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OPTICAL AND KINETIC PROPERTIES OF SEMIREDUCED PLASTOQUINONE AND UBIQUINONE: ELECTRON ACCEPTORS IN PHOTOSYNTHESIS

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SUMMARY

1. Semiquinone anion *minus* quinone ($\dot{Q}^-$–Q) and neutral semiquinone *minus* quinone ($\dot{Q}$H–Q) difference spectra have been measured for plastoquinone-9, ubiquinone-10 and ubiquinone-0, using pulse radiolysis in methanolic solution. The difference spectra were used to determine the absolute spectra of each semiquinone.

2. The ($\dot{Q}^-$–Q) difference spectrum for plastoquinone-9 is similar to the difference spectrum detected at a time less than 20 ns after flash excitation of the photosynthetic light reaction Photosystem II in spinach chloroplasts [Stiehl, H. H. and Witt, H. T., (1968) *Z. Naturforsch.* 23b, 220–224; Witt, H. T. (1971) *Q. Rev. Biophys.* 4, 365–477].

3. The ubiquinone $\dot{Q}^-$–Q spectra are similar to difference spectra found on illumination of reaction centre preparations from *Rhodopseudomonas spheroides* and *Rhodospirillum rubrum* [Slooten, L. (1972) *Biochim. Biophys. Acta* 275, 208–218; Clayton, R. K. and Straley, S. C. (1972) *Biophys. J.* 12, 1221–1234]. Ubiquinone has been suggested to be closely associated with the primary electron acceptor of bacterial photosynthesis.

4. Rate constants were measured for formation of semiquinones by reaction of $e^-_{CH_3OH}$, $\dot{C}H_2OH$ and $\dot{C}H_2O^-$ with quinone, and for the decays of the neutral semiquinones. The semiquinone anions were stable for seconds. The ubisemiquinone-6 anion reacts only slowly with oxygen, $k \approx 10^3$ $M^{-1} \cdot s^{-1}$.

5. The effect of substituents upon semiquinone physicochemical properties is discussed.

INTRODUCTION

The isoprenoid quinone ubiquinone is involved in various biological systems, in particular, the electron transport chain of mitochondria. Recent evidence[1–3] suggests that ubiquinone is also closely associated with the primary electron acceptor of the bacterial photosystem. This evidence is in part based upon the detection of an absorption in the range 250–500 nm, very similar to that of the radiolytically-produced ubisemiquinone-6 anion radical[4,5], on illumination of reaction centre preparations from *Rhodopseudomonas spheroides* and *Rhodospirillum Rubrum*. Plastoquinone, another isoprenoid quinone, is present in the photosynthetic electron transport chains of higher plants. An absorption with maximum at 320 nm has been detected less than 20 ns after excitation of photosystem II in spinach chloroplasts[6,7] which was tentatively assigned to plastosemiquinone.

Previous studies[4,5] by pulse radiolysis of ubiquinone-6 in methanol have led to the determination of various physicochemical properties of the ubisemiquinone-6 neutral and anionic radicals. We now report absorption spectra and kinetic properties of semiquinones derived from two ubiquinones ($n=0$ and 10) which differ in their isoprenoid chain lengths from that studied previously ($n=6$). We also report absorption spectra and kinetic properties of anionic and neutral plastosemiquinone-9, determined by pulse radiolysis in methanolic solution.

METHODS

Plastoquinone-9 and ubiquinone-0 were gifts from Dr O. Isler, Hoffman-La Roche and Co., Basel, and Dr R. Azerad, Department de Biochemie, Université de Paris, Orsay, respectively. Methanol (Burroughs) was freed from carbonyl impurities by the method of Baxendale and Mellows[8]. All other chemicals were the purest available commercially.

The pulse radiolysis equipment[9] and methods used[4,5] were as previously described. Methanolic solutions of quinones, up to 10^{-4} M, were irradiated with 0.005–1-μs pulses of 9–12-MeV electrons in a cell of 4-mm optical path length. All solutions were deaerated by bubbling with argon for at least 30 min. At each wavelength optical absorptions were observed as a function of time before and after the pulse, with photoelectric detection. Photomultiplier signals were displayed on an oscilloscope and photographed on polaroid film. For the measurement of spectra deflections were noted at a particular time after the pulse. Such spectra correspond to the difference between the absorptions of quinone and semiquinone. The absolute absorption spectra of the semiquinones were obtained by subtracting from the observed differences the loss in quinone absorption resulting from semiquinone formation. These losses were estimated by assuming that all the solvated electrons and radicals formed on radiolysis of methanol ($G=6.3$)[8,10] give rise to ubisemiquinone radicals. Radiation doses were measured using the absorption of the hydrated electron, taking $\varepsilon_{720\,\mathrm{nm}} \cdot 1.8 \cdot 10^4$ $\mathrm{M^{-1} \cdot cm^{-1}}$ (ref. 11) together with a yield for formation of hydrated electrons in water, G (e^-_{aq}), of 2.6 (ref. 12).

