Indirect evidence for structural changes coupled with $Q_B^{-\bullet}$ formation in photosystem II

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Abstract The thermal blockage of $Q_A^{-\bullet}$ oxidation was analysed in PS II membrane fragments by monitoring flash-induced changes of the relative fluorescence quantum yield as a function of temperature. The results obtained reveal: (a) in dark-adapted samples the fraction of $Q_A^{-\bullet}$ that is not reoxidised within a time domain of 10 s after the actinic flash increases with lowering the temperature (half-maximum effect at 250–260 K), (b) at low temperatures where $Q_A^{-\bullet}$ generated in dark-adapted samples remains almost completely reduced, a significant extent of $Q_A^{-\bullet}$ reoxidation arises when samples are used that were preilluminated at room temperature by one saturating flash followed by rapid freezing before performing the experiment, and (c) the extent of $Q_A^{-\bullet}$ that is reoxidised at 258 K exhibits a characteristic binary oscillation as a function of the number of preillumination flashes given at room temperature. Based on these data it is inferred that Q_B and $Q_B^{-\bullet}$ are located at different equilibrium positions in the Q_B site. As a consequence the formation of $Q_B^{-\bullet}$ is coupled with significant structural changes that require sufficient flexibility of the protein matrix. This general feature corresponds with a recently proposed model for the acceptor side reactions of anoxygenic bacteria [Stowell, M.H.B., McPhillips, T.M., Rees, D.C., Soltis, S.M., Abresch, E. and Feher, G., Science 276 (1997) 812–816].

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Key words: Photosystem II; Plastoquinone formation; Thermal block

1. Introduction

Structure-function relationships are of paramount importance for most processes in enzymology. Proteins provide a unique material for optimisation of chemical reactions in order to achieve any degree of efficiency and regulatory control that is required for a particular biological function. The coupling of redox reactions with protein dynamics gives rise to characteristic temperature dependences. One prominent example is the quinol formation in reaction centres of anoxygenic purple bacteria and photosystem II (PS II) of all oxygen-evolving photosynthetic organisms. This process occurs via a sequence of two univalent reduction steps at a quinone molecule transiently bound to a special protein pocket referred to as Q_B site (for a review, see [1,2]). It was found that the first electron transfer to Q_B with $Q_A^{-\bullet}$ acting as reductant becomes completely blocked at lower temperatures in both purple bacteria [3,4] and PS II [5]. Typical values for half-maximum effects of about 210 K and 260 K were obtained for chromatophores of the purple bacterium *Rhodospirillum rubrum* [3] and PS II membrane fragments from spinach [6], respectively. The latter value is confirmed by independent measurements of flash-induced absorption changes [7]. Comparative Mössbauer spectroscopic studies revealed that these features correlate with significant changes of protein flexibility [3,8]. A strikingly different pattern was observed when using reaction centres from *Rhodobacter sphaeroides* that were frozen under illumination. In these samples the rate of $Q_A^{-\bullet}$ reoxidation was increased by several orders of magnitude [4]. These data strongly supported the idea that the electron transfer from $Q_A^{-\bullet}$ to Q_B is accompanied by significant structural change(s) that is (are) prevented when the protein flexibility is 'frozen out' at lower temperatures. Direct experimental evidence for this idea was provided by a recent report on structural analysis of high resolution X-ray diffraction data from crystals of *Rhodobacter sphaeroides* [9]. It was shown that the head of the ubiquinone molecule in the Q_B site is displaced by about 5 Å from its equilibrium position in state Q_B and rotates by about 180° around the fixed isoprenoid chain when $Q_B^{-\bullet}$ is formed. Analogous information is not yet available for PS II. However, for two reasons it appears very likely that the electron transfer from $Q_A^{-\bullet}$ to Q_B in PS II is coupled with significant structural changes analogous to those unravelled in purple bacteria: (i) there exist striking structural and functional similarities of the acceptor side in purple bacteria and PS II [10,11] and (ii) the temperature dependence of $Q_A^{-\bullet}$ reoxidation by Q_B exhibits virtually the same correlation with protein flexibility monitored via Mössbauer spectroscopy in both types of organisms [3,8].

