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Rapid report

Tyrosine Y_Z and Y_D of photosystem II Comparison of optical spectra to those of tyrosine oxidised by pulsed radiolysis

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Abstract

We have compared the optical spectrum of tyrosine oxidised in aqueous solution by pulsed radiolysis with spectra of redox active tyrosines Y_Z and Y_D of photosystem II. This indicates a ''tyrosinate'' state for these tyrosines and also casts doubt on the assumption that Y_Z and Y_D optical spectra are very similar in different photosystem II preparations. It suggests that further optical spectra of Y_Z in more intact oxygen-evolving preparations are needed before the role of Y_z in water oxidation can be clarified. © 1998 Elsevier Science B.V.

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Photosystem II (PSII) is the membrane–protein complex which catalyses electron transfer from water to plastoquinone [1–3]. Most cofactors are bound to core polypeptides termed D1 and D2. Absorption of light leads to photo-oxidation of the reaction center chlorophyll, P680. Oxidised P680 ($P680^+$) is reduced by electrons from the water oxidising complex (WOC) via the intermediate D1 tyrosine 161, Y_Z [4–6]. A second redox active tyrosine residue, Y_D, has been identified as tyrosine 161 of D2 [7,8]. D2His 190 probably forms a hydrogen bond to Y_D, which is located in a relatively hydrophobic pocket [9–11]. With Y_Z, which is located in D1 in a position symmetrical to Y_D [12], the situation is less clear. A hydrogen bond involving Y_Z is detected but it appears disordered in the Mn-depleted preparations used [13–15]. The mutation of D1His 190 suggested that it was not an acceptor for the Y_Z proton [16]. Y_Z is oxidised by $P680^+$ on the nanosecond time scale but this is dramatically slowed by removal of the WOC. During its cycle, the WOC passes through different redox states termed S-states, $S_0 \cdots S_4$, electrons being removed from S_0–S_4 and O_2 being evolved at S_3–S_0 [17]. Y_Z^+ reduction is S-state dependent, from 50 μs–1.5 ms respectively for the S-state transitions between S_0 and S_4 [18,19]. Oxidised Y_D is isolated and relatively stable, the E_m of Y_D^+/Y_D is estimated at +750 mV (vs. NHE) whilst the Y_Z^+/Y_Z couple is estimated at +950–1000 mV (vs. NHE) [20]. Hypotheses suggesting how Y_Z and the Mn cluster may interact in the mechanism of water [15,21,22] suggest that on oxidation, Y_Z deprotonates forming the neu-

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tral radical detected by EPR. This in turn is involved in a rate-limiting H-atom abstraction from water bound to the WOC. However for technical reasons most of the recent studies on Y_Z have been performed using samples where the WOC is inhibited or absent, which in turn affects the properties of Y_Z. The role for Y_Z in these recent models is difficult to rationalise with the rapid electron transfer role which would be expect to occur with low reorganisation energy and also with the detection of electrochromic shifts near 440 nm which accompany oxidation of Y_Z and Y_D [23]. The latter indicates that the charge i.e. proton is retained near Y_Z or Y_D.

In PSII, the hydroxyl group of Y_Z and Y_D is involved in hydrogen bonding. The properties of the O–H bond in tyrosine will depend, among other factors, on the basicity of the bound H^+-acceptor. Here, we hypothesise that the changes in optical spectra observed on oxidation of the tyrosine residues in PSII reflect mainly the extent of dissociation of the O–H group prior to oxidation.

