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EPR PROPERTIES OF IMMOBILIZED QUINONE CATION RADICALS AND THE MOLECULAR ORIGIN OF SIGNAL II IN SPINACH CHLOROPLASTS

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In Photosystem II, Z reduces P-680$^+$ and gives rise to a characteristic EPR signal, termed II_{vf} in oxygen-evolving chloroplasts and II_f in non-oxygen-evolving chloroplasts. Previous model compound studies of Signal II have centered on the immobilized anionic and neutral forms of semiquinone radicals. These radicals, however, exhibit an essentially structureless band shape in constrast to the partially resolved hyperfine pattern observed for Signal II. In the experiments reported here, we show that some cationic semiquinone radicals (e.g., 2-methyl-5-isopropylhydroquinone cation radical) exhibit band shape and microwave power saturation characteristics upon immobilization which are similar to Signal II. Examination of a series of quinone cation radicals shows that a Signal-II-like spectrum is observed when significant unpaired spin density occurs at a ring carbon to which a methyl group is bound. Whether this will occur for a specific quinone depends on the extent to which the peripheral substituent pattern favors a contribution from the antisymmetric benzenoid molecular orbital to the ground state of the radical. For the 2-methyl-5-isopropylhydroquinone cation radical, for example, a 26% contribution of this orbital is estimated. A plastoquinone cation radical in which the electron-donating ability of the quinol-OH groups has been decreased is compatible with antisymmetric orbital stabilization and, therefore, is identified as the Z^+ species. Hydrogen bonding of the quinol oxygen to hydrogen-donating amino acid residues in vivo plus an out-of-plane geometry for the quinol-OH groups is proposed to stabilize the antisymmetric orbital. The partially resolved structure of Signal II indicates that the antisymmetric orbital is the major contributor to the ground state; the principal hyperfine splitting in the spectrum arises from the 2-CH_3 group of the plastoquinone cation radical. The estimated electrode potential of the Z^+ radical is in close agreement with the in vitro electrode potential of quinone cation radicals.

Introduction

The broad, partially resolved structure of Signal II was first observed by Commoner et al. in 1956 [1]. In recent years, the discovery of fast transient components of this signal has indicated that the radical species giving rise to it acts as an intermediary electron carrier (Z^+) between the oxygen-evolving complex and P-680$^+$ [2]. The molecular origin of this signal still remains an open question, although various molecular structures, principally connected with the plastosemiquinone anion radical, have been proposed.

A plastoquinone precursor for Signal II was

Abbreviations: $TMQH_2^+$, trimethylhydroquinone cation radical; PQH_2^+, plastoquinone cation radical; P-680$^+$, primary donor chlorophyll of Photosystem II; PS II, Photosystem II; ENDOR, electron nuclear double resonance.

originally suggested by Weaver [3]. Subsequently, Kohl and Wood [4] showed that Signal II could be eliminated and reconstituted by extracting and adding back plastoquinone; they suggested that Signal II arose from a plastochromenoxyl radical [5]. More recently, Hales and Gupta [6,7] have shown that the spin density distribution of Signal II is closely related to the spin density distribution of a neutral plastosemiquinone radical and have proposed that a divalent alkaline earth ion in ion pair equilibrium with a plastosemiquinone anion radical sufficiently perturbs the spin density distribution to give rise to a Signal-II-type structure on immobilization.

The structural models proposed thus far, however, have limitations. While a plastochromenoxyl radical remains plausible, the model compound data are somewhat ambiquous (discussed in Ref. 5); moreover, its mode of generation from plastoquinone in vivo and its redox properties are uncertain. Despite the fact that computer simulation of the spin density distribution outlined by Hales and Gupta [6] does give rise to Signal-II-type structure, no experimental evidence exists which demonstrates that a perturbing divalent ion will produce such a redistribution of spin density in the semiquinone anion. Indeed, recent experimental studies of divalent ion pairing with the 2,6-di-*t*-butyl semiquinone anion [8] argue strongly against such a redistribution of spin density. In addition, the isotropic saturation characteristics of the neutral radical species [7] suggests that the divalent metal ion-semiquinone anion would not be expected to exhibit the anisotropic saturation properties characteristic of Signal II (see below). Finally, the electrode potential of the couple Z^{+}/Z has been estimated at +1.1 V [9]. The electrode potential of the $PQ^{-\cdot}/PQ$ couple has a value of −0.165 V [10], which renders $PQ^{-\cdot}$ an unlikely candidate as an oxidant of the oxygen-evolving complex.