In order to provide further evidence for this idea the temperature dependence of $Q_A^{-\bullet}$ reoxidation was analysed in PS II membrane fragments as a function of the redox state of Q_B. The results obtained strongly support a mechanism where the formation of $Q_B^{-\bullet}$ is accompanied by significant structural changes.

2. Materials and methods

PS II membrane fragments were prepared from spinach according to the procedure of Berthold et al. [12] with significant modifications outlined by Völker et al. [13]. After the final isolation step the material was resuspended in 10 mM MES/NaOH (pH = 6.5), 15 mM NaCl, 4 mM $MgCl_2$ and 0.4 M sucrose to Chl concentrations of about 5 mg/ml. These samples were frozen in small aliquots in liquid nitrogen and stored for at least 6 months at −80°C until used. During this very long storage time long living radicals (Y_D^{ox}, $Q_B^{-\bullet}$) decay to a large extent.

Flash-induced changes of the relative fluorescence quantum yield were monitored with home-built equipment [14].

The calculation of the normalised $Q_A^{-\bullet}$ population from the fluorescence data was performed on the basis of the non-linear relationship

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Abbreviations: P680, photochemically active primary donor of PS II; Pheo, pheophytin; PS II, photosystem II; Q_A, Q_B, primary and secondary plastoquinones-9 of PS II; UQ, ubiquinone

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described by Joliot and Joliot [15] as outlined in [16]. The same values of the fitted parameters were used as in [16], i.e. the fraction of photosynthetic units that are connected by excitation energy transfer ($a = 0.7$) and its probability of emerging from PS II centres in the 'closed' state P680PheoQ$_A^-$ ($P = 0.5$).

3. Results and discussion

The left side of Fig. 1 shows transients of the normalised $Q_A^{-\bullet}$ population that were calculated from the flash-induced changes of the fluorescence yield measured in dark-adapted samples at different temperatures. As previously shown, the time course of $[Q_A^{-\bullet}(t)]$ can be described by a triexponential decay and a fraction that does not relax within a time domain of 10 s after the actinic flash (for further details, see [6,17]):

$$\frac{[Q_A^{-\bullet}(t)]}{[Q_A^{-\bullet}(t=0)]} = \sum_{i=1}^{3} a_i \exp(-t/\tau_i) + a_4 \quad (1)$$

where a_i are the normalised amplitudes with $\sum_{i=1}^{4} a_i = 1$ and t_i ($i = 1,2,3$) the corresponding lifetimes.

The different kinetics are ascribed to $Q_A^{-\bullet}$ reoxidation by (i) Q_B ($Q_B^{-\bullet}$) in an occupied site (a_1, τ_1), (ii) a PQ molecule which diffuses to an empty Q_B site (a_2, τ_2) and (iii) by recombination reaction with the donor side (a_3, τ_3). The first reaction dominates in samples with an intact Q_B site and water-oxidising complex [17].

The extent of a_4 in dark-adapted samples reflects the equilibrium constant of the reaction $Q_A^{-\bullet}Q_B \leftrightharpoons Q_A Q_B^{-\bullet}$ at room temperature (for details, see [6,17]). The marked increase of a_4 at lower temperatures was interpreted as reflecting the thermal blockage of $Q_A^{-\bullet}$ reoxidation by Q_B [6]. A thermodynamic analysis of the temperature dependence of the presumed equilibrium between the active (A) and inactive state (I) for $Q_A^{-\bullet}$ reoxidation revealed that the transition from I to A is an entropy-driven process [6]. The increase of the entropy in state A is indicative of increased protein flexibility. Accordingly, the electron transfer from $Q_A^{-\bullet}$ to Q_B was inferred to depend strongly on the dynamics of protein environment of Q_B. This idea is supported by recent Mössbauer studies ([8], Garbers et al., unpublished results). If the formation of $Q_B^{-\bullet}$ is accompanied by a change of the equilibrium position of the plastoquinone molecule this structure should be retained when the protein flexibility becomes restrained at lower temperatures. Therefore the reoxidation of $Q_A^{-\bullet}$ by $Q_B^{-\bullet}$ is expected to remain intact at temperatures where the transfer from $Q_A^{-\bullet}$ to Q_B is already blocked. The population of $Q_B^{-\bullet}$ is drastically increased by illumination of dark-adapted samples with one saturating flash at room temperature and consequently a_4 should be smaller in these samples at low temperatures. To test this idea, experiments were performed with samples that were preilluminated by one saturating flash at room temperature, rapidly frozen in liquid nitrogen and subsequently poised at the described temperature in the range of $-55 \leq \vartheta \leq +25$°C. After reaching this temperature the sample was illuminated by an actinic flash and the fluorescence transient monitored. Typical traces of $[Q_A^{-\bullet}(t)]$ calculated from these measurements are depicted on the right side of Fig. 1.

