Biochimica et Biophysica Acta, 637 (1981) 303–311
Elsevier/North-Holland Biomedical Press

BBA 48113

SUPPOSITION OF THE ORIGIN OF SIGNAL II FROM RANDOM AND ORIENTED CHLOROPLASTS

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(Received October 8th, 1980)
(Revised manuscript received April 7th, 1981)

Key words: ESR; Plastosemiquinone; Signal II; Photosynthesis; (Chloroplast)

From previous studies of biological semiquinones in different solvents, the origin of Signal II in chloroplasts is hypothesized to be a plastosemiquinone anion radical perturbed by a metal cation. Assuming this model, theoretical principal *g* factors and hyperfine splitting constants were calculated and used to simulate the random spectrum of spinach Signal II. Oriented chloroplasts were used to determine the principal angles of this model. Oriented chloroplasts from collard greens showed a different angular dependency of Signal II from those of spinach as well as the presence of added fine structure.

When green plant photosynthesis was first investigated by ESR spectroscopy [1], two room-temperature signals were observed. One signal, called Signal I, is light-induced with a *g* factor of 2.0025 ± 0.0001 and linewidth of $7.5 \cdot 10^{-4}$ T. This signal has been shown to be due to chlorophyll (probably a dimer) serving as the primary electron donor of Photosystem I.

The identity of the second signal (Signal II) is much more ambiguous. Although initially thought to be not associated with any light-driven electron transport, it has been shown recently [2–7] to have mixed photo-induced formation and decay kinetics. The association of this radical (*g* factor 2.0046 ± 0.0002, linewidth approx. $19 \cdot 10^{-4}$ T) with the photosynthetic electron-transport system is unknown although it is felt to be closely linked to the oxygen-evolving site (Photosystem II) probably on the oxidizing side.

In the previous paper [8], we presented evidence obtained from model semiquinone systems to suggest that Signal II was due to a plastosemiquinone radical with spin distribution similar to that of the neutral protonated radical. This hypothesis is further expanded in this paper by calculating the probable anisotropic *g* factors and hyperfine splitting constants of such a radical, then showing that these theoretical parameters are consistent with both the solution and oriented spectra of Signal II.

Materials and Methods

Chloroplasts were isolated by grinding market spinach or collard greens for 10 s in a Waring blendor using an isolation medium consisting of 0.4 M sucrose, 0.1 M Tricine (pH 7.6), and 0.01 M NaCl. The suspension was filtered through eight layers of cheesecloth and the filtrate centrifuged for 1 min at $3\,000 \times g$ (Sorvall RC-2B centrifuge). The pelleted chloroplasts were resuspended in the isolation solution. All operations were carried out at 4°C.

All ESR spectra were recorded on a Varian E-109 (X-band) ESR spectrometer equipped with a TM_{110} cavity slotted for illumination. Solution spectra (chlorophyll concentration 0.9 mg/ml) were recorded using a quartz ESR flat cell. Oriented samples were prepared [9] by layering 200 μl of a chloroplast suspension (.13 mg/ml) on a 50 × 5 mm quartz slide and

Abbreviation: Tricine, *N*-tris(hydroxymethyl)methylglycine.

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dried over water-saturated N_2. The quartz slide, which was fused to a quartz rod, was held by a goniometer to allow an accurate measurement of the angle subtended by the slide with the direction of the external magnetic field. Illumination was provided by a 600 W quartz halogen movie-light focused onto the sample in the cavity through a 6 inch water-filter. Unless otherwise stated, all ESR spectra were recorded at room temperature with the following spectrometer settings: modulation amplitude 0.8 G, filter time constant 2.0 s, microwave power 0.5 mW, sweep width $\pm 10^{-2}$ T and scanning time 8 min. For both solution and oriented studies, chloroplasts were illuminated in situ in the cavity for 20 s prior to recording the spectra in order to generate a large concentration of Signal II. ESR spectra were simulated as previously described [10].

Results

Powder spectra

The typical Signal II spectrum (Fig. 1) consists of an asymmetric signal containing five lines where the separation between the maximum and minimum first-derivative inflections is approx. $19 \cdot 10^{-4}$ T and the apparent g factor is 2.0046 ± 0.0002. In none of our spectra (either first or second derivative) do we observe six hyperfine lines as reported by Warden and Bolton [7]. In discussing Signal II, it is important to remember that it is probably a membrane-associated radical. Since it is observed mainly in isolated whole chloroplasts, destruction of the integrity of the chloroplasts usually destroys Signal II. The fact that the Signal II radical is membrane bound means that its ESR spectrum is that of an immobilized radical, i.e., a powder spectrum. Isotropic g factor and hyperfine splitting constants cannot be used to simulate such a spectrum, the anisotope parameters must be used.

