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Charge-transfer complexes of plastoquinone and α-tocopherol quinone in vitro

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It has been found that plastoquinone (PQ) and α-tocopherol quinone (α-TQ) can form quinhydrone-type charge-transfer complexes on PQH_2 and α-TQH_2, respectively, both in the crystalline state and in solutions of organic solvents. The charge-transfer spectra of PQ/PQH_2 mixtures in hydrophobic solvents showed two bands: one at 349–358 nm, the other at 430–440 nm, one charge-transfer band at 351–355 nm occurring in water-miscible solvents. The intensity ratio of these two bands varied with changing PQ/PQH_2 ratio. The charge-transfer spectra of α-TQ/α-TQH_2 mixtures in all solvents investigated showed one peak at 361–367 nm and a broad shoulder within the range 400–540 nm, whose shape varied depending on the solvent used. In the infrared spectrum of PQ and α-TQ (1700–1600 cm^{-1}) splitting of the carbonyl band occurred and was caused by the presence of two peaks. In the spectra of quinhydrones the splitting disappeared, this being brought about by the appearance of a new peak at the position of splitting, which originated from the complexed quinones. The possibility of the formation of such complexes in thylakoid membranes is discussed.

1. Introduction

Several kinds of prenylquinones are found in chloroplasts [1]: plastoquinones A, B and C, tocopherol quinones and phylloquinone. Plastoquinone A (PQ-9, PQ) functions at at least five sites in the photosynthetic electron-transport chain: as the secondary electron donor to photosystem II (Z) [2], the primary (Q_A) and secondary (Q_B) electron acceptors of photosystem II [3,4], a component of the cytochrome b_6-f complex (Q_z) [5] and as a hydrogen carrier across the thylakoid membrane (PQ pool) [6], Q_A, Q_B and Q_z are noncovalently protein-bound PQ molecules, and PQ-pool molecules occur in a free form in the hydrophobic layer of the thylakoid membrane. PQ and plastoquinol (PQH_2) molecules show a high degree of both lateral and transverse mobility in thylakoid membranes [7]. Experiments in model systems led to the conclusion [8] that prenylquinones with a side chain of at least three prenyl units (ubiquinone homologues, PQ), incorporated into liposome membranes, effectively catalyzed proton transport across the membrane. Because the length of PQ or ubiquinone-10 molecules is comparable to the thickness of the membrane, diffusion of these molecules across the membrane might be hindered. Understanding of this problem at the molecular level is unsatisfactory.

Like PQ, α-tocopherol quinone (α-TQ) always occurs in thylakoid membranes, but in smaller quantities [1,9]. Since α-TQ was not detected in photosystem II [10] or photosystem I [11] particles, it may be supposed that α-TQ is located primarily in the lipid part of the membrane. The presence of α-TQ in thylakoid membranes, and its

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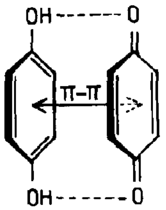
redox properties and capacity to restore activity of the Hill reaction may suggest that α-TQ is an intermediate in photosynthetic electron transfer [12].

Quinhydrone (structure I) is one of the best known examples of charge-transfer complexes. The stabilization of the quinhydrone molecule is due mainly to π-π interaction between the two rings [13,14]. Additionally, hydrogen bridges are formed between the quinol –OH groups, and the quinone –C=O groups [15]. The two rings in the complex are parallel to each other [16]. Quinhydrone is a classic example of weak charge transfer [17]. The most characteristic feature of charge-transfer complexes is the appearance of a new absorption band (charge-transfer band) [18], usually in the visible range, which is responsible for their characteristic colours. Since PQ and α-TQ are derivatives of *p*-benzoquinone and occur in vivo in equilibrium with their reduced forms (PQH_2, α-TQH_2) we attempted in this study to answer the question as to whether both these prenylquinones form quinhydrone-type charge-transfer complexes in the crystalline state or dissolved in hydrophobic solvents. Theoretically, there is steric hindrance to the approach of PQ and PQH_2 molecules or α-TQ and α-TQH_2 molecules, because of the presence of substituents in both rings. Rubrogliocladin, however, which was isolated from *Gliocladium* sp. and identified as a natural quinhydrone consisting of one molecule of 5,6-dimethoxy-2,3-dimethylbenzoquinone and one molecule of the corresponding quinol [19,20], is stable despite the presence of four substituents in both rings.