An inspection of the data in Fig. 1 readily reveals two striking features: (i) the relaxation becomes slower and the fraction of the non-relaxing $Q_A^{-\bullet}$ (a_4) drastically increases with decreasing temperature, and (ii) the contribution of the non-relaxing fraction at subzero temperatures is markedly reduced in samples that were preilluminated by a saturating flash before freezing. In order to illustrate this effect more clearly the temperature dependence of a_4 was calculated for both sample types. The results obtained are depicted in Fig. 2. A pronounced difference is observed. This general feature is in agreement with the idea that the equilibrium positions of Q_B and $Q_B^{-\bullet}$ in the binding site are different. After fixing them by 'freezing out' the protein flexibility, electron transfer from $Q_A^{-\bullet}$ can only occur in PS II centres which contain $Q_B^{-\bullet}$ in its Q_B site. Accordingly, in the ideal limit of this simple mechanism, in all centres attaining the electronic configuration $Q_A Q_B^{-\bullet}$, the

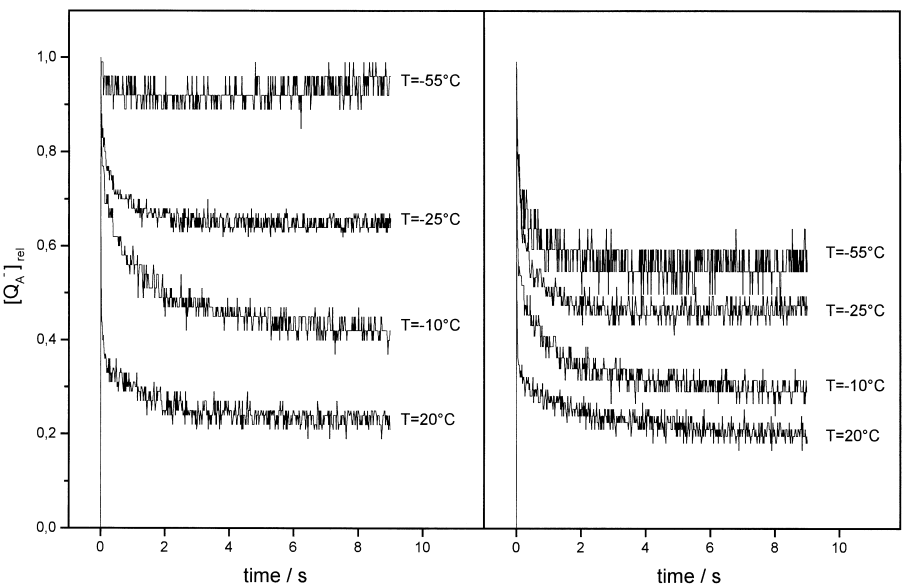

Fig. 1. Normalised $Q_A^{-\bullet}$ concentration as a function of time, calculated from traces of flash-induced transients of relative fluorescence yield measured in PS II membrane fragments that were equilibrated at the indicated temperatures. Left side: measurements with dark-adapted samples. Right side: measurements with samples that were preilluminated by one flash at room temperature, rapidly frozen in liquid nitrogen and subsequently equilibrated at the desired temperature. For experimental conditions and further details, see Section 2 and text.