We now propose an alternative explanation for the molecular origin of Signal II which involves a plastoquinone cation radical [11]. Certain hydroquinone cation radicals in which partial stabilization of the antisymmetric benzene molecular orbital occurs exhibit partially resolved structure and anisotropic saturation properties similar to Signal II. A plastoquinone cation radical in which a substantial stabilization of the antisymmetric orbital occurs is compatible with a Signal-II-type spectrum. The in vitro electrode potential estimated for the QH_2^{+}/QH_2 couple at pH 7, +920 mV [12], is also in accord with the known close involvement of Signal II with the oxygen-evolving complex of Photosystem II [2]. In the present experiments we have focused on Signal IIs but the identical lineshape and orientation of Signals IIs and IIf [15] indicate that the results and interpretations developed below apply to both species. Preliminary reports of this work have appeared [11,13].

Experimental

Spinach chloroplasts and Photosystem-II particles were prepared as previously described [14,15]. Chlorophyll concentrations were in the range 4–6 mg/ml. The cation radicals were prepared by treating the parent quinone (Aldrich reagent grade) with sodium dithionite in concentrated H_2SO_4 [16]. The 2-methyl-5-isopropylhydroquinone was a gift from Dr. Roger Prince. Concentrations of the hydroquinones were in the range 10^{-2}–10^{-3} M; the concentrations of radicals which formed were estimated to be 10^{-4}–10^{-5} M. After formation of the radical occurred, the sample was immobilized by freezing in liquid N_2. Formation of the correct radical species was established by recording spectra at room temperature which were checked against previously published data [16]. EPR spectra were recorded at X-band by using a Bruker ER200D spectrometer operating at temperatures and instruments settings indicated in the figure captions.

Results and discussion

In Fig. 1 the characteristic spectral shape of Signal II and the relationship of this shape to incident microwave power at room temperature are illustrated. The microwave power dependence of Signal II is unusual in that the center of the spectrum saturates at a faster rate than the wings. This initially led to the conclusion that more than one radical species was involved [17]. However, Hales and Gupta [6] showed that the unusual saturation behavior of Signal II arises from an orientation dependence in the saturation rate. This

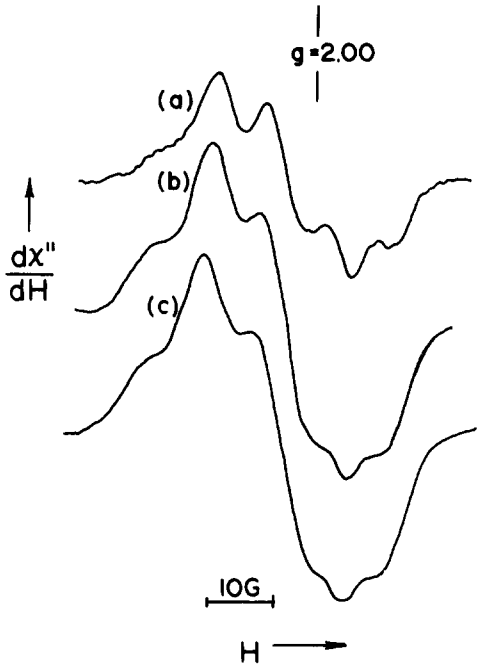

Fig. 1. Dependence of spectral shape of Signal II in chloroplast membranes as a function of microwave power. Temperature, 298 K. Under the conditions of this experiment the sample is not oriented significantly by the magnetic field. Modulation, 3.2 G. (a) Power, 1.0 mW; time constant, 200 ms; sweep time, 100 s; (b) power, 12.6 mW; time constant, 10 s; sweept time, 500 s; (c) power, 31.0 mW; time constant, 10 s; sweep time, 500 s.

is apparent in unoriented (powder) samples but is not observed in oriented samples. They suggested that Signal II arises from a single radical species which has anisotropic microwave power saturation characteristics. These results are confirmed by the data on Signal II in PS-II particles in Figs. 2 and 3. For PS-II particles at room temperature (Fig. 2), the magnetic field causes orientation of the membranes [15]. In this sample, anisotropic saturation is not observed. Freezing the same particles in the absence of a field removes the orientation effect and one observes Signal II saturation behavior (Fig. 3) similar to that observed in chloroplasts which do not orient at X-band under most conditions [15]. These experiments, combined with the data of Hales and Gupta [6], represent good evidence for the conclusion that the unique saturation properties of Signal II are derived from anisotropic saturation properties for different orientations of a single radical species.