The technique used for forming semiquinones was as follows. Dilute solutions of quinones were irradiated in methanol with short pulses of fast electrons. High energy irradiation of methanol results in the formation of solvated electrons $e^-_{CH_3OH}$, $\dot{C}H_2OH$, as well as several other radicals including $H^\bullet$, $OH^\bullet$, $CH_3^\bullet$ and $CH_3O^\bullet$. All these other radicals react rapidly with methanol to form additional $\dot{C}H_2OH$ (ref. 8). Thus, for the present purposes, the radiation 'primary act' may be regarded as

$$CH_3OH \xrightarrow{\wedge\!\!\vee\!\!\wedge\!\!\vee\!\!\wedge} e^-_{CH_3OH} + \dot{C}H_2OH$$

Both these radicals reduce quinones to semiquinones. In neutral methanol a mixture of both anionic and neutral forms of the semiquinone are produced. Thus for studies of the semiquinone anions alone, solutions were made alkaline by addition of NaOH up to 10^{-2} M. In such solutions $\dot{C}H_2OH$ becomes deprotonated and the semiquinone anions are formed *via* the reactions:

$$e^-_{CH_3OH} + Q \rightarrow \dot{Q}^-$$

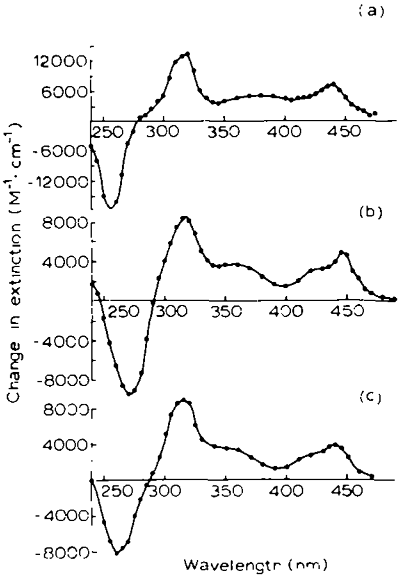
$$\dot{C}H_2O^- + Q \rightarrow HCHO + \dot{Q}^-$$

For studies of the neutral semiquinone radicals alone, solutions were made acidic by addition of H_2SO_4 up to 10^{-2} M. In such solutions $e^-_{CH_3OH}$ is protonated to form $H^{\cdot}$ which reacts with MeOH to form more $\dot{C}H_2OH$. The neutral semiquinone is then formed *via* the single reaction:

$$\dot{C}H_2OH + Q \rightarrow HCHO + \dot{Q}H$$

Difference and absolute spectra

Figs 1a–1c show the difference spectra in the range 240–490 nm, 100 μs after irradiation of 10^{-4} M solutions of plastoquinone-9, ubiquinone-10 and ubiquinone-0 in alkaline methanol. These spectra are the differences between the semiquinone anion absorptions and the corresponding quinones. Figs 2a–2c show the difference spectra 50 μs after irradiation of the same quinone solutions in acidic methanol. Such spectra are the corresponding neutral semiquinone–



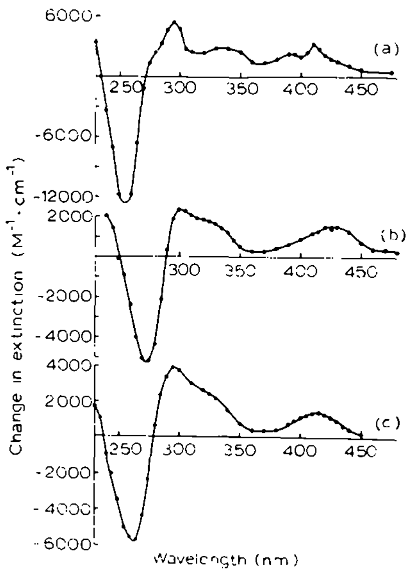


Fig. 1. Semiquinone anion radical–quinone difference spectra. (a) Plastosemiquinone-9 anion *minus* plastoquinone-9. (b) Ubisemiquinone-10 *minus* ubiquinone-10, (c) Ubisemiquinone-0 *minus* ubiquinone-0.

Fig. 2. Neutral semiquinone radical–quinone difference spectra. (a) Neutral plastosemiquinone-9 *minus* plastoquinone-9. (b) Neutral ubisemiquinone-10 *minus* ubiquinone-10, (c) Neutral ubisemiquinone-0 *minus* ubiquinone-0.

quinone absorption differences. Figs 3 and 4 show the absolute spectra of the various semiquinones subtracted from the difference spectra of Figs 1 and 2 as described in the previous section.