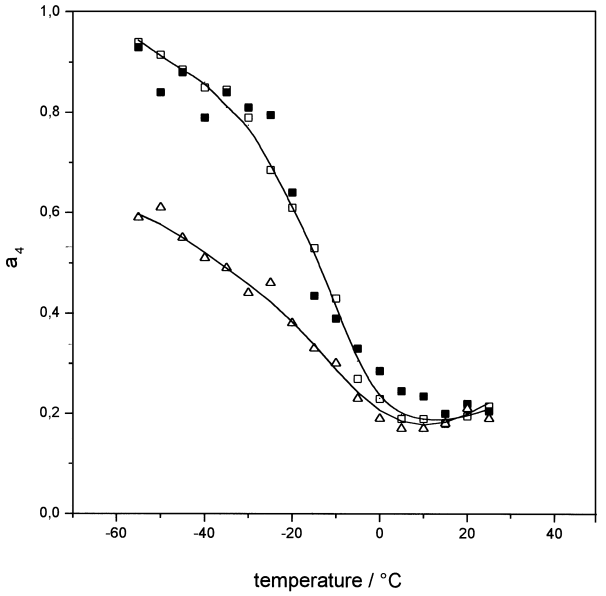

Fig. 2. Temperature dependence of the normalised amplitude a_4 of $[Q_A^{-\bullet}(t)]$ that remains reduced at 10 s after the actinic flash in PS II membrane fragments. Open squares: dark-adapted samples suspended in H_2O buffer; filled squares: dark-adapted samples suspended in D_2O buffer; open triangles: samples suspended in H_2O buffer and preilluminated by one saturating flash as described in Fig. 1.

$Q_A^{-\bullet}$ reoxidation should sustain at low temperatures and a_4 is expected to remain constant. This is obviously only partially satisfied. Different mechanisms could be responsible for this phenomenon (e.g. partial $Q_B^{-\bullet}$ reoxidation during the freezing procedure following the preillumination flash, incomplete Q_B reduction, sample heterogeneity, etc.). At present no unambiguous answer can be offered and therefore we will refrain from further speculation on this particular point because it is not essential for the general conclusions of this study

The dynamics of proteins can be affected by hydrogen bonds. It has previously been shown that the kinetics of $Q_A^{-\bullet}$ reoxidation are retarded by a factor of about 2 when exchangeable protons are replaced by deuterons [17] while the reactions leading to formation of the stabilised radical pair $P680^{+\bullet}Q_A^{-\bullet}$ and the reduction of $P680^+$ by Y_Z in the submicrosecond time domain exhibit only marginal kinetic H/D isotope exchange effects [18,19]. Based on the finding that the thermal stability of the oxygen evolution capacity is enhanced by about 5°C when thylakoids or PS II membrane fragments are suspended in D_2O [20,21], 6–7 hydrogen bonds were inferred to stabilise the protein matrix. In order to check if an H/D isotope exchange effect also modulates the protein mobility that is assumed to be required for Q_B reduction, comparative experiments were performed with dark samples suspended in D_2O. The results obtained are also shown in Fig. 2. An inspection of the data does not reveal any significant difference. Taking into account the experimental error of the data, at most two hydrogen bonds of moderate strength can be involved in the protein dynamics that is coupled with the formation of $Q_B^{-\bullet}$.