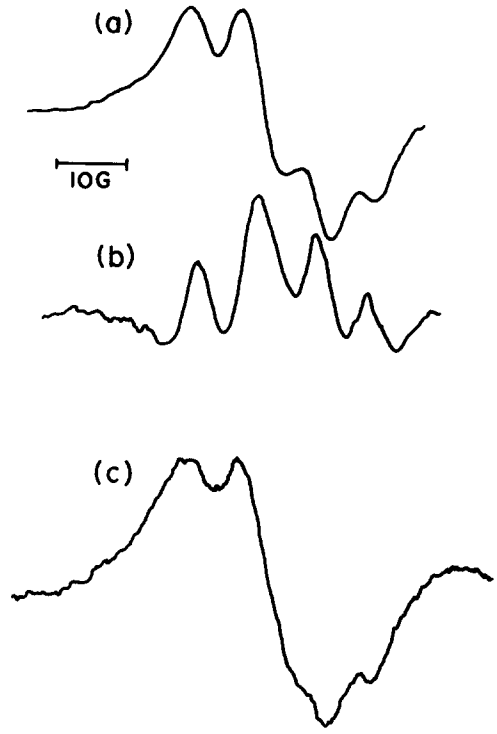

Fig. 2. Dependence of the spectral shape of Signal II in Photosystem-II particles in an EPR flat cell at room temperature as a function of microwave power. Under these conditions orientation of the membranes by the applied field occurs [15]. Modulation, 3.2 G. (a) 1st and (b) 2nd derivative spectra; power, 0.63 mW; time constant, 5 s; sweep time, 500 s. (c) power, 63 mW; time constant, 500 ms; sweep time, 200 s.

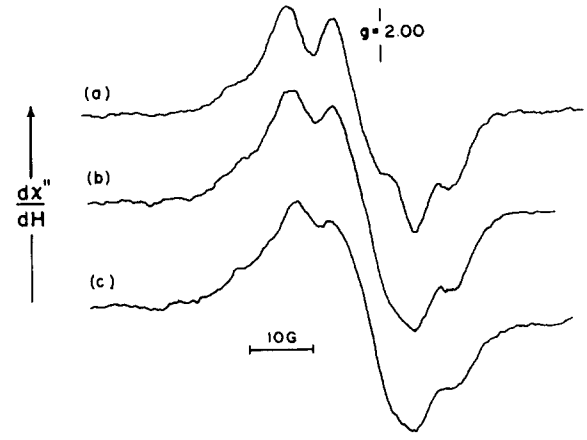

Fig. 3. Dependence of Signal II shape in Photosystem-II particles frozen at zero magnetic field as a function of microwave power. Temperature, 123 K. Under the conditions of this experiments, no sample orientation is expected. Modulation, 3.2 G; time constant, 500 ms; sweep time, 100 s. power, 0.5 mW (a); 6.3 mW (b); 15.8 mW (c).

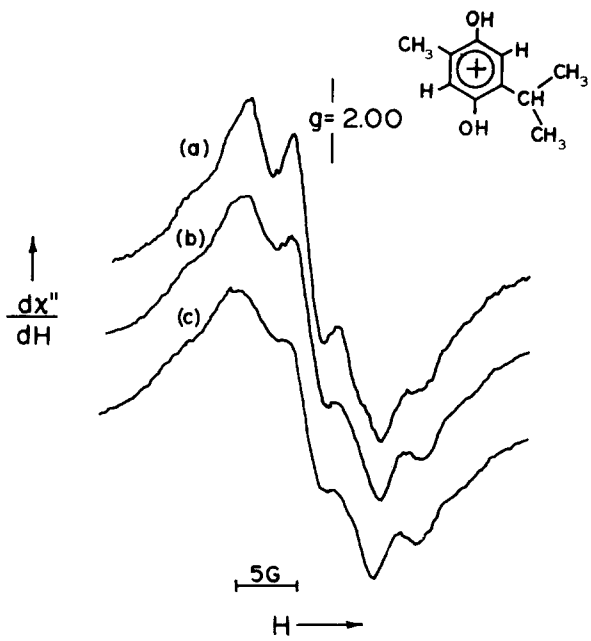

Fig. 4. Dependence of spectral shape of 2 methyl-5-isopropylhydroquinone cation radical on microwave power. Temperature, 123 K; modulation, 0.16 G; time constant, 500 ms; sweep time, 200 s. Power, 0.063 mW (a); 0.63 mW (b); 2.0 mW (c).

