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Protein Quinone Complexes. Bovine Plasma Albumin and Halogenated *p*-Quinones

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Abstract

A colloidal dispersion of chloranil in water or an aqueous solution of an amino acid shows an ESR signal characteristic of the semiquinone radical anion. The signal is broadened in the presence of bovine plasma albumin, and the available evidence supports the idea that the freedom of the free radical is restricted by a weak association with a specific site in the protein.

Introduction

Davis et al. [1] have shown that electron accepting quinones, when present to about 5% by weight in a bovine plasma albumin film, raise the conductivity of the film by a factor of 10^5 at room temperature and lower the energy gap $\Delta\varepsilon$ from 2.80 to 1.06 eV. By analogy with earlier work on chloranil charge-transfer complexes with nitrogen bases [2], it was assumed that the chloranil, by accepting electrons, inject positive holes into

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the protein which thereby showed *p*-type semiconduction. Subsequent admission of the electron donors water or ammonia to the chloranil protein complex gave a twelvefold decrease in conductance, and it was inferred that the water or ammonia was donating electrons into the positive holes and thus reducing the number of charge carriers in the first instance. It was estimated that there are about three chloranil molecules to every BPA molecule in the complex which also shows a weak electron spin resonance (ESR) signal having a line width of about 6 G and a *g* value of 1.998. Similar results were obtained using bromanil and iodanil, but no optical absorption corresponding to $\Delta\varepsilon$ 1.06 eV was detected. It was found that the ESR signal produced in the chloranil/BPA/water system persisted in the solid after evaporation of the water. It was thought that the ESR signal could have arisen from the species involved in charge transfer since such a process could theoretically produce two radicals. However, the characteristics of the signal were determined primarily by the electron acceptor and modified only slightly by change in the supposed electron donor. Thus, similar signals were obtained using tyrosine, β-alanine, and tryptophan as electron donors. Beales [3], however, noted that when a weak solution of BPA was left in contact with chloranil, a suspension of the acceptor in the solution gave a single-line ESR absorption which disappeared after the solid had been centrifuged off. He suggested that the signal detected by Davis et al. is first formed at the solid surface of the acceptor. This work has been repeated and, because of its increased solubility in water, trichloro-*p*-benzoquinone (TCBQ) was used instead of *p*-chloranil (CHL) in some experiments. In this case, possible hyperfine splitting in the signal due to the TCBQ semiquinone radical anion was an added advantage to its use.

Experimental

Reagents

Bovine Plasma Albumin (BPA) from Armour Pharmaceuticals Co. Ltd., Fraction V, was used without further treatment. British Drug Houses reagent grade *p*-chloranil (CHL) was used. A sample was recrystallized twice from Analar toluene and then sublimed at 3×10^{-6} torr at 85°C. Analysis: C = 29.4%, Cl = 57.4% (theoretical: C = 29.3%, Cl = 57.7%). Trichloro-*p*-benzoquinone (TCBQ) was recrystallized four times from petroleum ether (60/80). Analysis: C = 35.5%, Cl = 50.0% (theo-

retical: C = 34%, Cl = 50.35%). Fluoranil (FL) from K and K Labs Inc. was used without further purification. The water used was distilled water passed through a Bio-deminrolit mixed bed ion-exchange resin, distilled from alkaline potassium permanganate and further distilled twice, the whole process being carried out under a stream of N_2 gas.

Procedure

Either solid CHL was added to a solution of BPA in water and then dispersed, or an aqueous BPA solution was added to a colloidal dispersion of CHL in water. The ESR spectrum of the resultant solutions was determined using a Decca X3 ESR Spectrometer. An ultrasonic generator (Dawe Instruments, Soniclean 500 W) was used to produce the colloidal dispersions of CHL in water. Its use enables the ESR spectra to be examined minutes after mixing the reactants; the ESR signal produced was the same as that formed in several hours in the absence of ultrasonics.