The results reported so far support the proposal of a structural change which is coupled with $Q_B^{-\bullet}$ formation. Straightforward and convincing evidence for this idea can be presented by showing that the pattern is specific for $Q_B^{-\bullet}$. One characteristic feature of the acceptor side reactions in purple bacteria and PS II is the binary oscillation of the $Q_B^{-\bullet}$ population when dark-adapted samples with a high initial Q_B population probability are excited with a train of saturating flashes at room temperature (for a review, see [1,2]). This pattern can therefore be used as a fingerprint to label $Q_B^{-\bullet}$ as the species that is responsible for sustaining electron transfer from $Q_A^{-\bullet}$ to the Q_B site at low temperatures. To check this most important point, dark-adapted PS II membrane fragments were illuminated at room temperature with a definite number of saturating flashes, rapidly frozen in liquid nitrogen and subsequently thermally equilibrated at the temperature where the flash-induced transient of the fluorescence quantum yield is monitored. The selection of this temperature is essential because two indispensable prerequisites have to be satisfied simultaneously in order to permit an unambiguous interpretation of the data: (i) a significant fraction of the electron transfer from $Q_A^{-\bullet}$ to Q_B is thermally blocked and (ii) the freezing of the S_i state transitions in the water-oxidising complex is negligibly small. A temperature of 258 K is a suitable compromise for conditions (i) [6,7] and (ii) [22]. Furthermore, at this temperature the recombination reaction of $Q_A^{-\bullet}S_2$ is of the order of a few minutes [23] and therefore this reaction does not affect the measurements. A possible reduction of S_2 by Y_D [24] would even lengthen the lifetime of $Q_A^{-\bullet}$ because the S_i state cannot reoxidise $Q_A^{-\bullet}$ [23]. Another important parameter is the PQ pool size in PS II membrane fragments. Its value was found to be 2–3 molecules per PS II [25] so that no limitations occur when the samples are preilluminated by four flashes. Fig. 3 shows the a_4 values gathered from data analysis (see above) as a function of the number of preillumination flashes. A pronounced binary oscillation pattern is obtained. This provides strong support for the idea that in PS II complexes kept in the state $Q_AQ_B^{-\bullet}$ the $Q_A^{-\bullet}$ formed by an actinic flash becomes reoxidised at subfreezing temperatures, where the electron transfer from $Q_A^{-\bullet}$ to Q_B is already blocked. This conclusion is corroborated by analogous measurements in thylakoids which exhibit the same characteristic binary oscillation pattern (data not shown).

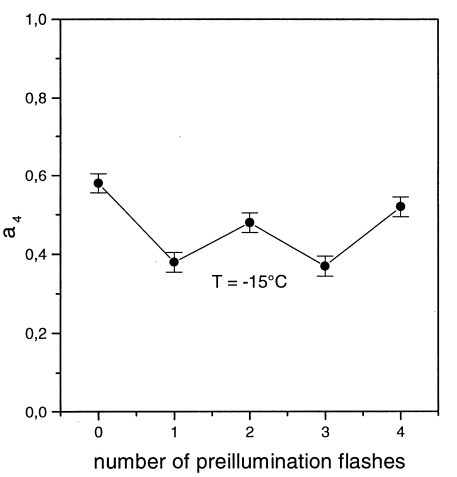

Fig. 3. Normalised amplitude a_4 of $[Q_A^{-\bullet}(t)]$ as a function of the number of preflashes given to PS II membrane fragments at room temperature followed by rapid freezing and thermal equilibration at −15°C. Other experimental conditions as in Fig. 1.

4. Conclusions

The results reported in this study show that in PS II membrane fragments the electron transfer from $Q_A^{-\bullet}$ to $Q_B^{-\bullet}$ can occur at low temperatures where the corresponding reaction from $Q_A^{-\bullet}$ to Q_B is already thermally blocked. As a consequence it is inferred that Q_B and $Q_B^{-\bullet}$ are located at different equilibrium positions within the Q_B site. This assumption implies that the reduction of Q_B to $Q_B^{-\bullet}$ in PS II is accompanied by significant structural changes. These general features can be fully explained by a model analogous to that recently proposed (on the basis of X-ray crystallographic structural analysis) for the reaction pattern of the acceptor side in purple bacteria [9]. In this respect it has to be emphasised that in spite of striking similarities neither the structure of the reacting molecules that affects the conformation of semiquinones (UQ in purple bacteria versus PQ in PS II [26]) nor the detailed structure of the Q_B site (as deduced from model studies [27] and reflected inter alia by marked differences in the affinity to herbicides [28]) are identical in both types of organisms. Therefore quantitative differences are expected to arise. However, the basic principle of a coupling between $Q_B^{-\bullet}$ formation and protein flexibility appears to be the same.

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