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Review

Protein dynamics and reactions of Photosystem II

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Abstract

The kinetics of charge recombination by electron transfer from $Q_A^{\cdot -}$ to $P680^{\cdot +}$ on the reducing branch of PSII is likely to be strongly dependent on protein dynamics, in analogy with the kinetics of the corresponding reaction in the reaction center of purple bacteria [Biophys. J. 74 (1998) 2567]. On the oxidizing branch of PSII, the kinetics of electron hole transfer from $P680^{\cdot +}$ to Y_Z is known to be multiexponential. This transfer is in the Babcock model of the reactions of the water-oxidizing complex coupled with proton transfer from Y_Z. The proton is via switching hydrogen bonds in the protein transferred to the thylakoid lumen. The demand for successive proton transfers requires rearrangement of the hydrogen bonds, which in turn requires a flexible protein making fluctuating excursions among all its conformations. In the equilibrated protein, only a fractional part of the molecules is in a conformation that is able to support the proton transfer from Y_Z. The kinetics of the rearrangement to this active conformation will be multiexponential and dependent on the distribution among all conformations, which is likely to be sensitive to various influences, in particular from changes in the protein coordination to the $(Mn)_4$ cluster between the different S states.

Keywords: Photosystem II, PSII; Reaction kinetics; Protein dynamics; Protein conformation

1. Preliminaries

My first encounter with Jerry Babcock was in August 1985. I was working with the enzyme ribonucleotide reductase, RNR, in which we had discovered a tyrosyl radical necessary for the activity [1,2]. Burning questions were to find out whether this tyrosyl radical was cationic or neutral, and whether it is hydrogen bonded or not. In an earlier work with flavin radicals [3], I had found ENDOR to be useful in studies on protein bound radicals and I thought now it could be a helpful technique also in the RNR case. Our old ENDOR instrument from 1970 was not in good condition, but I knew of Jerry's work on semiquinones and that he had a modern ENDOR spectrometer. So I went to see him in East Lansing and try to persuade him to take a look at the tyrosyl radical of RNR. In my seminar, I described how we had discovered the radical, demonstrated that it was a tyrosyl radical, and at what site in the protein it was located. I also showed how sensitive the EPR spectrum was to small changes of the dihedral angles of the β hydrogen atoms. During my stay, we discussed the experiments that should be planned for RNR. It took quite some time to have all that work organized and done, but 4 years later we had a common paper published with many collaborators involved [4]. There we demonstrated that the tyrosyl radical of *E. coli* RNR was a neutral radical with no detectable hydrogen bonding to the oxygen.

About 25 years earlier, I had, together with Bob Whatley, done some EPR work on light-induced radicals in spinach chloroplasts. We obtained a lot of interesting results, but Bob had to leave Stockholm and go back to Berkeley, and I had a number of things to do before I could think of joining him there. But then, before we could finish up the work and much to our despair, a paper by Calvin and Androes [5] appeared, reviewing, among other things, already published results similar to what we had obtained. Hence, I was quite receptive to the updating Jerry gave me on the actual state of knowledge on the various radicals of PSII. At that time, the radicals were believed to be some sort of semiquinones. We had a long and fascinating discussion about the protein bound radicals known at the time. When we talked about how the tyrosyl radical EPR spectrum could change when the dihedral angle was varied, I could see the intense glimpses in Jerry's eyes, so I am quite sure he already had alerted the thought in his brain, the thought that a little later was (or was not, my memory is not sharp here) formulated by the words: Why not check whether the PSII radicals are tyrosyl radicals? Indeed, Jerry

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soon organized the work and did everything in a most elegant manner. We all know the fascinating results and the new possibilities it opened up.

More recently, I used the present knowledge about protein dynamics and conformational substates [6] for making a realistic molecular model describing the reversible transfer of the RNR radical from its resting tyrosine site to the substrate site, a distance of 35 Å, and back again [7,8]. In parallel, I started to consider the possibility of how protein dynamics could play a role in the reactions of the tyrosyl radicals of PSII. Every time I met Jerry, I tested my thoughts along these lines on him. He encouraged me to continue, read what I scribbled down on the subject, and gave me invaluable hints to help me orient in the vast literature on PSII properties and mechanisms. The following discussion is a first report on the theme of protein dynamics and PSII reactions.