Pulse radiolysis. Optical spectra of $Y_Z^{\cdot}$–Y_Z and $Y_D^{\cdot}$–Y_D have been compared previously to a spectrum attributed to the tyrosine neutral radical which was obtained by UV flash photolysis [24]. However the photolysis initiates other reactions in addition to ionization and therefore the spectrum in [24] contains contributions from other species such as the triplet and *p*-hydroxy benzyl radical. Therefore, we decided to characterise spectroscopically the tyrosyl radical using the clean one-electron oxidation by the azidyl radical ($N_3^{\cdot}$) generated radiolytically. The experiments were carried out with a pulse radiolysis system based on a computer-controlled 6 MeV linear accelerator that delivered electron pulses of 0.7 ms. Doses/pulse of ca. 2.5 Gy were used, which generated $< 2\,\mu M$ radicals. L-Tyrosine from Sigma was used without further purification. Solutions were prepared using water purified by a Millipore Milli-Q system. Experiments were performed at room temperature. Before adding tyrosine, the solutions were purged with nitrogen to prevent auto-oxidation. Before irradiation, solutions were saturated for ca. 30 min with oxygen-free nitrous oxide (British Oxygen Company). The tyrosine solutions (0.2 mM) contained sodium azide (0.05 M) and either 10 mM sodium hydroxide or 10 mM phosphate, for experiments at pH 12 and 7.8, respectively. Under these conditions, irradiation generates the azidyl radical ($N_3^{\cdot}$) in $< 1\,\mu s$ [25]. The latter reacts with tyrosine to yield the tyrosyl radical with rate constant $k = 1.0 \times 10^8\,M^{-1}\,s^{-1}$ [26]. The radical has an absorbance maximum at 410 nm which was used to monitor the reaction. Under the conditions of our experiment, the reaction was complete in $< 100\,\mu s$. At pH 12, tyrosine deprotonates to tyrosinate and the reaction rate was increased. From the observed rate of build-up of absorbance at 410 nm, we estimate the rate of reaction of $N_3^{\cdot}$ with tyrosinate as ca. $3 \times 10^9\,M^{-1}\,s^{-1}$, i.e. diffusion limited. The absorption changes after completion of the reaction were measured which yielded the difference spectra shown. Calibration in extinction coefficient units was done assuming complete conversion of $N_3^{\cdot}$ to tyrosyl radical and radiation chemical yield of $N_3^{\cdot}$ $0.7\,\mu mol\,J^{-1}$. At pH 7.8, the transient absorption spectrum was very similar in the visible range. However, the apparent yield was only ca. 44% of that measured at pH 12. We attribute this to the slower oxidation of tyrosine (relative to tyrosinate) which allows a contribution from radical–radical decay. In order to compensate for this, the spectra were normalised to the same absorption at 410 nm.

Sources of the spectra. $Y^{\cdot}$–YH and $Y^{\cdot}$–Y^- by pulse radiolysis and [24]; $Y_D^{\cdot}$–Y_D [23]; $Y_Z^{\cdot}$–Y_Z [23,27–36]. Spectra were digitised manually from the published spectra.

Fitting procedure. Two gaussian lines were fitted to the UV portion of the spectra, in energy scale by a non-linear least squares procedure. The equation used was

$$y = \frac{A_1}{w_1\sqrt{\pi/2}}\,e^{-2(E-E_1)^2/w_1^2} + \frac{A_2}{w_2\sqrt{\pi/2}}\,e^{-2(E-E_2)^2/w_2^2} + c \qquad (1)$$

where E_1 and E_2 are the central energies, A_1 and A_2 are the peak areas, w_1 and w_2 are the peak widths and c is a baseline correction. Values of w of 1700–5000 cm^{-1} resulted from the fits.

The behaviour of phenoxyl radicals in aqueous solution is well characterised. Phenols have pK_a values ≈ 10 ($pK_a = 10.1$ for tyrosine) but on one-electron oxidation they yield radical cations with dissociation constants that are over 10 orders of magnitude

higher (e.g. the phenol radical cation $pK_a = -2$) [37]. Therefore, oxidation of phenols or phenolates (such as tyrosine) in aqueous solution at pH > 0 is accompanied by deprotonation to give neutral phenoxyl radicals.

Difference spectra (relative to the parent tyrosine or tyrosinate) obtained by pulse radiolysis at pH 7.8 and pH 12 are shown in Fig. 1. The differences largely reflect those of the tyrosine (YH) and tyrosinate (Y^-) spectra, which is red-shifted, rather than the tyrosine radical which is neutral in both cases. The spectrum is quite distinct from that obtained by UV flash photolysis in [24].

