V at a reasonable binding energy value of H_2O_2 about 0.5 eV. Transfer of the fourth electron makes possible the second two-electron step of type (7) leading to liberation of two hydrogen ions.

Taking account of the role of bases, which improves the energy balance both of two- and four-electron reactions, promotes one-electron steps too. Therefore, the mechanism involving only one-electron oxidations cannot be in principle excluded. In this case, however, one still has to deal with the difficulty of exact matching of the binding energies of intermediates mentioned above, since the steps of formation and consumption of intermediate require opposite trends in binding energies. That is the reason why the pathway involving four one-electron oxidations of water molecules seems to us less probable than the mechanism exploiting multi-electron steps.

In our analysis we proceeded from the ΔG_c values characteristic of reactions in the bulk of solution and then introduced corrections for binding energies. The latter were estimated by order of magnitude as similar to the energies of complexes formation for uncharged saturated molecules or, for radicals and ions, such values were just chosen to meet the requirements of suitable energetical relations for each reaction step or of an effective use of the enzyme's active site. Remember that any substantial deviation from the binding energy values chosen for some definite reaction path will significantly hinder the reaction rate.

We need much more information on the structure of the oxygen-evolving enzyme to be in a position to give a well-founded independent estimate of binding energies. Besides usual problems with calculating short-range interactions we meet here a new difficulty specific of enzymes: the protein forms the polar medium of reaction bringing about a substantial change in long-range forces as compared to an aqueous medium. The specific features of proteins as polar media are their low dielectric constant and a high concentration of fixed dipolar groups setting up a strong intraglobular electrostatic field (see, e.g., Refs. 24 and 25). The low dielectric constant makes unfavourable the transfer of ions from water into protein. Hence, this factor raises the redox potential for reaction (1) (formation of free H^+) and lowers it for reaction (4) (disappearance of X^-), etc. The intraglobular field may affect the ions energy in both direction; this effect cannot be predicted without data on the protein structure. Therefore, we have to give up at present any attempt of an independent calculation of binding energies and to confine ourselves with a formal analysis of the possible limits of these energies.

In the overall process the initial reactant (H_2O) is taken from the bulk of solution and the products (O_2 and H^+) are transferred ultimately from the enzyme back into solution. That means that if ΔG_c in solution has a large positive value the value of ΔG_c for some of the reaction steps is to be large and positive: this may be for redox reactions inside the enzyme or for the transfer of reactants to or from the bulk of solution. The energy deficiency is to be distributed between different steps of reaction. A formal analysis of different versions of this distribution given in this section allows us to make some conclusions on the possible reaction pathways. The mechanisms including recombination of two bound radicals (OH or O) are least probable. The routes exploiting multi-electron water oxidations seems to be more probable than the mechanisms involving only one-electron steps. The oxidation can be facilitated via simultaneous proton detachment by some base coordinated to manganese, its basicity being enhanced upon manganese reduction.

In the foregoing we considered the possibilities of simultaneous transfer of two or even four electrons. In aqueous solutions such reactions are relatively uncommon, since the reorganization energy of the polar medium, to a large degree determining the activation energy of the process, is proportional to the square of the charge being transferred. Recently, we analyzed the factors promoting multielectron enzymatic processes [26]. We showed that these factors, especially a simultaneous use of several donors and/or acceptors, reduce the reorganization energy of multielectron enzymatic reactions to a quite acceptable level.

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