The data above indicate that Signal II arises from a unique chemical species and we now consider its molecular identity. In Fig. 4, the immobilized spectrum of the 2-methyl-5-isopropylhydroquinone cation radical is presented along with the dependence of the spectrum on microwave power. This radical has spectral shape and power dependence characteristics similar to that observed for Signal II. In Fig. 5, we also illustrate the similar spectral shape and power dependence characteristics of the 2,5-dimethylhydroquinone cation radical. In contrast to the above, however, Fig. 6 indicates that the cation radicals of 2,6-dimethylhydroquinone, 2,3,6-trimethylhydroquinone and plastoquinol exhibit essentially structureless band shapes on immobilization *, although anisotropic

* For the $TMQH_2^+$ radical some fine structure is observed. However, it is negligible in comparison to that observed for 2-methyl-5-isopropyl and 2,5-dimethyl hydroquinone radicals. For PQH_2^+ no such structure is observed. This may be due to extra fine structure due to γ proton interactions from the isoprenoid chain. These small splittings will give rise to extra broadening of the EPR band on immobilization and hence will mask any fine structure that would be observed.

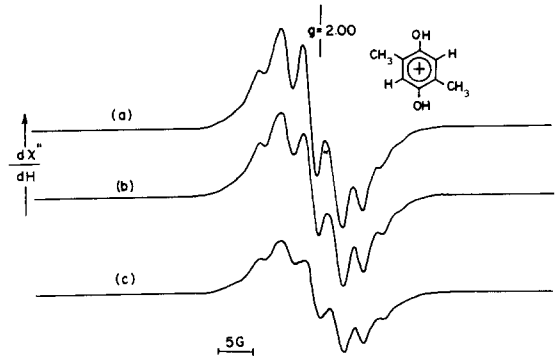

Fig. 5. Dependence of spectral shape of 2,5-dimethylhydroquinone cation radical on microwave power. Temperature, 123 K; modulation, 2.0 G; time constant, 100 ms; sweep time, 100 s. Power, 200 μW (a); 0.63 mW (b); 2.00 mW (c).

saturation properties were observed for these radical species.

These data show that certain quinone cation radicals reproduce the Signal II shape well and suggest that such a species is responsible for the in vivo spectrum. However, only some cationic quinone radicals exhibit partially resolved, Signal-II-like fine structure which indicates that it is necessary to examine how the spin density in the

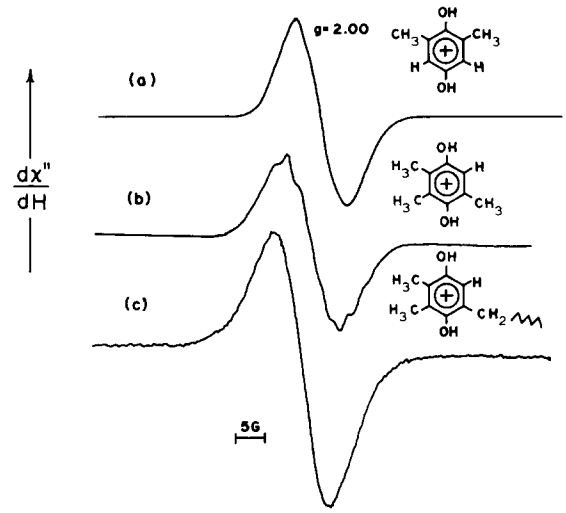

Fig. 6. Immobilized cation radical spectral for (a) 2,6-dimethylhydroquinone, (b) trimethylhydroquinone and (c) plastoquinone. Temperature, 123 K; power, 200 μW. (a) Modulation 1.6 G; time constant, 200 ms; sweep time, 200 s; (b) modulation, 1.6 G; time constant, 100 ms; sweep time, 100 s; (c) modulation, 0.5 G; time constant, 200 ms; sweep time, 100 s.

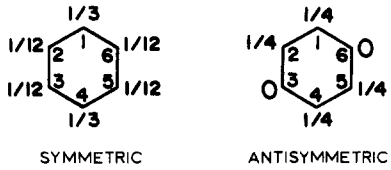

Fig. 7. Spin density distribution for the antisymmetric (A) and symmetric (S) molecular orbitals of benzene. Note that the numbering scheme for the antisymmtric orbital is altered compared to that normally used (e.g., Ref. 25). This has been done here in order to facilitate the discussion of *p*-semiquinone electron density distribution (-OH groups at the 1 and 4 positions) in terms of these orbitals (see also Ref 16).