Of course, before anisotropic parameters can be assigned to Signal II, its identity and structure must be known. Signal II has been suggested [11,12] to be due to a radical form of plastoquinone (1, Fig. 2). The strongest argument for this assignment has come from the work of Kohl and co-workers [11,12] who showed that plastoquinone could be extracted from chloroplasts and eliminate Signal II. Furthermore, back addition of deuterated plastoquinone led to a narrowed regenerated Signal II. The obvious choice of such a radical would be the plastoquinone anion (2). It has been shown [8,11], however, that the spectrum of the immobilized anion is a symmetric structureless line of width $9.5 \cdot 10^{-4}$ T. In fact, all immobilized semiquinone anions observed [8] so far have linewidths much less than the $19 \cdot 10^{-4}$ T of Signal II.

It has also been suggested [13,14] that Signal II is due to the overlap of several different radical species. Using microwave saturation techniques [13] and electron spin echo [14] experiments, several different relaxation times for Signal II were detected. The presence of these different relaxation times was interpreted to mean the presence of several different radicals. Another possible interpretation of these results is discussed in this paper where the radical yielding Signal II possesses an aniotropic relaxation behavior. However, whether Signal II arises from one radical species or several closely related species does

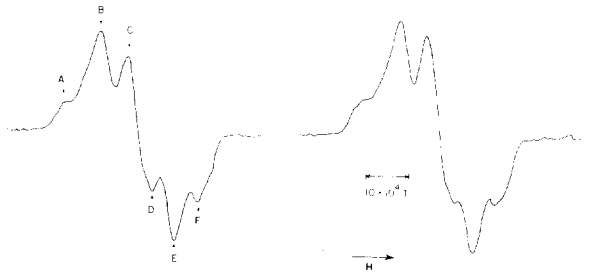

Fig. 1. ESR spectra of Signal II in chloroplasts at room temperature from spinach (left) and collard greens (right). Spinach Signal II has inflections labelled. Note that C and D correspond to maximum at the same line.

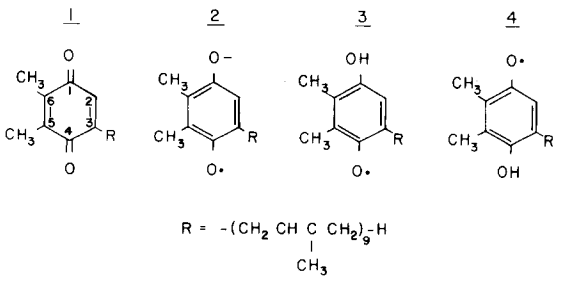

Fig. 2. Structures of plastoquinone (1), its semiquinone anion (2) and the neutral semiquinone (3 and 4). Of the neutral semiquinones, only form 3 has been observed by us in solution at room temperature.

not effect the basic theme of this paper which is to understand how a quinone radical could have a spectrum with the general shape of Signal II.

We previously demonstrated [8] that the spectra of immobilized neutral semiquinones have linewidths much larger than those of their related anions. In fact, the photochemically generated neutral plastosemiquinone (3) in ethanol and cyclohexane has a linewidth of $18.0 \cdot 10^{-4}$ and $17.5 \cdot 10^{-4}$ T, respectively. Although the spectrum of 3 in ethanol shows no fine structure, in cyclohexane there are at least eight observable inflections approx. $6 \cdot 10^{-4}$ T apart. This demonstrates that the redistribution of spin density of 3 compared to 2 produces a spectrum of greater linewidth comparable to that of Signal II. From this fact, we proposed that Signal II was a plastosemiquinone radical with the asymmetric spin distribution similar to that of a neutral semiquinone. The cause of this unique spin distribution need not be protonation but could be due to the presence of a metal cation (e.g., ion pair).

A good example of this effect can be seen with the benzosemiquinone radical. The spectrum of the anion in ethanol contains considerable structure and a maximum-to-minimum linewidth of $5.4 \cdot 10^{-4}$ T, while the neutral radical shows no fine structure and linewidths of $15.5 \cdot 10^{-4}$ and $12.4 \cdot 10^{-4}$ T in ethanol and cyclohexane, respectively [8]. It is well known that the anion in ethanol does not form stable ion pairs with a metal cation. On the other hand, in dimethoxyethane, ion pairing is strong enough to produce observable linewidth variations [15] even in the room-temperature spectrum of the anion while the immobilized spectrum [15] of the anion in dimethoxyethane contains no fine structure and is more similar to that of the neutral semiquinone in cyclohexane than the anion with no iron pairing in ethanol. This is understandable, since the metal cation probably resides over one of the two phenolate oxygens inducing a spin density similar to that of a neutral semiquinone.