2. Why protein dynamics?

Detailed spectroscopic studies of the dynamics of the "simple" protein myoglobin was introduced by Frauenfelder et al. about 30 years ago (for references, see Refs. [6,9]). The ligand CO was dissociated off by a flash of light and its rebinding was followed as function of temperature and other parameters. It was necessary to formulate new concepts to understand the nature of the dynamics of a small but still very complex protein [6,9]. For myoglobin, much insight has been gained about how the dynamics of a protein may be connected with and control function. Many questions have been answered and more new ones have been raised. The field is still in a phase of strong and lively development [9]. So far, essentially only systems where a reaction may be started by a light flash, like in myoglobin [9] and bacterial reaction centers, vide infra, have been studied experimentally, but new experimental approaches are being developed. In general, any enzyme function should in a more or less obvious manner be dependent on protein dynamics. Therefore, it appears important to discuss new systems with regard to how protein dynamics may be important for function [7,8, this paper], which could inspire experimentalists to find ways on how to examine these questions in more detail.

3. Reactions in the reducing part of PSII

The reactions of PSII are started by the excitation of the chlorophyll complex P680 and the following charge separation by very fast reduction of pheophytin, forming $Pheo^{\cdot -}P680^{\cdot +}$. The charge separation is stabilized by fast reduction of the primary quinone Q_A:

$$Q_A Pheo^{\cdot -} P680^{\cdot +} \rightarrow Q_A^{\cdot -} Phe\ P680^{\cdot +} \quad (1)$$

$Q_A^{\cdot -}$ then reduces the secondary quinone Q_B. After four such reductions, two molecules of the hydroquinone H_2Q_B have been released to the stroma and four protons taken up.

There are analogous reactions in the photosynthetic reaction center complex of purple bacteria. For this latter system, the reactions have been investigated in great detail. In particular, the reversal of Reaction (1) has been studied in detail, and it has been shown (a) that there is a structural difference between the proteins before and after the reaction [10,11], and (b) that the distribution among the conformational substates of the protein and the fluctuating relaxation between them is of great importance for the reaction kinetics [11]. The experiments of McMahon et al. [11] were made in a glycerol/water mixture (3:1, v/v) over the temperature range 5–280 K. Their theoretical model permitted extrapolation to physiological conditions, which suggests that at 300 K the electron transfer (ET) from $Q_A^{\cdot -}$ to $P^{\cdot +}$ ($P^{\cdot +}$ in bacterial reaction centers corresponds to $P680^{\cdot +}$ in PSII) involves relaxations in the time range from 1 ps to 1 ms. There are good reasons to believe that the general principles of the reaction kinetics and conformational changes in the reducing part of PSII are very similar to those of the bacterial reaction center. The PSII system is, however, less straightforward to investigate because of the coupling and interactions between the reducing and oxidizing parts.

4. Reactions in the oxidizing part of PSII

In the oxidizing part of PSII, four successive electron holes created at P680 are moved to the water-oxidizing complex containing the Y_Z tyrosine, a tetranuclear manganese cluster, a calcium ion, and possibly chloride. Following the absorption of four photons, two water molecules are oxidized to O_2 and four protons released to the thylakoid lumen. A very suggestive mechanistic scheme for how the oxidation of the $(Mn)_4$ cluster with its bound water molecules is coupled to the proton transfer through the protein has been formulated by Babcock et al. [12,13]. In the following, the kinetics of the various steps of these reactions will be considered and it will be outlined how they most likely depend on the protein dynamics.