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From examination of the difference spectra in the visible range and infrared spectra, it was concluded that PQ and α-TQ form quinhydrone-type charge-transfer complexes in vitro.

2. Materials and methods

PQ was isolated from maple leaves (*Acer platanoides*) on a preparative scale according to the method described in ref. 9. PQ was additionally purified chromatographically on a silica gel column (Merck, $60F_{254}$) in chloroform/heptane (50 : 50, v/v) or heptane/benzene (15 : 85, v/v) [1]. In order to remove traces of vitamin K_1 the impure PQ was crystallized at −25 °C from absolute ethanol and separated from vitamin K_1 in the supernatant solution by centrifugation while cold. The purity of PQ was assayed spectrophotometrically and chromatographically. PQH_2 was prepared by reducing PQ with $NaBH_4$ (BDH Chemicals) in acetone/ethanol/methanol (20 : 60 : 40, v/v). It was purified by repeated crystallization at −25 °C from hexane and centrifugation while cold. The purity of PQH_2 was assayed spectrophotometrically. α-TQ was obtained from α-tocopherol acetate (Merck). The acetate was hydrolysed using concentrated KOH solution. The crude α-tocopherol obtained was oxidized with $FeCl_3$ [21]. α-TQ was separated from the other products of α-tocopherol oxidation on a silica gel column, using benzene/acetone/heptane (96 : 4 : 2, v/v) for elution [1]. The purity of α-TQ was assayed similarly to that of PQ. α-TQH_2 was made by reducing α-TQ with $NaBH_4$ in methanol. α-TQH_2 was purified and assayed similarly to PQH_2.

Solutions for spectrophotometric measurements were prepared under nitrogen, from freshly purified samples and were used as quickly as possible. Using hexane solution of PQ (10.7 mM) and PQH_2 (32.1 mM), three mixtures were prepared for spectroscopic measurements with PQH_2/PQ molar ratios of 3 : 1, 2 : 1 and 1 : 1. The charge-transfer spectra were obtained by subtracting from the spectra of the mixtures the spectra of the components, thus leaving the new bands in evidence. In order to compare the stability of the PQ-PQH_2 complex in various solvents, mixtures of PQ and PQH_2 were prepared in hexane, CCl_4, chloroform, diethyl ether, acetone, 1,4-dioxane and their charge-transfer spectra were recorded. Using solutions of α-TQ (17.9 mM) and α-TQH_2 (35.7 mM) in hexane, three samples were prepared with α-

TQ/α-TQH$_2$ molar ratios of 2 : 1, 1.5 : 1 and 1 : 1. Subsequently, the charge-transfer spectra of these mixtures were recorded in the same way as those of PQ and PQH$_2$ mixtures. The mixtures of α-TQ and α-TQH$_2$ (1 : 2) were prepared in CCl$_4$, chloroform, acetone, diethyl ether, 1,4-dioxane, methanol and their charge-transfer spectra were recorded. All absorption spectra were recorded on a Specord M40 UV-VIS spectrophotometer (Carl Zeiss, G.D.R.) at room temperature.

The infrared spectra were recorded on a Specord M80 IR spectrophotometer at room temperature. Crystals of the PQ-PQH$_2$ complex were obtained by preparing a mixture of PQ and PQH$_2$ (1 : 3) in ethanol and allowing the solvent to evaporate.

3. Results

Purified PQ samples in hexane showed absorption maxima at 253.7, 261.3 and 314 nm and a shoulder in the range 400–515 nm. The absorbance ratio $A_{253.7}/A_{226.9}$ was 6.23. The spectrum of PQH$_2$ in hexane had an absorption peak at 290 nm. Purified α-TQ in hexane showed maxima at 259.8, 268.4 and 325.6 nm and a shoulder in the range 400–515 nm. The $A_{268.4}/A_{225.2}$ absorbance ratio was 11. α-TQH$_2$ exhibited an absorption maximum at 293 nm. The charge-transfer spectra of PQ/PQH$_2$ mixtures in hexane showed the presence of two bands, for which the ratio of the relative intensities was not constant (fig. 1). The position of λ_{max} for these two bands was also variable (table 1). Neither of the bands obeyed the Beer-Lambert law and therefore it was not possible to determine the association constant K_a of the complex by the method of Benesi and Hildebrand [22]. The concentrated hexane solutions of PQ/PQH$_2$ mixture were orange in colour (solutions of PQ appear yellow, those of PQH$_2$ being colourless). The absorption spectra of mixtures of PQ and PQH$_2$ (2 : 1) exhibited charge-transfer bands in all solvents used, but their intensity and number varied considerably (table 2). If we assume that the molar extinction coefficient ϵ for the PQ-PQH$_2$ complex remains constant in various solvents, as in the case of quinhydrone [23], then the K_a value for the PQ-PQH$_2$ complex should be proportional to A_{max} of the charge-transfer band. There was, however, no apparent correlation between K_a of the complex and the dielectric constant, dipole moment or surface tension. It is interesting to note that only one charge-transfer band can be distinguished in the spectra of PQ-PQH$_2$ complexes in solvents miscible with water (dioxane, acetone) or slightly miscible (diethyl ether) (fig. 2). Moreover, the intensity of this band in acetone, dioxane or diethyl ether (see table 2) was higher than in solvents that are immiscible with water (hexane, CCl$_4$, chloroform). The absorption maximum of the PQ-PQH$_2$ complex is located within the range 350–358 nm, its