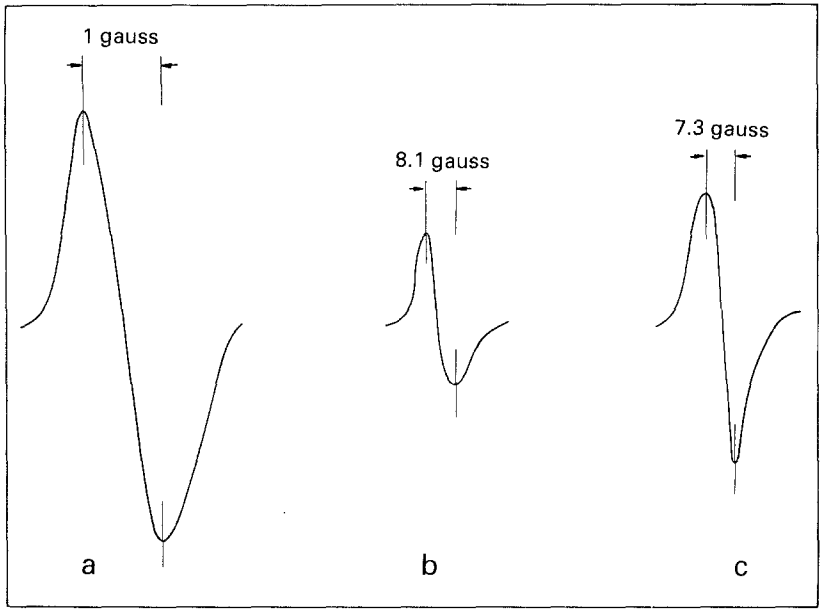

Figure 1. ESR spectra for the systems: (a) CHL/H_2O; (b) BPA/CHL/H_2O; (c) BPA/CHL/H_2O(solid).

Results and Discussion

Preliminary investigation failed to detect any ESR absorption from BPA, H_2O, CHL, or from a solution of BPA in water. However, a signal was detected from a colloidal dispersion of CHL in water. ESR signals were also given by a colloidal dispersion of CHL in BPA/H_2O solution and from the solid obtained by centrifugation of such a mixture. These signals, all obtained at room temperature, are shown in Fig. 1. The results obtained using TCBQ and FL are given in Fig. 2. The broadening found in the presence of BPA was sufficient to obscure the splitting in the TCBQ spectrum but not in the case of FL.

The width of the ESR absorption for CHL/H_2O was determined as a function of pH, and no significant change in g or ΔH was found in the pH range from 5 to 10. The solution pH was altered by the addition of HCl or KOH, and the pH was monitored with a pH meter. Solutions of CHL and several amino acids in water were also found to give ESR signals of similar g values and line widths. A dispersion of CHL in water was passed through a column of alumina (Spence type H). The dried alumina give ESR signals similar to those found for the other CHL-containing systems. These results are given in Table I.

The agreement between these results suggests that the ESR absorption is due to the same species in all cases and that this could be the semiquinone radical anion derived from CHL. It was only in the presence of BPA that the broader signals were obtained. That this broad signal could be attributed to a free radical species derived from CHL was illustrated by observing the ESR absorption as a function of time after adding a few drops of BPA solution to the CHL/H_2O system. There was a rapid transition

TABLE 1. g values and line widths of solutions of CHL and amino acids in water

System	g	ΔH	Isoelectric point	Reference
Histidine/CHL/H_2O	2.006	1.07	7.59	This work
Arginine/CHL/H_2O	2.005	1.03	10.76	This work
Aspartic acid/CHL/H_2O	2.006	0.9	2.77	This work
Alumina/CHL/H_2O(solid)	2.008	1.0		This work
CHL/H_2O	2.005	1.0		This work
Na^+CHL^-/H_2O	2.005	1.0		Beales