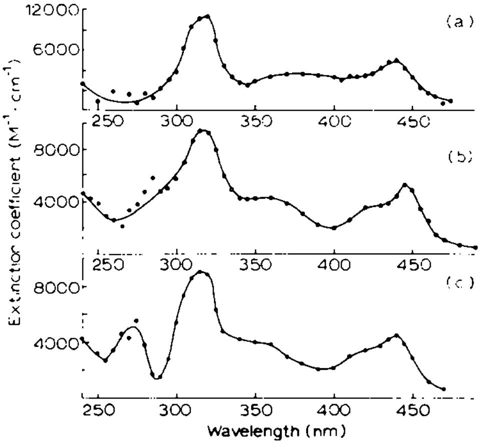


Fig. 3. Absolute spectra of semiquinone anions. (a) Plastosemiquinone-9 anion. (b) Ubisemiquinone-10 anion. (c) Ubisemiquinone-0 anion.

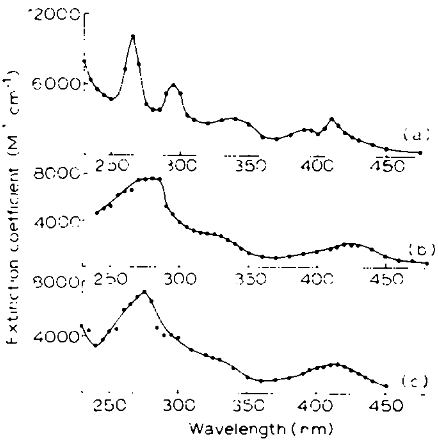


Fig. 4. Absolute spectra of neutral semiquinones. (a) Neutral plastosemiquinone-9 (b) Neutral ubisemiquinone-10. (c) Neutral ubisemiquinone-0.

Rate constants

The rate constants for formation of semiquinones by reaction of $e^-_{CH_3OH}$, $\dot{C}H_2OH$ and $\dot{C}H_2O^-$ with the various quinones, and for the decays of the neutral semiquinones, are listed in Table I. The solvated electron rates were obtained from measurements of the decay of $e^-_{CH_3OH}$ absorption at 650 nm in pure methanol. The $\dot{C}H_2OH$ rates were obtained from observations of QH$^\bullet$ formation around 420 nm in acidic solution. The $\dot{C}H_2O^-$ rates were obtained from observations

TABLE I

RATE CONSTANTS IN METHANOL FOR REACTIONS OF VARIOUS RADICALS WITH QUINONES (Q) AND FOR THE SUBSEQUENT NEUTRAL SEMIQUINONE ($\dot{Q}$H) DECAYS

Quinone	*$k \times 10^{-10}(M^{-1}\cdot s^{-1})$*			*$2k \times 10^{-8}$ $(M^{-1}\cdot s^{-1})$*
	$e^- + Q$	*$\dot{C}H_2OH + Q$*	*$\dot{C}H_2O^- + Q$*	*$\dot{Q}H + \dot{Q}H$*
Plastoquinone-9	1.6	0.21	0.06	3.9
Ubiquinone-10	1.2	0.10	0.12	1.7
Ubiquinone-0	2.6	0.17	0.13	4.4
Ubiquinone-6[4]	1.7	0.14	0.20	1.9*
Duroquinone[4]	≈ 1	0.10	0.33	4.4

* The previous estimate[4] of this rate constant was lower by the factor 4 due to an arithmetic error.

of $\dot{Q}^-$ formation around 445 nm in alkaline solution, subsequent to the initial rapid $\dot{Q}^-$ formation from the reaction of $e^-_{CH_3OH}$. The QH radicals decayed over milliseconds by second-order kinetics, whereas the $\dot{Q}^-$ radicals were stable over periods of seconds. Saturation of 10^{-4} M ubiquinone-6 in alkaline methanol with oxygen instead of argon reduced the corresponding $\dot{Q}^-$ half life to approx. 0.1 s. This indicates that the rate constant for the reaction of this radical anion with oxygen is about 10^3 $M^{-1} \cdot s^{-1}$.