It is this asymmetric spin density which produces the broader immobilized spectrum compared to that of the anion. Is Signal II due to a neutral plastosemiquinone radical? We think not. First of all, protonation adds extra fine structure which tends to mask out the fine structure of the ring protons. The photochemically generated neutral radical has very broad fine structure [8] compared to Signal II (Fig. 1). This comparison is not completely valid since protonation of 2 in both ethanol and cyclohexane has been observed by us to produce 3 where protonation has occurred primarily at the oxygen *meta* to the long isoprenoid side chain. The neutral radical with protonation *ortho* to the side chain (4) is not observed, but may, in fact, have a different better resolved immobilized spectrum.

Secondly, the spectrum of Signal II is unaffected by high base concentrations up to pH 9.5. This value is typically at or above the pK for most semiquinone anion/neutral semiquinone equilibria. If Signal II is due to a protonated semiquinone, and if this radical is accessible to deprotonation by the aqueous medium, one would expect its spectrum to shift towards that of the anion at high pH.

Finally, resuspending and washing chloroplasts in ^2H_2O had no observable effect on the spectrum of Signal II. Since a protonated hydroxyl radical should exchange with a deuterated protic solvent, one would expect signal narrowing in the deuterated medium again, assuming that the Signal II radical is accessible to the aqeous medium. This is not observed. None of these data show definitively that Signal II is not a protonated semiquinone, they merely suggest it.

Our first hypothesis, therefore, is that Signal II is due to a plastosemiquinone anion radical (or radicals [13,14]) perturbed by a metal cation. (The metal cation should also stabilize the anion radical preventing its decay by disproportionation.) This model has two possible sites for cation interaction, i.e., one at each phenolate oxygen. To determine which oxygen interacts with the cation, it is important to note that the cation will perturb the anion radical's spin density causing an increase in density at sites *meta* to the cation interaction. The structure in Signal II's spectrum is mainly due to the largest hyperfine interaction in the radical. A note of caution should be added here. A powder spectrum is the sum of the spectra of all possible orientations of the radical in the magnetic field. It is often asymmetric due to the radical's anisotropic Zeeman and hyperfine interactions. It is, therefore, incorrect to attempt to simulate such a spectrum using only isotropic terms. On the other hand, it often happens that the major structure of the powder spectrum of an organic radical is due predominantly to the largest anisotropic hyperfine inter-

action while the smaller interactions mainly broaden the signal.

If such is the case, then the five lines of Signal II imply that the largest spin density is at carbon positions 2 and 6 (1, Fig. 2). In such a radical the three methyl protons will have splitting constants similar to that of the lone ring proton. Obviously, if the major spin density were at positions 3 and 5, a six-line spectrum would be produced. This statement can be made because it has been shown experimentally [16] that the spin densities at the ring carbons in a given semiquinone radical are basically symmetric with respect to the semiquinone's phenolate groups. Therefore, to a first approximation, plastoquinone's methyl group and proton at positions 6 and 2, respectively, will have similar splitting constants and yield a five-line pattern. Such a spin density can be achieved by cation perturbation of the phenolate *ortho* to the side chain.

This phenomenon of similar spin densities is shown in Fig. 3. Even though the radicals shown in this figure are all protonated, one would expect similar spin distributions for semiquinone-metal complexes with, of course, different magnitudes depending on the effective charge of the cation.

Now that the radical giving rise to Signal II has been hypothesized, its isotropic and anisotropic parameters can be predicted. We previously measured [10] the anisotropic g factors of the benzosemiquinone anion in methanol to be g^{xx} 2.0065, g^{yy} 2.0053 and g^{zz} 2.0023 using the axis system where the x-axis lies along the quinone's oxygen atoms and the z-axis is perpendicular to the ring. It is well known that the isotropic g factors of anionic and neutral semiquinones are all very similar, typically ranging from 2.0046 to 2.0052. This implies that the anisotropic factors are probably also very similar. Since methylated quinones typically have isotropic g factors at the high end of this range and since the g factors for any of the plastosemiquinone radicals PQ^- have not been measured, we will predict the anisotropic g factors for a PQ^-M^+ complex (M, metal) to be slightly larger than those of the benzosemiquinone anion with values of g^{xx} 2.0067, g^{yy} 2.0055 and g^{zz} 2.0023 using the same axis system as described above for benzoquinone. Since these g factors are close to one another and since we are observing Signal II at x-band fequencies, large deviations from these predictions should not dramatically effect the spectrum.