aromatic ring varies with substitution on the ring. For quinone radicals, this can be understood by using McLachlan perturbation corrections to the Hückel LCAO method or by simply considering the radicals in terms of benzene-like molecular orbitals [16]. The Hückel LCAO approach predicts two degenerate molecular orbitals for the benzene molecule. These are usually designated as antisymmetric (A) and symmetric (S) and the spin density distribution for these orbitals is outlined in Fig. 7. For the benzene cation radical these orbitals are degenerate and the electron spends an equal amount of time in both orbitals. Introduction of substituents on the aromatic ring removes this degeneracy making one orbital more stable than the other. For a cation radical with a positive electron hole, four approximately equal electron-donating substituents (e.g., -OH, $-OCH_3$ or $-CH_3$) at the 1, 2, 4 and 5 positions will result in the highest occupied molecular orbital being of the antisymmetric (A) type. An interesting facet of this type of stabilization is that although the orbital has a node at the 3 and 6 positions, electron correlation effects give rise to a negative spin density at these positions. For unequally electron-donating substituents, one expects a different situation to occur. If the 1,4 substituents have a much greater effect one would expect the highest occupied molecular orbital to be of the symmetric type. However, as the electron-donating ability of the other pair of substituents at the 2 and 5 positions increases, a gradual transition from the symmetric type orbital to the antisymmetric type occurs. During this process, a mixing of both orbitals, with the spin density at each carbon being a weighted average dependent on the contribution from each orbital, takes place. An example of antisymmetric orbital stabilization is given by 1,2,4,5-tetramethylbenzene (durene) cation radical which exhibits methyl proton coupling values of 10.7 G with negligible values for the 3 and 6 α protons [18]. Using a Q^{CCH_3} value of 39.2 G [19] and the relationship $a_i = Q\rho_i$ [16], one obtains spin densities as the 1, 2, 4 and 5 ring carbons of 0.27 which are in excellent agreement with the prediction of Fig. 7.

For the unsubstituted 1,4-hydroquinone cation radical [16] the -OH substituents at the 1 and 4 positions would be expected to lead to stabilization of the symmetric orbital which is indeed confirmed by the ring proton splittings of 2.3 G($Q^{CH} = 27$ G) corresponding to a ring carbon spin density of 0.08, again in agreement with theoretical considerations (Fig. 7).

For 2,5-dimethylhydroquinone and 2-methyl-5-isopropylhydroquinone cation radicals we have a situation where there are unequally electron-donating substituents around the ring and, as mentioned above, a mixture of the two orbital might be expected. It is well known that the electron-donating ability of an -OH group is greater than a methyl group [16], and, as the hydroxyls are situated *para* to each other, the symmetric orbital should be more favored in the hybrid orbital which results. Experimentally, $-CH_3$ coupling values of 3.8 G and α proton values of 0.85 G have been observed [16]. Using a Q^{CCH_3} value of 29.9 G estimated by Sullivan et al. [20], one calculates a spin density value of 0.13 at the 2 and 5 positions. Based on the S orbital value for this ring position of 0.081 and the A orbital values of 0.27 estimated by Bullock and Howard [21] (this value allows for electron correlation effects), a 26% contribution of the A orbital to the spin density distribution can be estimated. The relatively large methyl coupling value, together with the low anisotropy of methyl splittings [22] and the small proton value, gives rise to the partially resolved hyperfine structure observed for the immobilized 2,5-dimethylhydroquinone cation radical at 123 K (Fig. 5). The splitting between the partially resolved peaks (approx. 4.0 G) in the immobilized spectrum is in good agreement with the coupling value observed for the methyl groups at room temperature and is

in accord with the above interpretation.

For the 2-methyl-5-isopropylhydroquinone cation radical the partially resolved structure can be explained in a similar fashion. Partial stabilization of the antisymmetric orbital occurs and, as outlined for the 2,5-dimethylhydroquinone radical, this gives rise to the partially resolved structure observed *. As noted for various semiquinone anions [23], the presence of a long chain side group such as isopropyl does not lead to any noticeable change in the spin density distribution around the ring.

In contrast to the previous two cases, Fig. 6 shows that for the 2,6-dimethylhydroquinone cation radical an essentially structureless band shape is observed on immobilization. Consideration of the orbital stabilization by ring substitution also gives a satisfactory explanation for this effect. From Fig. 7, it is apparent that 1,2,4,5 substitution leads to A orbital stabilization whereas 1,2,4,6 or 1,2,3,4 substitution does not. For 2,6-dimethyl substitution, then, the electron donating effect of the -OH groups will predominate and the A orbital will have a negligible contribution to the spin density distribution. This is confirmed experimentally by the room temperature spectrum which gives $-CH_3$ coupling values of 2.2 G and ring proton values of 2.0 G [16] which are in good agreement with values expected from the S orbital distribution. Therefore, both the ring protons and the methyl groups have similar coupling values