To compare the spectra shown in Fig. 1 with those obtained from the tyrosine radicals of PSII we have digitised the spectra in the literature which consist of one $Y_D^{\cdot}-Y_D$ spectrum [23] and several $Y_Z^{\cdot}-Y_Z$ spectra. The latter include those from a variety of preparations, varying from oxygen-evolving thylakoid membranes to Mn-depleted detergent-treated PSII preparations [23,27–36]. The spectra were converted to energy scale and the two main bands were analysed. In Fig. 2, the aqueous solution, the $Y^{\cdot}-Y^-$ (Fig. 2A), $Y^{\cdot}-YH$ (Fig. 2B) difference spectra are compared to spectra of $Y_D^{\cdot}-Y_D$ (Fig. 2(G)) [23] and $Y_Z^{\cdot}-Y_Z$ (Fig. 2C–F, H) [23,28,31,33,36] obtained from the literature. These show differences when comparing Mn-containing and Mn-depleted preparations (Fig. 2C–E vs. F–H).

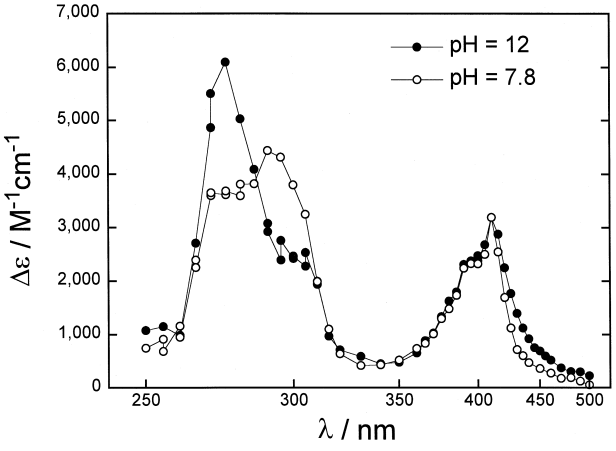

Fig. 1. Oxidised minus reduced optical difference spectrum of tyrosine obtained by pulsed radiolysis at pH 7.8 and pH 12. See text for details.

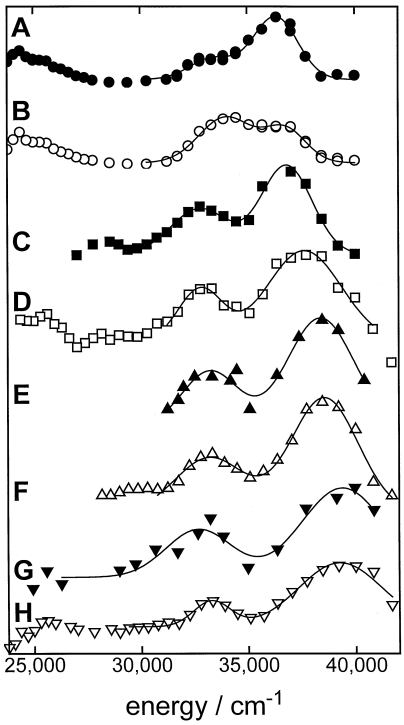

Fig. 2. Energy scale optical spectra of PSII tyrosine radicals from the literature compared to those obtained by pulsed radiolysis of tyrosine in this study. (A) Pulse radiolysis at pH 12, $Y^{\cdot}-Y^-$. (B) Pulse radiolysis at pH 7.8, $Y^{\cdot}-YH$. (C–F)&(H) $Y_Z^{\cdot}-Y_Z$ spectra from (C) [31]. (D) [36]. (E) [33]. (F) [28]. (H) [23]. (G) $Y_D^{\cdot}-Y_D$ spectrum from [23]. See text for further details.

The results of the fit of two gaussian bands to the experimental spectra are summarised in Table 1. The two apparent bands have similar energies in the $Y^{\cdot}-YH$ and $Y^{\cdot}-Y^-$ spectra but the ratio of the intensities of the low energy to high energy band is far higher in the $Y^{\cdot}-YH$ spectrum, reflecting the underlying absorption of tyrosinate. It can be seen

Table 1
Results of the fitting of a two-Gaussian function to the experimental difference spectra