Determination of the radical hyperfine splitting constants is not as straightforward. We must first of all determine the isotropic value of each hyperfine component. There is a nearly linear relationship [16] between the magnitude of the *ortho* and *meta* splitting constants of benzosemiquinones in solution. Fig. 4 shows a plot of this relationship with various

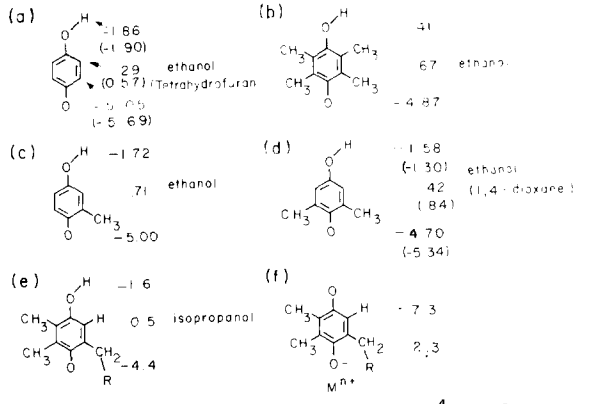

Fig. 3. Hyperfine splitting constants (in 10^{-4} T) of various neutral benzosemiquinones at room temperature in different solvents. (a) *p*-Benzoquinone in ethanol and tetrahydrofuran (in parentheses); (b) duroquinone in ethanol; (c) methyl-*p*-benzoquinone in ethanol; (d) 2,6-dimethyl-*p*-benzoquinone in ethanol and 1,4-dioxane (in parentheses); (e) plastoquinone in isopropanol; (f) proposed identity of Signal II with predicted splitting constants. Data for a–d were taken from Ref. 16.

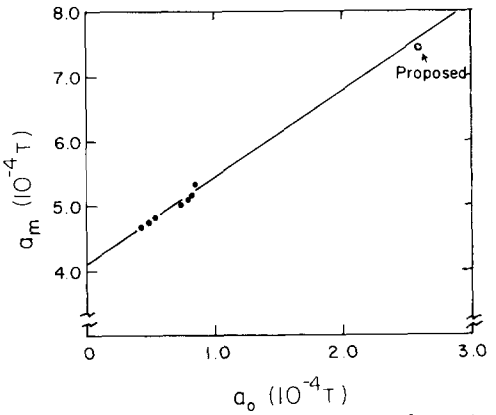

Fig. 4. Relation of splitting constants *ortho* (a_o) and *meta* (a_m) to protonation of various neutral semiquinones. Data were taken from Ref. 22. Also plotted is proposed splitting of plastoquinone-metal pair giving rise to Signal II.

representative data. If Signal II is a plastosemiquinone-like radical, the *ortho* and *meta* splittings should lie very close to the least-squares line of these data. Computer simulation of Signal II (Fig. 5) suggests that the best values for the *ortho* and *meta* constants are $2.2 \cdot 10^{-4}$ and $-7.3\ 10^{-4}$ T, respectively. As seen in Fig. 4, these values lie very close to the predicted linear relationship. The fact that these values are far from the group of neutral semiquinones implies that the metal ion has a stronger perturbing influence on the spin density of Signal II than does a proton on a typical semiquinone.

As mentioned above, the Signal II spectrum is that of an immobilized radical. Therefore, the correct computer simulation (as was done for Fig. 5) must contain the radical's isotropic and anisotropic splitting constants. We have already shown [10] that semiquinone splittings can be closely predicted from its structure and the magnitude of its isotropic splitting. Due to the large number of ring protons on plastoquinone, this would be a formidible task. Luckily, this calculation can be greatly simplified. In general, α-protons on conjugated radicals have greater anisotropic hyperfine splitting constants than do β-protons. Since the broad lines of Signal II generally will mask out anisotropic splittings of small magnitude, we will make another general assumption. This assumption is that the only spectrally significant anisotropic hyperfine splitting occurs at the single α-proton *meta* to the perturbing cation (Fig. 3f). In other words, since all the other ring protons are β-protons, we will not calculate their anisotropic contributions.