The first reaction to consider is the oxidation of Y_Z by $P680^{\cdot +}$:

$$\begin{aligned} P680^{\cdot +} \ldots\ldots Y_Z - H \cdots His - H \cdots X_1 - H \cdots \vdots \cdots \\ X_2 - H \rightarrow P680 \ldots\ldots Y_Z^{\cdot} \cdots H - His - H^{+} \cdots \\ X_1 - H \ldots\ldots \vdots \cdots X_2 - H \end{aligned} \quad (2)$$

The experiments demonstrating that Y_Z is a tyrosine residue were initiated by Babcock [14]. He also showed that $Y_Z^{\cdot}$ is a neutral radical [15]. This is indicated in Reaction (2), where the phenolic proton is moving away via a nearby base, probably histidine, along a chain of hydrogen bonds within the protein. The oxidation of Y_Z by $P680^{\cdot +}$ may be

considered as an ET reaction from Y_Z to $P680^{\cdot+}$, but this ET may only take place if at the same time the phenolic proton of Y_Z will move away. The reduction of $P680^{\cdot+}$ to P680 is accompanied by a decay of light absorption at 830 nm. Since $P680^{\cdot+}$ is a strong quencher of chlorophyll fluorescence, Reaction (2) is also accompanied by a fluorescence increase. Both types of effects have been monitored in studies of the kinetics of $P680^{\cdot+}$ reduction, i.e., Y_Z oxidation. The back-reaction of Reaction (1) has to be hindered or corrected for. The kinetics observed by Jeans et al. [16] from the light absorption decay are representative for the type of results presented: The decay curves are fitted with several exponentials until the residual does not decrease, which, in Ref. [16], results in four exponentials and one constant term [τ(time constant)/A(amplitude): 14 ns/0.37, 150 ns/0.25, 0.7 μs/0.11, 8 μs/0.19, and $A=0.08$]. The response time of their experimental system is reported as 10 ns (fwhm) and the sampling time is 2.5 ns. The 14-ns component is about the best they could expect to resolve, and it might as well also represent parts of faster components with time constants <14 ns. The constant term probably represents slow components stretching up in the millisecond region. This is typical for a system with kinetics of the same general kind as analyzed by McMahon et al. [11]: Each protein molecule follows its own dynamic trajectory; some relax very fast to a state allowing ET to take place, others relax more slowly and give slower ET. Such nonexponential kinetics is often well fitted by a stretched exponential [17] and the system is best described as a distribution between protein conformational substates with different activation energies [17].

Other experiments of similar kind as in Ref. [16] have indicated that the "main kinetic component" is dependent on the redox state (the S state) of the $(Mn)_4$ cluster, when Reaction (2) is started. The subscript of the S state S_n denotes the number of electrons extracted from the $(Mn)_4$ cluster including the two substrate water molecules. Typical data are as follows (see Refs. [16,18]): (starting S state/main kinetic component: S_0/20–60 ns, S_1/20–60 ns, S_2/50 ns, and S_3/300 ns). The results referred to in the previous paragraph represent an average for all starting S states with a main component of 14–150 ns. The properties of Y_Z and $Y_{\dot{Z}}$ could be influenced directly by the different charge distributions within the $(Mn)_4$ cluster in the different S states and/or the conformational substates and their relaxation could depend on the state of the cluster through the coordinating protein side chains.

The next reaction to consider is the oxidation of the water-oxidizing complex by $Y_{\dot{Z}}$, which in the Babcock model is directly coupled with a proton transfer from the $(Mn)_4$–substrate complex to Y_Z, a first step toward the thylakoid lumen:

$$S_n - H\cdots Y_{\dot{Z}}\cdots H - His\cdots \rightarrow S_{n+1}\cdots H - Y_Z\cdots H - His\cdots \quad (3)$$

The proton of an H_2O or an OH^- in the coordination sphere of the $(Mn)_4$ cluster of the water-oxidizing complex, which is H-bonded to $Y_{\dot{Z}}$, has been indicated. Also, the rate of Reaction (3) has been shown to be S-state-dependent with $Y_{\dot{Z}}$ lifetimes of <3–250 μs for the step S_0/S_1, 30–140 μs for S_1/S_2, 100–600 μs for S_2/S_3, and 1–4.5 ms for S_3/S_0 (see Ref. [19] and references therein). Such a strong dependence on the S state transition may have more than one cause: A direct influence from the changing charge distribution within the water-oxidizing complex, the changing organization of the H bond between S_n and $Y_{\dot{Z}}$, or influence on the protein conformational substates from changes of the Mn-coordinating groups of the protein.