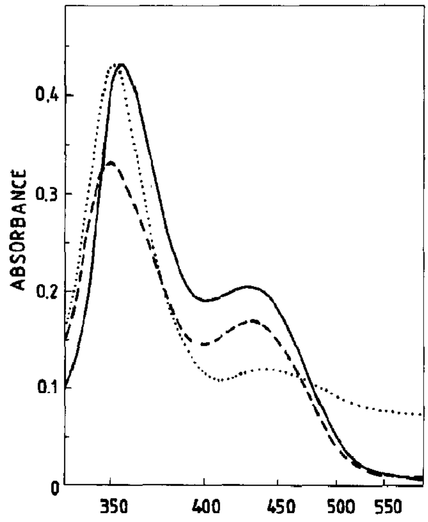


Fig. 1. Absorption spectra of PQ and PQH$_2$ mixtures in hexane after subtracting the absorption of its components (difference spectra); (———) 5.35 mM PQ and 16.05 mM PQH$_2$ (1 : 3), (······) 5.35 mM PQ and 10.7 mM PQH$_2$ (1 : 2), (- - - - -) 5.35 mM PQ and 5.35 mM PQH$_2$ (1 : 1).

Table 1

Characteristic charge-transfer bands of the PQ-PQH$_2$ complex for various PQ/PQH$_2$ molar ratios

Molar ratio, PQ/PQH$_2$	λ_{max} (nm)	A_{max}		A_1/A_2
		A_1	A_2	
1 : 1	349, 430	0.331	0.169	1.96
1 : 2	350, 440	0.432	0.118	3.67
1 : 3	356, 430	0.432	0.215	2.01

Table 2

Characteristic charge-transfer bands of the PQ-PQH_2 complex in various solvents (25.4 mM PQ, 12.7 mM PQH_2)

Solvent	A_{max}	λ_{max} (nm)
n-Hexane	0.18	350, 439
1,4-Dioxane	0.26	352
CCl_4	0.22	353, 440
Diethyl ether	0.28	351
Chloroform	0.22	358, 440
Acetone	0.30	355

position depending on the solvent used. The charge-transfer spectra of mixtures of α-TQ and α-TQH_2 in hexane exhibited one charge-transfer band with an absorption peak at 365–367 nm and a broad shoulder in the range 435–545 nm (fig. 3 and table 3). This band showed slight variability in λ_{max} with changing α-TQ/α-TQH_2 molar ratio and its intensity did not obey the Beer-Lambert law. Concentrated mixtures of α-TQ and α-TQH_2 in hexane were red-brown (solutions of α-TQ are yellow and those of α-TQH_2 colourless). The presence of a charge-transfer band in the absorption spectrum of α-TQ/α-TQH_2 mixtures was observed in all solvents investigated, however its

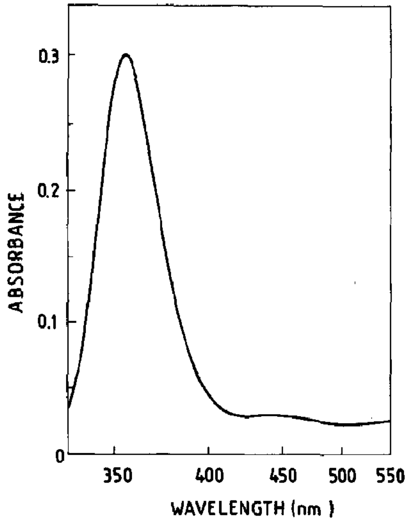


Fig. 2. Absorption spectrum of PQ and PQH_2 mixture in acetone after subtracting the absorption of the components (difference spectrum): 25.4 mM PQ and 12.7 mM PQH_2 (2:1).