As has been shown previously [10], the theoretical anisotropic hyperfine splitting constants for α-protons on a semiquinone ring are:

$$t^{xx} = 8.1\rho_c^{\pi} + 76.9\rho_c^{\sigma}$$

$$t^{yy} = -6.4\rho_c^{\pi} - 10.6\rho_c^{\sigma}$$

$$t^{zz} = -1.7\rho_c^{\pi} - 66.3\rho_c^{\sigma}$$

where ρ_c^{π} and ρ_c^{σ} are the spin densities of the carbon $2p_z$ and σ orbitals, respectively, adjacent to the proton. Assuming $\rho_c^{\sigma} \simeq 0.05\rho_c^{\pi}$, the best-fit simulation occurs with $\rho_c^{\pi} = 0.18$, or

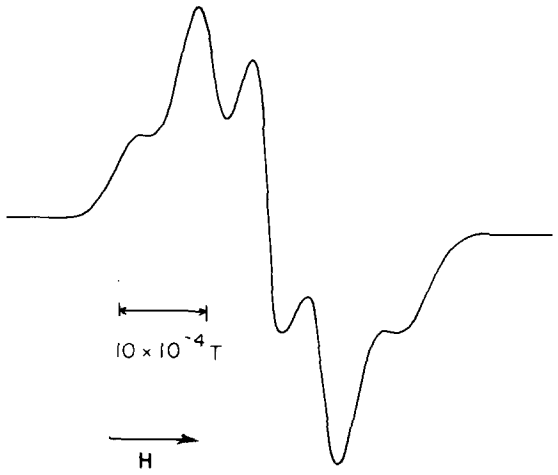

Fig. 5. Computer simulation of spinach Signal II spectrum using anisotropic parameters listed in text.

$$t^{xx} = 2.2 \cdot 10^{-4}\ \text{T}$$

$$t^{yy} = -1.2 \cdot 10^{-4}\ \text{T}$$

$$t^{zz} = -0.9 \cdot 10^{-4}\ \text{T}$$

Using these anisotropic splitting constants with the anisotropic g factors mentioned above, we can simulate the spectrum of our hypothesized plastosemiquinone-metal complex. This is shown in Fig. 5.

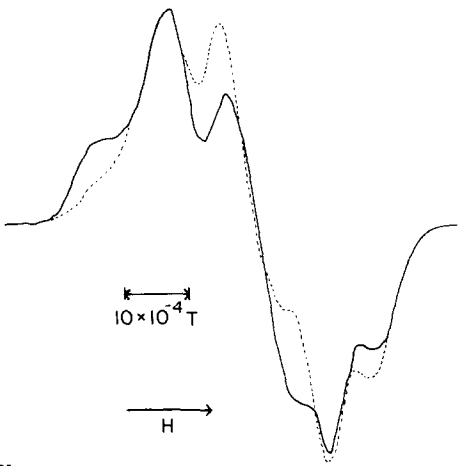

Fig. 6. Room-temperature spectra of Signal II from spinach chloroplasts oriented by drying on quartz slides. (- - - - -) Spectrum with slide oriented parallel (normal perpendicular or 90°) to external magnetic field; (———) spectrum with slide perpendicular (normal parallel to 0°) to field.

Oriented spectrum

Dismukes et al. [16] showed that membrane-bound radicals of chloroplast suspensions frozen in the presence of a large magnetic field flux exhibit an orientation-dependent spectrum. Fig. 6 shows the spectra of Signal II obtained by orienting whole chloroplasts using a drying technique on quartz slides. As will be discussed below, these samples yield phenomena previously unobserved with this system.

First of all, the parallel and perpendicular oriented spectra have different power saturation properties (Fig. 7). While the Signal II spectrum from a suspension of chloroplasts yields a saturation profile (Fig. 7) characteristic of an inhomogeneously broadened system, the oriented spectra saturate more like a homogeneous signal. The difference could be due partly to the differences in sample preparations, although the multilayer preparation is hydrated and Signal II in it should not be in an environment greatly different from the aqueous suspension of chloroplasts. The saturation profile is also orientation dependent with the signal saturating more easily when the membrane plane is parallel (normal is 90°) to the direction of the external magnetic field than when it is perpendicular. This anisotropic power saturation behavior is not unique and has been observed previously by us [15] in model semiquinone systems. Esser [17] has also observed a power dependency of the spectrum of chloroplast suspensions. This effect can be explained in terms of a directional dependency of the relaxation behavior of the radical. This dependency apparently arises in quinones because of the strong interactions of the quinonoid carboxyls with the environment.