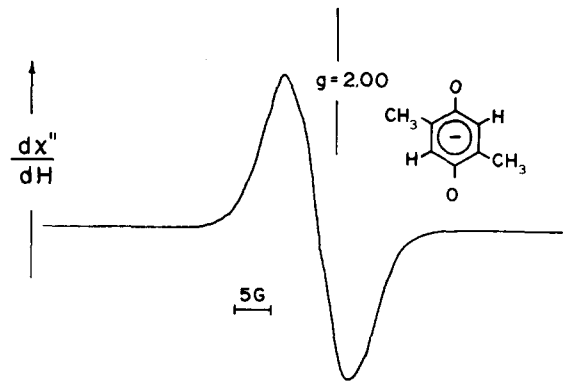

Fig. 8. Immobilized anion radical spectrum of 2,5-dimethylbenzosemiquinone. Temperature, 123 K; power, 0.63 mW; modulation, 1.25 G; time constant, 100 ms; sweep time, 100 s.

and, upon immobilization, a broadening of the total radical fine structure will occur giving rise to a symmetric band shape for the immobilized radical spectrum. In this case, the absence of large methyl groups splittings relative to the ring protons prevents the observation of any partially resolved structure.

In no case have we observed partially resolved structure in frozen solutions of methyl-subsituted benzosemiquinone anions. For example, in Fig. 8 we illustrate the immobilized anion spectrum of the 2,5-dimethyl-benzosemiquinone anion radical. For the anion radicals the O^- group has a greater electron donating ability than the -OH group [24] and the methyl substituents at positions 2 and 5 are unable to induce a mixing of the A orbital. This is confirmed by the room-temperature hyperfine coupling value of $a_{CH_3} = 2.25$ G and $a_H = 1.8$ G [16]. Again immobilization results in a broadening of the total hyperfine structure giving rise to the structureless band shape observed. Hales and

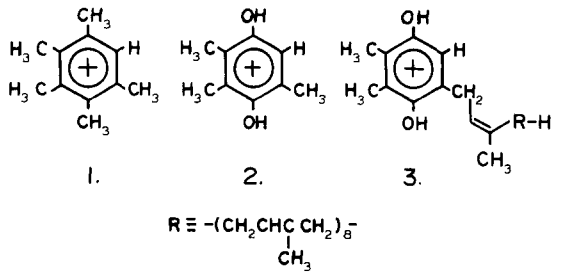

Fig. 9. 1. pentamethylbenzene; 2. trimethylhydroquinone; 3. plastoquinone.

* From Fig. 5, it can be seen that extra fine structure is observed on the wings of 2,5-dimethyl hydroquinone radical spectrum in comparison to the 2-methyl-5-isopropyl hydroquinone radical (Fig. 4). This arises from the greater number of protons contributing to the partially resolved structure of the 2,5-dimethyl hydroquinone radical spectrum. Due to the expected non-rotation of the bulky isopropyl group at low temperatures for the 2-methyl-5-isopropyl hydroquinone radical it can be concluded that the partially resolved structure observed for this radical is principally due to proton hyperfine interaction of the $2\text{-}CH_3$ group (see Fig. 4). The similar spectral characteristics of this radical to that observed for Signal II suggests that the Signal-II-partially-resolved structure is principally determined by hyperfine interaction with one rotating methyl group (i.e., the $2\text{-}CH_3$ group of plastoquinol, see Fig. 9). Recent orientation studies in our laboratory have confirmed this and these results will be discussed more fully in a future publication [41].

Gupta [6] have observed similar structureless band shapes for various neutral semiquinone radicals.

The extraction reconstitution experiments of Kohl and Wood [4] suggest that the precursor for Signal II is a plastoquinone. Moreover, the average splitting between the partially resolved peaks of Signal II, as determined from the second derivative spectrum (Fig. 2b), is approx. 8 G. For the model cation radicals of Figs. 4 and 5 the splittings are approx. 4 G. This suggests that if Signal II arizes from PQH_2^+ in vivo then the antisymmetric orbital is substantially stabilized and makes a greater than 50% contribution to the spin density distribution. However, oxidation of plastoquinol and its closely related analog, trimethylhydroquinone, in H_2SO_4 (Fig. 6) give rise to essentially structureless EPR band shapes (see first footnote in this section, p. 373). Based on arguments outlined above this would suggest that the spin density distribution is determined principally by the S orbital for these two radicals in vitro. The extraction/reconstitution data of Kohl and Wood [4] and the close similarity between the Signal-II shape and that observed for cation radicals in which partial stabilization of the A orbital has occurred, however, raise the question as to whether stabilization of the A orbital occurs for a plastoquinone cation radical in the membrane environment.