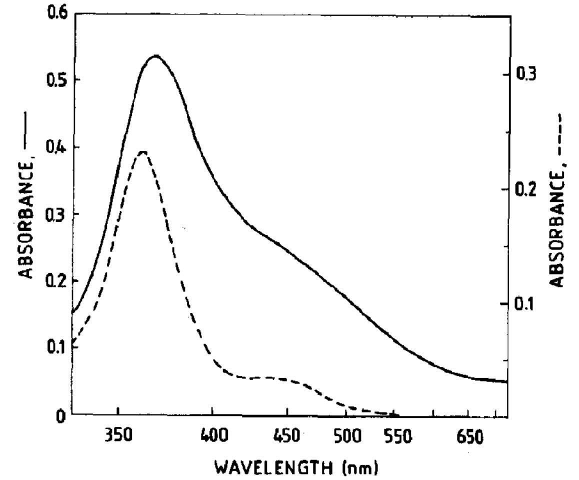


Fig. 3. Absorption spectra of α-TQ and α-TQH_2 mixtures after subtracting the absorption of the components (difference spectra): (———) 8.95 mM α-TQ and 17.9 mM α-TQH_2 (1:2) in hexane, (- - - - - -) 8.95 mM α-TQ and 17.9 mM α-TQH_2 (1:2) in diethyl ether.

Table 3

Characteristic charge-transfer bands of the α-TQ–α-TQH_2 complex at various α-TQ/α-TQH_2 molar ratios

Molar ratio α-TQ/α-TQH_2	λ_{max} (nm)	A_{max}
1:1	367.2	0.275
1:1.5	364.9	0.45
1:2	366.3	0.4–0.6 (0.47)

Table 4

Characteristic charge-transfer bands of the α-TQ–α-TQH_2 complex in various solvents (8.95 mM α-TQ, 17.9 mM α-TQH_2)

Solvent	A_{max}	λ_{max} (nm)	Shoulder (nm)
n-Hexane	0.47	366.3	broad 425–545
1,4-Dioxane	0.28	364.1	400–500
CCl_4	0.22	363.6	broad 410–545
Diethyl ether	0.23	361.0	flat 415–490, λ_{max} = 440
Chloroform	0.26	366.3	flat 415–490
Acetone	0.23	366.3	flat 415–490
Methanol	0.18	366.3	–

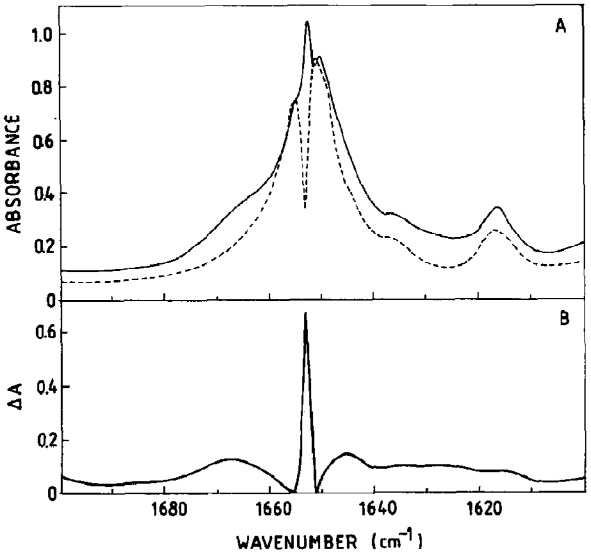


Fig. 4. (A) Infrared spectra of PQ in CCl_4 (------) and of PQ/PQH_2 mixture in CCl_4 (———). The absorption of CCl_4 was subtracted from both spectra. (B) Difference spectrum: PQ/PQH_2 mixture (1 : 3) minus PQ. PQH_2 does not absorb in the range examined.

intensity, λ_{max} and shape were variable (fig. 3 and table 4). No correlation was found between K_a and various physicochemical constants of the solvent used. The position of λ_{max} of the charge-transfer band fell within the range 361–366 nm.