	$E_1/10^3\,cm^{-1}$	$E_2/10^3\,cm^{-1}$	A_1/A_2	
$Y^{\cdot}-YH$	34.0±0.1	36.8±0.1	1.7 ±0.2	This work
$Y^{\cdot}-Y^-$	33.4±0.2	36.4±0.1	0.4 ±0.1	This work
$Y_D^{\cdot}-Y_D$	32.7±0.4	39.4±0.3	0.4 ±0.1	[23]
$Y_Z^{\cdot}-Y_Z$	32.8±0.1	36.8±0.1	0.6 ±0.2	[31]
$Y_Z^{\cdot}-Y_Z$	32.8±0.2	37.6±0.1	0.4 ±0.3	[36]
$Y_Z^{\cdot}-Y_Z$	33.3±0.3	38.4±0.2	0.4 ±2.6	[33]
$Y_Z^{\cdot}-Y_Z$	33.3±0.1	39.3±0.1	0.18±0.03	[23]
$Y^{\cdot}-YH$	33.7±0.1	40.1±0.1	0.2 ±0.1	[24]

that the photosystem II spectra show a difference in both lineshape and position (shift to higher energy) compared to the aqueous $Y^{\cdot}$. The spectra of Y_D and Y_Z as well as their radicals are influenced by the environment. Spectral shifts and broadening can result from interaction with nearby aromatic residues, nearby charges on protein side chains or cofactors and by hydrogen bonding. The relative intensities and maxima of the two apparent bands (Table 1) suggests that $Y_D^{\cdot}$–Y_D resembles a blue shifted $Y^{\cdot}$–Y^{-} spectrum. The ratio of intensities of the two bands in $Y_D^{\cdot}$–Y_D is similar to that of $Y^{\cdot}$–Y^{-} and far lower than that in $Y^{\cdot}$–YH. In $Y_Z^{\cdot}$–Y_Z the intensity ratio in Mn-depleted samples is even lower than that in $Y^{\cdot}$–Y^{-}. The low ratio of the intensities of the two bands in the PSII spectra possibly indicates the engagement of the tyrosine proton in a strong hydrogen bond that makes the residue resemble tyrosinate. The intensity ratios change for $Y_D^{\cdot}$–Y_D and $Y_Z^{\cdot}$–Y_Z, possibly reflecting differences in the hydrogen bonding of the hydroxyl group, increasing the ''tyrosinate-like'' state for Y_Z.

In comparing Y_D and Y_Z, Diner [23] suggested three ways that the redox differences between Y_D and Y_Z could be achieved. These are by changes in the hydrogen bond strength of the oxidised/reduced forms, the pK_a of proton acceptor or in the charge stabilisation. A similar electrochromic shift is observed upon oxidation of either Y_D and Y_Z indicating that the charge is retained nearby in both. Differences in hydrogen bonding were discounted as experimental data indicates hydrogen bonding of similar strength, but more delocalised in Y_Z [13–15]. However this was only measured for the $Y_Z^{\cdot}$ species and hydrogen bonding involving Y_Z may involve different amino acid side chains, the polypeptide backbone or perhaps a cofactor (Cl^{-}) which could result in a lower pK_a for the proton acceptor. The proton acceptor itself may also be involved in further hydrogen bonding or protonation which could be the source of the proton observed to be released on oxidation of Y_Z. One of the main problems with analysis of $Y_D^{\cdot}$–Y_D or $Y_Z^{\cdot}$–Y_Z spectra is that most of the work has been done on samples where some PSII polypeptides and the Mn cluster of the WOC are absent. As the Mn cluster is thought to be near Y_Z, the environment of Y_Z is probably disturbed in these samples. This may depend on the how the sample is purified and prepared as the most intact preparation (inside out thylakoids) shows the spectrum more closely resembling the aqueous solution spectrum (Fig. 2B and C). It is clear that more studies on Y_Z in oxygen evolving preparations are needed to establish the optical spectrum in oxygen-evolving membranes.

The self-exchange rates of phenoxyl radical/phenolate couples are higher than those for phenoxyl radical/phenol. Therefore, the more ''tyrosinate like'' state for Y_Z suggested in this study may help explain the fast rate of electron transfer from Y_Z. The hydrogen bonding explains the evidence for slow proton exchange and that proton release occurs from a group/s with a pK near 7 i.e. not from Y_Z [1,2]. This would better support a mechanism where oxidation of (tyrosinate-like) Y_Z causes a deprotonation elsewhere by an electrostatic effect of the charge retained near Y_Z. This deprotonation lowers the redox potential of the WOC and allows electron transfer to $Y_Z^{\cdot}$. With the proton strongly influenced by the nearby base, the tyrosine is effectively negative in the reduced form and neutral when oxidised, accounting for EPR and electrochromic shift data previously thought to be contradictory.

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