To examine this possibility, we consider the substitution pattern of the aromatic ring in plastoquinone. Fig. 9 shows the structures of the PQH_2, $TMQH_2$ and pentamethylbenzene cation radicals. For the pentamethylbenzene radical the ring substituents all have equal electron-donating abilities, total stabilization of the A orbital occurs, and methyl group coupling values at the 1,2,4 and 5 positions of 10.05 G with negligible values at the 3 and 6 positions are observed [25]. Similar stabilization of the A orbital has been shown to occur for the pentamethoxybenzene cation radical [26]. The stabilization of the S orbital for $TMQH_2^+$ and PQH_2^+ in vitro suggests that for these cases the greater electron-donating effect of the -OH group overrides the pentasubstitution pattern. If in vivo, however, the electron-donating ability of the CH_3 groups and isoprenoid chain is increased considerably and/or the electron-withdrawing ability of the -OH groups is decreased, then a situation would arise in which five approximately equally electron-donating substituents were situated on the aromatic ring and would lead to stabilization of the A orbital.

The first of these possibilities, increased electron donation by the methyl groups, is unlikely; however, a pertubation of the -OH group such that its electron donating ability is decreased is quite plausible. For example, binding of the PQH_2^+ radical can occur via hydrogen bonding of the -OH group to the protein membrane structure. This hydrogen bonding can occur via hydrogen donation by the quinone -OH group or, alternatively, the -OH group can act as a hydrogen acceptor. This will be determined by the presence of basic, neutral or acidic amino acid residues at the quinone binding site. If the quinone acted as a hydrogen donor (bonding to proton accepting amino acid residues), then this would result in increased spin density at the oxygen atom which would lead to increased electron donation from the -OH group to the aromatic ring. Enhanced stabilization of the S orbital would result. However, with the -OH group acting as a hydrogen acceptor (bonding to proton donating amino acid residues), a decrease in the spin density at the oxygen atom would occur which, in turn, would lead to a corresponding decrease in the electron donating ability of the -OH group. This would enhance stabilization of the A orbital. The postulated stabilization of the A orbital in vivo suggests that the second situation prevails and that the quinone is hydrogen bonded principally to proton-donating amino acid residues (e.g., protonated lysine, arginine or histidine). A second effect which may lead to stabilization of the antisymmetric molecular orbital is steric interaction of the -OH groups in the protein environment. In the durosemiquinone neutral radical, for example, hydrogen bonding of the -OH group in bulky solvents such as tri-*n*-butyl phosphate has been shown to force the hydroxyl group out of the ring plane [27]. Because the electron-donating ability of the -OH group is governed by resonance strucures in which double bond formation between the oxygen and the ring carbon atom occurs [24], an out-of-plane geometry for the -OH group will decrease considerably the partial double bond character of the carbon oxygen bond and decrease the electron donating ability of the -OH group. Therefore, both a specific hydrogen bond

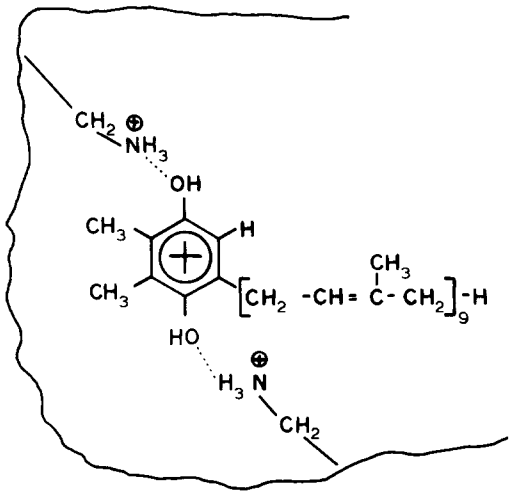

Fig. 10. Proposed structure and membrane environment of Z^{+}.

accepting environment for the -OH group and an out-of-plane geometry for this substituent may contribute to the high degree of stabilization of the A orbital proposed for Signal II in vivo. These considerations also provide an explanation for the apparent stabilization of the S orbital for PQH_2^+ observed in vitro [Fig. 6), as in the sulfuric acid solvent used in these studies we expect an in-plane -OH geometry with this group acting as both a hydrogen donor and acceptor [28] to solvent molecules. We are currently exploring other solvent systems for the generation of PQH_2^+, so that these intermolecular interaction effects can be studied in more detail.