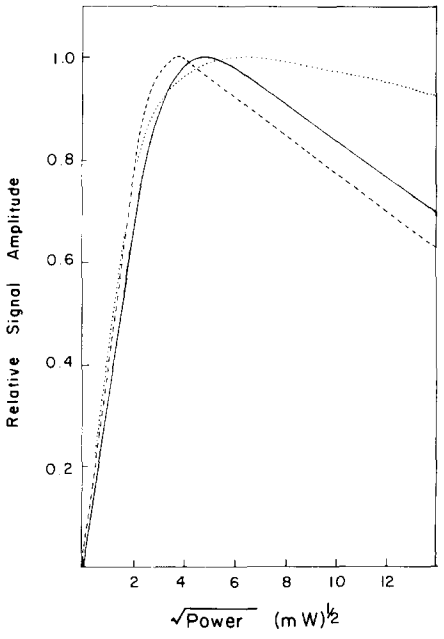

Fig. 7. Dependency of relative signal amplitude of spinach Signal II on microwave power in randomly oriented samples (· · · ·) and oriented samples with normal parallel (0°, ———) and perpendicular (90°, - - - - - -) to external field. All measurements were taken at room temperature.

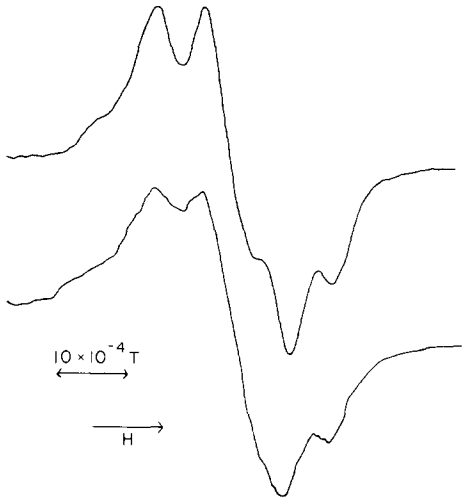

Fig. 8. Spectra of spinach chloroplast Signal II oriented with normal to multilayers perpendicular (90°) to external field showing effects of microwave power saturation broadening. Top spectrum recorded at an incident power of 0.2 mW while bottom spectrum was recorded at 100 mW.

Because of the ease with which Signal II saturates even at room temperatures in these oriented environments, all spectra were recorded at microwave powers below 1 mW. Spectra recorded at these powers (Fig. 8) were observed to be absent of saturation-broadening effects and extremely well resolved and therefore, necessitated the use of low modulation amplitudes.

Ruuge et al. [13] and Nishi et al. [14] also have investigated the power dependency and relaxation times of Signal II. Both groups conclude that Signal II is a composite of several different signals, possibly the same radical in different environments. Our work on the saturation profile of oriented Signal II along with our model semiquinone studies [15] now brings

up a second possible explanation in terms of an anisotropic saturation behavior. In such a system, the spectrum is due to a single species in a single environment. The environment, however, interacts with the radical nonuniformly and produces a saturation behavior which is different along the radical's three principal axes. In a powder sample, different parts of the radical spectrum will saturate at different rates making the spectrum power dependent. Therefore, the spectrum at low power levels represents the true powder spectrum while the spectrum at high power represents the spectrum of the sample with the magnetic field along the axis which is the most difficult to saturate. In Signal II the 0° spectrum (Fig. 6) saturates less easily than the 90° spectrum. Therefore, if our hypothesis is correct, at high power levels the Signal II spectrum should look like more like the 0° spectrum than the low-power powder spectrum. This change, in fact, has been observed both by us (Fig. 8) and Ruuge et al. [13]. Finally, if Signal II were due to several species, the saturation profile would probably not be orientation dependent. In our system, however, the oriented samples saturate uniformly without a change in spectral shape while the random sample saturated nonuniformly with a spectral change.

The orientation dependency of the spectrum of Signal II implies that the radical giving rise to Signal II is membrane bound, since it is oriented when the membrane is oriented. As mentioned above, Dismukes et al [16] first observed this orientation dependency in magnetically oriented chloroplasts. Fig. 9 shows the angular dependencies of the relative amplitudes of the six peaks labelled in Fig. 1. It should be remembered that the center line of Signal II has two labels, C and D, where C represents the magnitude of the low-field maximum and D the high-field minimum of the same line. C + D, therefore, represents the total amplitude of the center line.

Fig. 9 shows that the amplitude of only four of the six lines clearly possesses strong orientation dependency. Lines A and F have opposite dependencies. Similarly, C and D have opposite amplitude dependencies but, interestingly, C + D has virtually no dependency. In other words, the central line of Signal II does not change in magnitude but simply shifts from one side of the spectrum to the other.