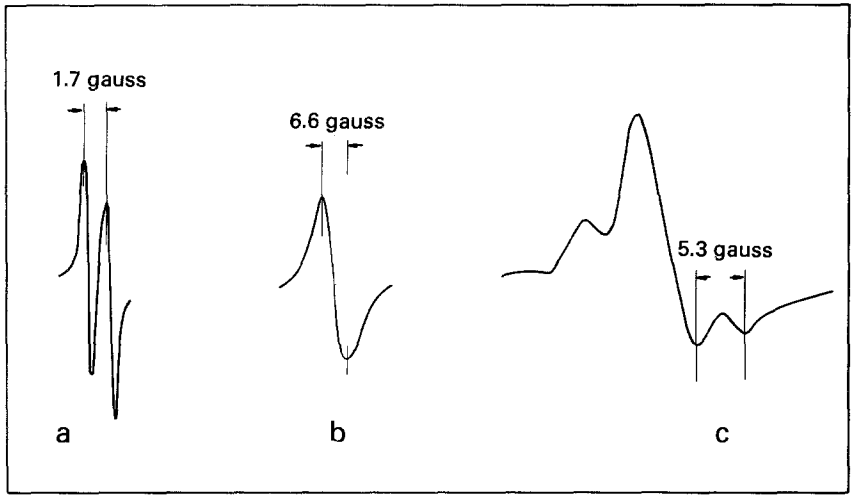

Figure 2. ESR spectra for the systems: (a) TCBQ/H_2O; (b) BPA/TCBQ/H_2O; (c) BPA/FL/H_2O(solid).

from the narrow to broad linewidth signal normally associated with the presence of BPA, and there was no significant change in g value. Similar signals were found with TCBQ, and the details are shown in Table II.

With FL, the ESR spectrum of the radical anion shows hyperfine splitting due to the interaction of the ^{19}F nucleus with the unpaired electron.

TABLE II. Result of adding BPA and TCBQ to the CHL/water system

System	g	ΔH	Reference
BPA/CHL/H_2O(solid)	1.998	6	1
BPA/CHL/H_2O(solid)	2.003	7.3	This work
BPA/CHL/H_2O	—	2 to 3	2
BPA/CHL/H_2O	2.007	6	3
BPA/CHL/H_2O	2.003	8.1	This work
BPA/TCBQ/H_2O	2.003	6.6	This work
BPA/TCBQ/H_2O	2.005	7 to 8	3
Tyrosine/CHL/H_2O	—	1	2
β-alanine/CHL/H_2O	—	5	2

The splitting, $a_F = 4.14$ G [4], is sufficiently large for the hyperfine splitting not to be completely obscured by the broadening found in the presence of the BPA. This suggests that the quinone radical interacts with a specific site on the protein and that, since the value of the g factor is unchanged, the interaction is weak. It follows then that the broadening of the signal is a consequence of restriction of the freedom of the free radical by association with the protein. The lack of any additional hyperfine splitting in the spectrum is further indication that the interaction with the protein is weak. The detection of an ESR signal attributable to a free radical species adsorbed on the protein surface does not always give information about the origin of adsorption from solution of the free radical species or the product of reaction between the surface and an adsorbed nonradical to give a radical. This latter process could markedly affect the solid-state properties of the protein since the concentration of positive holes or free electrons would be disturbed. Rose-Innes [5] has postulated the stabilization of free radicals by interaction with acidic or basic sites on surfaces, and the interaction discussed here could indicate such a process. A lower limit of 10^{-8} cm^2 V^{-1} sec^{-1} for mobility, as in polyvinyl carbazole [6], would correspond to an upper limit of concentration of charge carriers of 2×10^{14} cm^{-3}, too small to give rise to the observed ESR signal on the basis of simple electron transfer from BPA to chloranil.

Calvin et al. [7] have reported the reversible production of photoinduced ESR signals in charge-transfer complexes formed between chloranil and organic solvents. They found subtle variations in the signal amplitude, depending on the exact sequence of light irradiation, which led them to suggest that at least two competing photoinduced processes were taking place, one producing free radicals and the other causing recombination or destruction of radicals. Similar results have been found using the BPA/TCBQ/H_2O system,[3] as shown in Fig. 3. Calvin's work shows a similar rise and decay of photo ESR signals. Observed rise-times of order 10 msec were attributed to the initial process of electron transfer. Longer rise-times of minutes were associated with the subsequent formation of new chemical species. The observations of Fig. 3 fall into the latter category, as expected for semiquinone formation. Light irradiation changed only the amplitude of the ESR absorption and not the position or width of the signal, implying changes in the concentration of adsorbed semiquinone radicals. It is not possible from these results to decide whether more semi-

[3]Using a Bausch & Lomb super-pressure Hg lamp, and protecting the sample by heat filters.