In the infrared spectrum of PQ in CCl_4 three absorption peaks occurred in the range 1700–1600 cm^{-1} (fig. 4A). Two peaks originated from the stretching vibrations of carbonyl groups: $\nu_{as}(C{=}O)$ at 1654.3 cm^{-1} and $\nu_{sym}(C{=}O)$ at 1650.4 cm^{-1}. The maximum at 1616.6 cm^{-1} was due to the stretching vibrations of C=C bonds in the quinone ring. In the spectrum of the PQ/PQH_2 mixture in CCl_4 a new peak appeared at 1652.0 cm^{-1} (fig. 4A), which is clearly evident in the difference spectrum (fig. 4B). The maximum at 1649.8 cm^{-1} was due to $\nu_{sym}(C{=}O)$ vibrations of PQ, the weak shoulder at approx. 1654.5 cm^{-1} probably being derived from $\nu_{as}(C{=}O)$ vibrations, and the peak for $\nu(C{=}C)$ vibrations was located at 1616.2 cm^{-1}. The new band at 1652.0 cm^{-1} probably arises from $\nu(C{=}O)$ vibrations of PQ molecules in the complexed form. PQH_2 showed no absorption bands between 1700 and 1600 cm^{-1}. The carbonyl band of α-TQ was split slightly into two peaks at 1643.8 and 1641.0 cm^{-1}, which correspond to $\nu_{as}(C{=}O)$ and $\nu_{sym}(C{=}O)$ vibrations, respectively. The minimum between these two peaks occurred at 1642 cm^{-1}. In the spectrum of the α-TQ/α-TQH_2 mixture a new peak at 1642.4 cm^{-1} appeared, due probably to $\nu(C{=}O)$ vibrations of the α-TQ molecule in a complexed form. Absorption maxima arising from $\nu(C{=}O)$ vibrations of uncomplexed α-TQ molecules were evident only in the form of broadening of the main band.

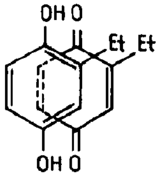
The PQ/PQH_2 and α-TQ/α-TQH_2 mixtures in hexane did not give EPR signals. This indicated that bands in the difference spectra of the complexes did not originate from semiquinone forms of quinones.

On freezing in liquid nitrogen the PQ/PQH_2 solution in hexane turned a violet colour, whereas that of α-TQ/α-TQH_2 became brown-yellow. The original light-yellow colours were restored on warming of these solutions (results not shown). A similar colour change has been reported for quinhydrone [17].

Crystals of plastoquinhydrone (complex of PQ and PQH_2) appeared pink-violet when observed in unpolarized light. In polarized light they demonstrated dichroism: some parts of the crystals were purple, while others were colourless or light-yellow. After rotating the polarizer by 90° the colours interchanged. Using two polarizers crossed at about 45° the crystals appeared partly purple and partly dark blue. After rotation of one of the polarizers by 90° the colours also interchanged (results not shown).

4. Discussion

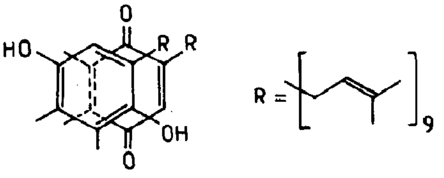
The results presented here led us to the conclusion that PQ and α-TQ form quinhydrone-type charge-transfer complexes both in the crystalline state and in solutions of many organic solvents. In the charge-transfer spectrum of plastoquinhydrone in hydrophobic solvents (hexane, CCl_4, chloroform) there occurred two bands, in contrast to the charge-transfer spectra of quinhydrone which showed one band with $\lambda_{max} = 440 \pm 2$ nm [23]. The presence of two charge-transfer bands was found to be a characteristic feature of many other charge-transfer complexes [18], including ethylquinhydrone [24]. The charge-transfer spectrum of ethylquinhydrone shows two maxima at about 360 and 450 nm, i.e., in similar regions to those in the case of plastoquinhydrone. There are also other similarities between the spectra of both complexes: λ_{max} of ethylquinhydrone showed slight instability at various molar ratios of the components in the mixture, the intensity ratio of both bands was variable, and neither band obeyed the Beer-Lambert law. The instability of λ_{max} and of the shape of charge-transfer spectra in various solvents has also been observed for other quinhydrone-type charge-transfer complexes [24]. With respect to both shape and λ_{max}, the charge-transfer spectrum of α-tocopherol quinhydrone (α-TQ–α-TQH_2 complex) resembles those of quinhydrones obtained by semi-oxidation of 1,3-bis(2,5-dihydroxyphenyl)propane and 1,3,5-tris(2,5-dihydroxyphenyl)pentane which show absorption maxima at 370 nm and a broad shoulder around 440 nm [24]. The association constant K_a of quinhydrone increased proportionally to the surface tension of the solvent [23] which could not be observed in the case of plastoquinhydrone or α-tocopherol quinhydrone. However, the lack of such a correlation is also characteristic of many other charge-transfer complexes [18]. A few charge-transfer bands arise as a result of electron transfer from more than one energy level of the donor molecule, or to more than one energy level of an acceptor molecule. It is believed that the 360 nm absorption peak of ethylquinhydrone may originate from a complex in conformation with adjacent ethyl groups (structure II). In such a conformation the overlap of π-orbitals between donor and acceptor decreases, and more energy is needed to transfer an electron from one π-orbital to the other than in the case of the long-wavelength absorption band at 450 nm, probably resulting from one or more alternate conformations [24]. Since in the charge-transfer spectrum of plastoquinhydrone two bands occurred with a variable intensity ratio, each presumably originates from a different conformation of the components in the complex. It is likely that two conformations of the PQ-PQH_2 complex dominate in solvents in which there are two charge-transfer bands, and that one conformation dominates in solvents in which a single band occurs.