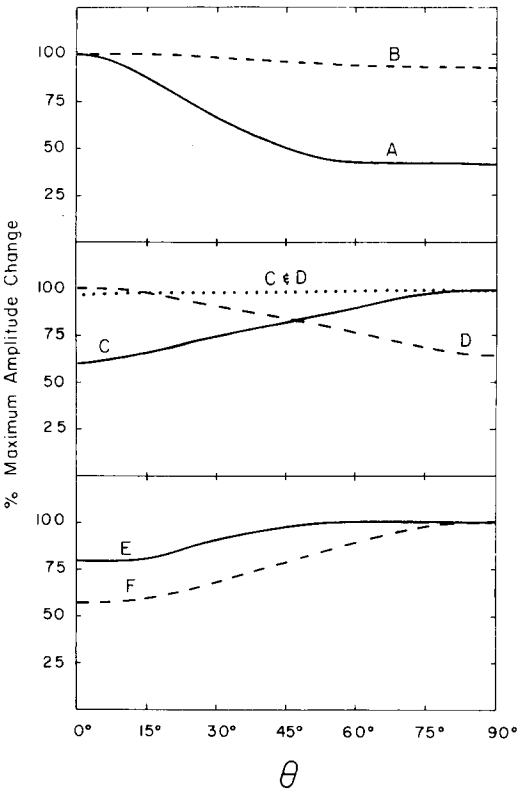

Fig. 9. Angular dependency of the relative amplitudes of spinach chloroplast Signal II spectral inflections. Inflection designations are those shown in Fig. 1. θ represents angle subtended by normal to multilayer slide and external magnetic field.

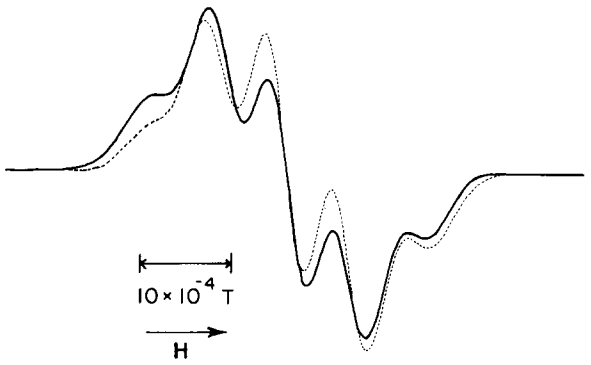

Fig. 10. Computer simulation of Signal II in oriented spinach chloroplasts with normal to membrane oriented perpendicular (- - - - -) and parallel (———) to external magnetic field. Spectral parameter used were the same as those in Fig. 5 and are defined in the text.

The strong test of our model is whether or not the g factors and anisotropic splitting constants derived from simulations of the powder spectrum can be used to simulate the oriented spectra. Fig. 10 shows the computer simulation of the oriented spectra of Signal II from spinach chloroplasts. The g factors, splitting constants and linewidths are the same as those discussed above for the powder spectrum. The additional parameters used were the angles between the principal axes and the membrane normal as well as the standard deviation of these axes about these angles. The best-fit values obtained via computer simulation are $\theta_x = 90°$, $\theta_y = 55°$ and $\theta_z = 35°$ with the angular standard deviation equal to 40°.

Finally, it is interesting to note the spectra of oriented Signal II observed in chloroplasts from another green plant system. Fig. 11 shows the 0 and 90° spectra of oriented chloroplasts obtained from collard greens. It is quite obvious that although the random powder spectra of Signal II (Fig. 1) from spinach and collard greens are nearly identical, the oriented spectra are clearly dissimilar. In terms of our model, this implies that Signal II is due to the same species in these two systems, but is oriented differently in the membrane in each.

The 90°-oriented spectrum of Signal II from collard greens shows the presence of hyperfine structure on the central line which is not detectable in the oriented chloroplasts from spinach. As the membrane normal is rotated from 90 to 0°, this fine structure shifts to the high-field end of the center line and becomes unobservable at angles below approx. 70°. However, at 90° four lines can be observed (see arrows in Fig, 11) with an average splitting of approx. $1.1 \cdot 10^{-4}$ T. In terms of our above model for the radical yielding Signal II, this splitting could possibly originate with the protons *ortho* to the perturbing cation.