The above arguments have been used to construct a model for the arrangement of Z^{+} on the membrane (Fig. 10). We envision Z^{+} as a plastosemiquinone cation radical in which the -OH groups act as hydrogen bond acceptors for proton donors ($-NH_3^+$ groups from lysine or arginine in this model but alternatively, perhaps, for protons from protonated histidines or neutral tyronsine or serine) in the binding site. Such a model rationalizes the binding of Z^{+} in its membrane environment and accounts for the ability to orient Z^{+} as one orients the membrane. The hydrogen bonding stabilizes the antisymmetric orbital so that substantial unpaired electron density occurs at the $-CH_3$ group at the 2 position which gives rise to the major splitting observed in the Z^{+} EPR spectrum. Recent orientation and ENDOR studies on Signal II provide additional evidence of a large methyl splitting in determining the line shape of Signal II [41]. An additional advantage of this model derives from the fact that semiquinone cation radicals are strong acids (this contributes substantially to their high redox potential at pH 7) and are likely to deprotonate if there are suitable bases in the vicinity. The absence of such species in Fig. 10 precludes this possibility. The cation nature of Z^{+} and its environment in Fig. 10 are also consistent with an earlier suggestion by Crofts and Wood [29] as to the structure around the site(s) of action of lipophilic anions in PS II. Recent work has identified Z^{+} as one of these sites [30]. Finally, recent optical work by Dekker, Van Gorkom and coworkers [38,39] and by DeVitry and Diner [40] has also implicated a cationic semiquinone origin for Z^{+}, although the latter group finds better agreement with a vitamin K_1 cation radical analog than with a PQH_2^+ model.

The observation of proton release in tris-washed chloroplasts by Renger and Voelker [31] has been attributed to deprotonation of Z^{+} upon its oxidation. This would appear to contradict the above assignment of Z^{+} to a quinone cation radical. However, reconciliation of this observation, if correct [32], with the model of Fig. 10 is straightforward if one suggests that the oxidation of Z results in a decrease in the pK_a of another group on the membrane rather than its own deprotonation. This interpretation is a form of a membrane Bohr effect [33] and its likelihood for Z is supported by the difference in kinetics reported by Renger and Voelker [31] for the oxidation of Z (less than 10 μs) and the appearance of the proton (approx. 1 ms). Such effects have previously been observed for the primary quinone acceptors of both photosynthetic bacteria [34] and of Photosystem II [35].

It is also of interest to observe that the anisotropic saturation characteristics of Signal II are displayed by the model quinone cation radical systems (Figs. 4 and 5). Anisotropic saturation behavior of this sort has previously been observed by Hales [36] for the *p*-benzosemiquinone anion radical and he has attributed this effect to strong directional hydrogen bonding along the radical's principal *X* direction which he defined as being parallel to the carbonyl groups. A similar effect is

likely to contribute to the anistropic saturation characteristics of the quinone cation radicals. If we use the same molecular axis system defined by Hales [36], in which the X axis is parallel to the C-OH groups (Fig. 9), the faster saturation rate for the central (g_{yy}) portion of the spectrum can be ascribed to hydrogen bonding along the radical's X direction *. Because of the direct coupling of the radical with its matrix environment along this direction, the spin lattice relaxation time will be directionally dependent thereby resulting in the observed directional dependency of the saturation profile. As the power dependence characteristics of Signal II are similar to the dependence observed for the model systems, we suggest that similar interactions of this radical species with its protein environment (i.e., interactions along the C-OH axis) give rise to its anisotropic saturation properties.

A final point concerns the electrode potential of in vivo Signal II and hydroquinone cation radicals. It has been shown [2,37] that fast transient components of Signal II generally referred to as II_{vf} (oxygen-evolving chloroplasts) or II_f (non-oxygen-evolving chloroplasts) arise from the radical species Z^+, which acts as the primary electron donor to P-680$^+$. Bouges-Bouquet [9] has estimated a redox potential for this species of +1.1 V. The redox potential of the $TMQH_2^+/TMQH_2$ couple at neutral pH has been estimated to have a value of +0.92 V [12] lending further evidence that the radical species giving rise to Signal II is a hydroquinone cation radical.

In conclusion, therefore, the bandshape characteristics, saturation properties, and redox potential values for hydroquinone cation radicals are all in accordance with the suggestion that Signal II is an EPR signal arising from an immobilized plastoquinone cation radical. The EPR data also suggest that the spin density distribution for this radical species is principally determined by the antisymmetric benzene molecular orbital.

* The center or g_{yy} portion of the quinone radical spectrum is determined by spin orbit coupling of the unpaired electron with excited state orbitals in the X direction [36]. Hence perturbations such as hydrogen bonding along the X direction will be reflected by this portion of the spectrum.

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