DISCUSSION

Plastosemiquinone

Photosynthesis in algae and higher plants is thought to be driven by two different light reactions, often referred to as Photosystems I and II (ref. 13). There is considerable interest in the identification of the primary electron donors and acceptors of these photosystems. The donors of both photosystems are believed to be different types of chlorophyll a molecules: P_{700} for Photosystem I and P_{680} for Photosystem II. The identities of the acceptors are much less certain. Plastoquinone is considered to be involved and the $\dot{Q}^-$–Q difference spectrum found here in methanol for plastoquinone-9 is similar to the difference spectrum detected between 230 and 350 nm by Witt and coworkers[6,7] <20 ns after photo excitation of Photosystem II in spinach chloroplasts. The chloroplast spectral changes were assigned to reduction of plastoquinone to the radical anion $\dot{Q}^-$ and the present results thus provide some support for this interpretation. The plastosemiquinone anion radical (Fig. 3) has a maximum at 320 nm and this can be clearly seen in the chloroplast spectral change. However, the 320 nm band is broader in the chloroplast difference spectrum than in the difference spectrum measured here. The radiolytic and photo-produced difference spectra also do not agree too well below 280 nm although, due to high parent quinone absorption, this is a difficult region in which to work. The absolute spectra of $\dot{Q}^-$ and $\dot{Q}H$ determined here are least accurate in this region since they originate from the subtraction of two relatively high extinctions. The protonated plastosemiquinone radical $\dot{Q}H$ (Fig. 4) does not exhibit a peak at 320 nm, suggesting that the semiquinone observed $<$ 20 ns after photo excitation by Stiehl and Witt[6,7] in chloroplasts is unprotonated. Knowledge of the spectrum of $\dot{Q}H$ will assist in deciding whether or not any $\dot{Q}^-$ subsequently takes up a proton during photosynthesis.

Ubisemiquinone

Bacterial photosynthesis is driven by only one light reaction[13]. Here the primary reaction is the photo-oxidation of a bacteriochlorophyll molecule which in purple bacteria is known as P_{870}. The difference spectra of light-induced absorption changes have been measured in reaction centre preparations from *Rhodopseudomonas spheroides*[1,2] and *Rhodospirillum rubrum*[1]. These difference spectra were resolved into two main components (P^+_{870}–P_{870}) and ($\dot{X}^-$–X), X being an electron acceptor. Experiments in the presence of an oxidising agent (ferricyanide) or reducing agent (ascorbate) enabled the contributions of (P^+_{870}–P_{870}) and ($\dot{X}^-$–X) to be separated.

The component of the total difference spectrum corresponding to ($\dot{X}^-$–X) is closely similar in the range 280–500 mn to the ubiquinone $\dot{Q}^-$–Q changes found using pulse radiolysis. Again below 280 nm the radiolytic and light-induced difference spectra agree less well. This is again more likely to be due to experimental error than to real spectral alterations due to the different environment of ubiquinone in the two sets of experiments. ESR evidence also supports the formation of ubisemiquinone anion radicals on illumination of *Rhodopseudomonas* reaction centre particles[3].

Effect of substituents upon physical properties of semiquinones

The length, and indeed the presence, of the isoprene chain appears to have little effect upon the absorption spectra of these semiquinones since the radicals derived from ubiquinone-0 and ubiquinone-10 found in this study are very similar to the radicals derived from ubiquinone-6 found previously in methanol[4,5]. Comparison of the various rate constants recorded in Table I shows that likewise the isoprene chain has little influence upon the reaction rates. Comparison of ubisemiquinone, plastosemiquinone and durosemiquinone[14] data shows that the methoxy groups also have a relatively minor influence upon the physical properties described here. The methoxy groups do however have a much more pronounced effect upon the corresponding triplet excited states (ref. 15 and Amouyal, E., Bensasson, R. and Land, E. J., unpublished).

Reactivities of semiquinone anions with oxygen

The radical anions of some quinones pass their electrons on to oxygen very rapidly:

$$\dot{Q}^- + O_2 \rightarrow Q + \dot{O}_2^-$$

for example, anthrasemiquinone 2-sulphonate ($4.6 \cdot 10^8$ $M^{-1} \cdot s^{-1}$)[16], anthrasemiquinone 2,6-disulphonate (approx. $5 \cdot 10^8$ $M^{-1} \cdot s^{-1}$)[17], whereas others appear to be extremely unreactive towards oxygen, for example, benzosemiquinone[17]. In the latter case, where the quinones have relatively high redox potentials, the reverse reaction is more efficient:

$$Q + \dot{O}_2^- \rightarrow \dot{Q}^- + O_2$$

In the case of benzoquinone the rate constant is $9.6 \cdot 10^8$ $M^{-1} \cdot s^{-1}$. Since the ubisemiquinone found in bacterial systems and the plastosemiquinone found in chloroplasts were detected in the presence of oxygen, it seems likely that both semiquinones are rather insensitive towards oxygen. This conclusion is supported by the low rate constant (approx. 10^3 $M^{-1} \cdot s^{-1}$) measured here for the reaction of the ubisemiquinone-6 anion with oxygen in methanol.

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