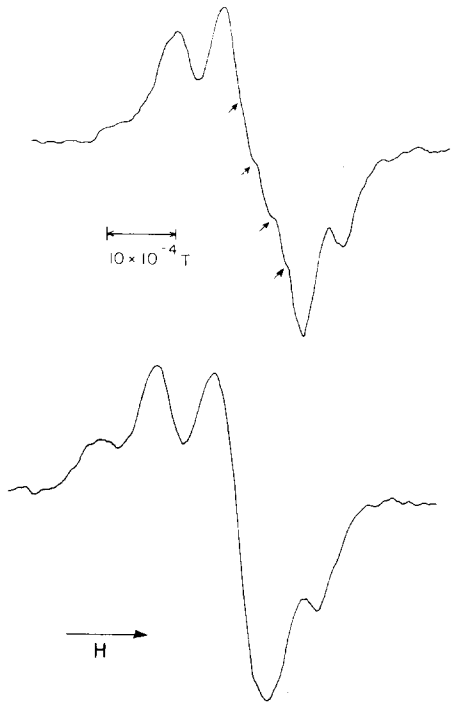

Fig. 11. Spectra of Signal II is oriented collard greens with normal to the membrane oriented perpendicular (top) and parallel (bottom) to the external magnetic field. Fine structure in top spectrum noted by arrows.

Discussion

In order to understand the results and implications of this paper, it is best to review first the basic assumptions made and the lines of reasoning used.

We first assumed Signal II to originate from a plastoquinone-type radical. This assumption is heavily based on the facts that the g factor of Signal II is very similar to that observed for semiquinones and the results of Kohl et al. [11,12] who showed that Signal II could be eliminated and reconstituted by extracting and adding back plastoquinone. Secondly, we assumed Signal II to arise from a semiquinone with a spin distribution more like that of a neutral radical than the anion. This is supported by our previous work [8] which shows that immobilized semiquinone anions have overall linewidths too small while protonated semiquinones yield spectra with linewidths much closer to those observed for Signal II.

The third assumption is that Signal II is due to a single radical species in a single environment. This assumption is contrary to the statements of Ruuge et al. [13] and Nishi et al. [14] who feel that Signal II is a composite of overlapping radicals. As stated in Results, we feel that their data can be reinterpreted in terms of our model. However, whether Signal II is due to one or several overlap species will have to be decided by more stringent testing. Regardless of the outcome, our first two assumptions concerning the possible identity of the radical(s) producing Signal II are still valid.

Is Signal II due to a membrane-bound protonated plastosemiquinone? We propose that it is not. For one thing, protonated semiquinones in solution, although broad, typically exhibit only minor structure. Furthermore, suspension in ^2H_2O or high base concentrations does not alter Signal II. Finally, the fine structure on Signal II has splitting constants much larger than any previously observed for a protonated semiquinone radical. This suggests that the perturbing ion is not a proton but possibly a multivalent diamagnetic cation such as Ca^{2+} or Mg^{2+}. Such ions should alter the semiquinone spectrum without broadening it by electron spin-spin interactions. Further, the metal ion probably does not have easily accessible d orbitals which when complexed with the semiquinone would contribute orbital angular momentum to the quinone's unpaired electron yielding principal *g* factors different from 2.

A good example of this last case where a semiquinone is highly perturbed by a neighboring metal ion is the primary acceptor complex of many photosynthetic bacteria. This acceptor is most probably a ubiquinone-iron complex. Mossbauer spectroscopy [18] suggests that the iron never changes oxidation state and is always high-spin Fe^{2+} while the quinone can be one-electron reduced by the photoexcited primary bacteriochlorophyll donor to a semiquinone. The ESR spectrum of the semiquinone-iron complex is broad [19,20], similar to the spectra of 2- and 4-iron ferredoxins with a *g* factor far from the *g* 2 region. The structure of this quinone-iron complex is still unknown.

Accepting the hypothesis that Signal II is due to a metal-quinone complex, what is its structure? One would expect a metal ion to interact most strongly with the carbonyl groups on the quinoid ring although metal-ring interactions [21] are also extremely common. With which carbonyl the cation interacts can be determined from the structure the Signal II. The five-line hyperfine structure can only be rationalized in terms of the cation interaction with the carbonyl *ortho* to the isoprenoid side chain. One of the functions of the side chain may be to hold and correctly position the cation next to the quinone ring.

Using this model anisotropic *g* factors and splitting constants were determined. In determining the latter, it was assumed that only protons α to the ring would yield large anisotropic terms. The fact that these theoretical values yield simulations reasonably similar to both the powder and oriented spectra of Signal II lends support to our model.

Acknowledgement

This research was supported by grant GM-25049 from the National Institute of General Medical Sciences of the public Health Services.

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