The charge of $P680^{\cdot+}$, which in Reaction (2) was transferred to a proton depicted still to be H-bonded to the His, ultimately has to be released to the lumen bulk. This proton charge inside the protein, with its low dielectric constant, will have a strong tendency to move to the protein surface. As soon as a chain of H bonds is available, this will happen and the proton will be released to the lumen aqueous phase depending on the pH of the bulk and the pK of the protein group X_2 exposing the protonic charge:

$$S_{n+1}\cdot\cdots H - Y_Z\cdots H - His\cdots H^+ - X_1 - H\cdots\cdots\cdots X_2 - H \rightarrow S_{n+1}\cdot\cdots H - Y_Z\cdots H - His\cdots H - X_1\cdots H - \cdots\cdots H - X_2 + H^+ \quad (4)$$

The availability of this chain of H bonds will depend on the fluctuating protein dynamics between the various conformational substates. Before the proton reaches the protein surface and is released to the lumen, it might even move around between sites within the protein. Already at this stage, a proton could be released from some acidic group at the protein surface due to electrostatic interaction. It is clear that Reaction (4) is likely to be multi-exponential. That protein relaxation processes might be important for the kinetics of Reaction (4) was already suggested by Tommos and Babcock [13]. The proton release of Reaction (4) is known to take place in a time span of 1–100 μs after the flash initiating Reactions (1) and (2) [12,13].

When we compare the organization of the chain of H bonds on the left side of Reaction (2) with the chain on the right side of Reaction (4), we see that in all the H bonds, the proton has switched from the end of the bond in direction of the water-oxidizing complex to the other end in direction of the lumenal surface. An important consequence of the Babcock mechanism is that the chain or web of H bonds from Y_Z to the protein surface, at least the steps nearest to the water-oxidizing complex, must be reorganized into the state depicted on the left side of Reaction (2), before Y_Z may be oxidized again. In the case of the His, this reorganization means either that

the imidazole ring has to rotate half a turn around its $C^{\beta} - C^{\gamma}$ bond, changing the partner of the H bond to Y_Z from the protonated $N^{\delta 1}$ to the unprotonated $N^{\delta 2}$, or vice versa, or that a network of H-bonded water molecules is formed as a transient linker between the two nitrogens, accepting the proton from one of them and donating a proton to the other one. In either case, a breathing mode of the protein is required to create enough volume around the imidazole ring. Similar mechanistic possibilities apply in the case of a Glu or an Asp residue as part of the H bond chain. A conclusion is that a considerable degree of flexibility and conformational freedom is needed in the protein.

Tyrosine Y_Z, where the H bond chain starts, of course also has to reorganize itself, most simply by flipping the proton from one side to the other by rotation of the C – O bond, which again requires that enough free space is created. Flexibility is also necessary since Y_Z has to form an H bond to the different ligand positions of the water-oxidizing complex, from which protons will be abstracted, as well as to either of the N atoms of the His imidazole if both orientations are possible.

The dynamic flexibility of the protein just described also means that the H bond chain is not stable in either of its limiting forms depicted on the left side of Reaction (2) and on the right side of Reaction (4). Every protein molecule will make stochastic conformational excursions among all its conformational substates. Only a fractional part of the molecules has at every instance the complete H bond chain functionally arranged as of the left side of Reaction (2), thus being able to have its Y_Z oxidized very rapidly. All the other conformational substates will relax to the functional state at different pace, leading to the observed multiexponential kinetics of the $P680^{\cdot +}$ reduction.

5. Comments

It appears important to include consideration of the protein as a dynamic equilibrium between conformational substates to obtain a realistic model explaining the kinetics observed for the reactions of PSII leading to water oxidation.

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