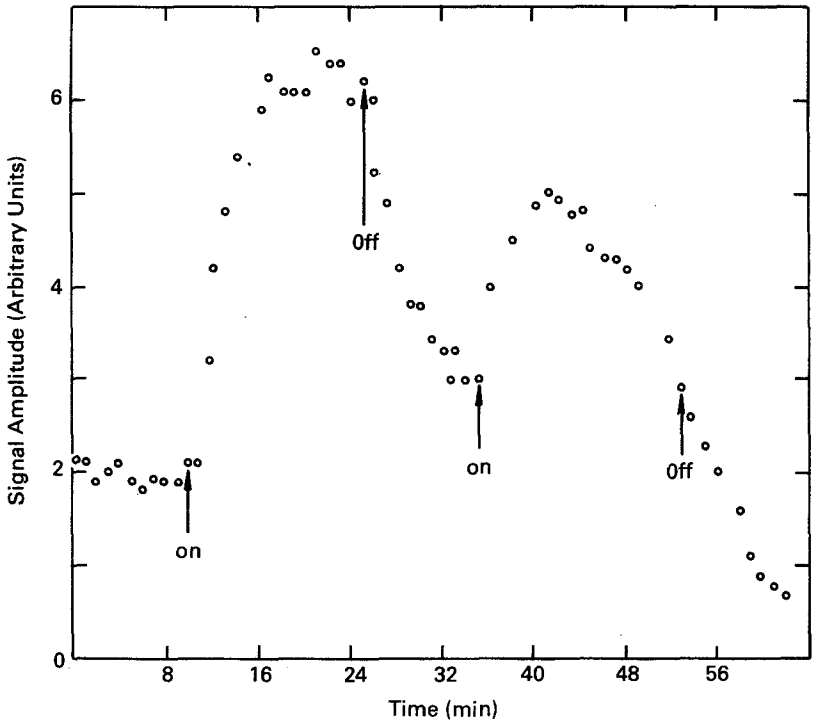

Figure 3. Change in amplitude of ESR absorption signal in the presence and absence of light irradiation for the system BPA/TCBQ/H_2O.

quinone radical species have been adsorbed on the protein or whether adsorbed quinone has reacted with the protein to form adsorbed semiquinone radicals.

Conclusion

In conclusion, this work shows that it is the chloranil semiquinone radical anion interacting with the protein that is responsible for the ESR signal observed in bovine plasma albumin films doped with chloranil by the aqueous solution method. Whether it is this radical anion, or the neutral chloranil molecule, which is responsible for lowering the room temperature resistivity of the said BPA film from 8×10^{17} to 3×10^{12} Ω cm [1] requires further experimental work. The presence of some neutral chlor-

anil in the film is not excluded by the present experiments. On the face of it, neutral chloranil seems more likely to be responsible for the increase in electrical conductivity if the mechanism is one of hole injection as is generally accepted for other organic semiconductors such as phthalocyanine and polyvinyl carbazole [8].

Acknowledgments

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References

1. K. M. C. Davis, D. D. Eley, and R. S. Snart, *Nature,* **188** (1960) 724; R. S. Snart, Ph.D. Thesis, University of Nottingham (1961); K. M. C. Davis, private communication.
2. D. D. Eley, H. Inokuchi, and M. R. Willis, *Disc. Faraday Soc.,* **28** (1959) 54.
3. K. J. Beales, Unpublished results, University of Nottingham (1967).
4. D. H. Anderson, P. J. Frank, and S. Gutowsky, *J. Chem. Phys.* **32** (1960) 196.
5. D. Bijl, H. Kainer, and A. C. Rose-Innes, *Nature,* **174** (1954) 830.
6. D. Lee, private communication.
7. D. F. Ilten, M. E. Kronenberg, and M. Calvin, *Photochem. Photobiol.,* **7** (1968) 331.
8. H. Meier, *Organic Semiconductors,* Verlag Chemie, Weinheim (1974), p. 368.