Et = C_2H_5 (II)

The infrared spectra of charge-transfer complexes can also provide some information about their conformations. For complexes of hydroquinone with benzoquinone or with halogeno-substituents of *p*-benzoquinone a new carbonyl band at 1633 cm^{-1} was observed in the infrared spectrum. This band was red-shifted by 20–50 cm^{-1} in relation to the carbonyl band of uncomplexed quinones [25]. The red-shift of the carbonyl band was brought about by the formation of hydrogen bonds between the carbonyl group of quinones and the hydroxyl group of hydroquinone. The spectra of weakly polar complexes, formed between aromatic hydrocarbons and tetrahalogenated quinones, show only slight differences in comparison with the superposition of the spectra of the components. In the case of perylene, *o*- or *p*-chloranil and *o*- or *p*-bromanil, the carbonyl absorption band occurs at a 5–10 cm^{-1} lower frequency [26]. In the chloranil spectrum (in KBr) the carbonyl band was split slightly, but in the complex with hexamethylbenzene the splitting disappeared [27]. A decrease in splitting of the carbonyl band may also be observed in the infrared spectrum of plastoquinhydrone, this being connected with the formation of a new band at the position of the split in the PQ spectrum. The absence of a new band at about 1633 cm^{-1} in the plastoquinhydrone spectrum indicates that hydrogen bonds are not formed between the components of the complex. In the spectrum of PQ there occurred two carbonyl bands, corresponding to the $\nu_{as}(C{=}O)$ and $\nu_{sym}(C{=}O)$ vibrations of coupled C=O oscillators. The appearance of a new band at intermediate frequency in the spectrum of the complex may result from interactions of PQ and PQH_2 rings, leading to 'uncoupling' of $\nu_{sym}(C{=}O)$

and ν_{as}(C=O) vibrations and the appearance of one ν(C=O) vibration, which is characteristic of both C=O oscillators. A similar situation may also take place in the case of α-TQ. On the basis of ultraviolet-visible and infrared absorption spectral analysis of plastoquinhydrone, the following conformation of this complex may be proposed:



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An analogous conformation may also occur in α-tocopherol quinhydrone. The lack of EPR signals in the charge-transfer complexes examined, as in the case of quinhydrone and the slight changes in the infrared spectrum of complexes in comparison with the superposition of the spectra of the components suggest that these are weak polar complexes (with poor charge transfer). The dichroism of plastoquinhydrone crystals, as in the case of quinhydrone [17,27] and some other charge-transfer complexes [18,29], showed that the ring planes of molecules forming the complex lie in parallel to each other.

The question arises as to whether PQ may form quinhydrone-type charge-transfer complexes in thylakoid membranes. It is known that PQ molecules move in the central, fluid, hydrophobic layer of the membrane and occur in equilibrium with PQH_2 molecules. Since, in hexane, which is a good approximation of the hydrophobic layer of the membrane, PQ forms charge-transfer complexes, theoretically a similar process might take place in thylakoid membranes. The same explanation may also hold true for α-TQ. This problem requires further experimentation.

Acknowledgements

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