Biophysical Chemistry, 32 (1988) 51–56
Elsevier

BPC 01310

Physicochemical properties of charge-transfer complexes of plastoquinone and α-tocopherol quinone, and their possible role in vivo

Jerzy Kruk

Department of Physiology and Biochemistry of Plants, Institute of Molecular Biology, Jagiellonian University, Al. Mickiewicza 3, 31-120 Cracow, Poland

Received 31 March 1988
Accepted 13 July 1988

Plastoquinone; α-Tocopherol quinone; Charge-transfer complex; Thylakoid membrane

It has been found that the solubility of PQH_2 and α-TQH_2 in hexane increased with the increase in PQ and α-TQ concentrations, respectively, that is connected with the formation of quinhydrone-type charge-transfer complexes. Measurements of the solubility of both prenylquinones and their reduced forms in hexane and acetone, at −30 °C and room temperature, showed a much higher affinity of the quinol forms for acetone than for hexane. In the case of quinones, the difference in affinity was not significant. The possibility of charge-transfer complex formation by PQ and α-TQ in thylakoid membranes and the influence of such complexes on the diffusion of PQH_2 and α-TQH_2 molecules have been considered.

1. Introduction

Plastoquinone (PQ) is an obligate intermediate in photosynthetic electron transport. It occurs in thylakoid membranes in a protein-bound form as Z, Q_A, Q_B and Q_z [1–4], and in a free form in the lipid matrix as a PQ pool. PQ of the pool functions as a proton pump carrying protons across the membrane [5] and as an electron carrier between photosystem II and the cytochrome b_6-*f* complex [6]. PQ and plastoquinol (PQH_2) molecules exhibit high lateral and transverse mobility [7,8], moving mainly within the fluid bilayer-midplane region of the membrane [7]. Like ubiquinones, PQ catalyses hydrogen transport across the liposome membrane [9]. α-Tocopherol quinone (α-TQ), which always accompanies PQ, although in smaller amounts [10,11], is probably an electron and proton carrier as well, but its site of action in photosynthetic electron transport is not well-known [12]. α-TQ is probably located in the lipid part of the membrane and is not membrane-bound. PQ and α-TQ occur in the thylakoid membrane in a state of equilibrium with their reduced forms. Physicochemical studies concerning the possible location of PQ and ubiquinones in membranes [13–18] suggest that they might be located relatively deep within the hydrophobic part of the lipid membrane close to the midplane region, nevertheless, the possibility exists of their penetration into regions closer to the membrane surface. Such a location of prenylquinones would make possible their long-range lateral diffusion, as well as simultaneous electron transfer between spatially separated protein complexes. However, the reduced PQ (PQH_2) in chloroplasts and ubiquinol in mitochondria are the real hydrogen carriers whose location in the membranes has not yet been thoroughly examined. Moreover, diffusion of PQH_2 is the rate-limiting step in photosynthetic electron transport. It was found that

Correspondence address: J. Kruk, Department of Physiology and Biochemistry of Plants, Institute of Molecular Biology, Jagiellonian University, Al. Mickiewicza 3, 31-120 Cracow, Poland.

ubiquinols showed a slightly increased tendency to leave the hydrophobic environment as compared to ubiquinones [16,18].

In organic solvents PQ and α-TQ can form quinhydrone-type charge-transfer complexes [19], which occur in one or more conformations depending on the kind of solvent used. Since, from a theoretical point of view, there exist conditions for the formation of such complexes in membranes, we decided to determine them in more detail by studying the solubility of these two prenylquinones and their reduced forms in different solvents, as well as the solubility of quinol forms as a function of PQ and α-TQ concentrations, in order to establish the possible influence of the formation of quinhydrone-type charge-transfer complexes in the thylakoid membrane on the physical state of PQ-pool and α-TQ molecules.

2. Materials and methods

PQ, α-TQ and their reduced forms were obtained as described in ref. 19. The measurements were carried out on freshly purified samples and in solvents deaerated with nitrogen. In order to determine the solubility of PQH_2 as a function of the concentration of PQ in hexane, solutions of PQ were prepared within the range 0–50 mg/ml. Then excess PQH_2 was added and the solution was left until equilibrium was attained. Subsequently, the excess PQH_2 was removed by centrifugation and the absorption spectrum of the supernatant in the range 220–350 nm was recorded. The equilibrium concentrations of PQ and PQH_2 were determined spectrophotometrically from absorbances in the maximum for PQ (254 nm) and PQH_2 (290 nm), by the method described in ref. 20. The molar extinction coefficients $\epsilon_{254} = 19\,050$ $M^{-1}\,cm^{-1}$ for PQ and $\epsilon_{290} = 4070$ $M^{-1}\,cm^{-1}$ for PQH_2 were taken from ref. 21, and ϵ_{290} for PQ and ϵ_{254} for PQH_2 were calculated from the absorption spectra of pure samples of PQ and PQH_2. The solubility of α-TQH_2 as a function of α-TQ concentration in hexane was determined as for PQH_2. For measurements hexane solutions of α-TQ within the range 0–200 mg/ml were used. The molar extinction coefficients of α-TQ and α-TQH_2 in hexane were determined spectrophotometrically from the absorption spectra of pure samples of α-TQ and α-TQH_2.

In order to demonstrate the influence of charge-transfer interaction on the solubility of a given substance the solubility of iodine was determined in hexane (noncomplexing solvent) and in benzene, with which I_2 forms a well-known charge-transfer complex [22]. The concentration of I_2 in the saturated solution was determined spectrophotometrically, following the estimation of the molar extinction coefficient of I_2 in both solvents.

The effect of PQ on the solubility of PQH_2 in acetone at −30 °C was also measured.

3. Results

Fig. 1 presents the effect of PQ on the solubility of PQH_2 in hexane. The molar extinction coefficients ϵ_{290} for PQ and ϵ_{254} for PQH_2 in hexane were 744 and 830 $M^{-1}\,cm^{-1}$, respectively. The plot shows that the solubility of PQH_2 increased with increase in PQ concentration, that is especially clear in the range of low PQ concentrations (below 5 mg/ml). In the range of concentrations examined, the solubility of PQH_2 increased nearly 3-fold from 10.8 mg/ml in pure hexane to 30 mg/ml in the PQ solution of 41.7 mg/ml. The increase in PQH_2 solubility indicates the interaction of PQ and PQH_2 molecules in a solution, that is connected with the formation of the charge-transfer complex under these conditions. The

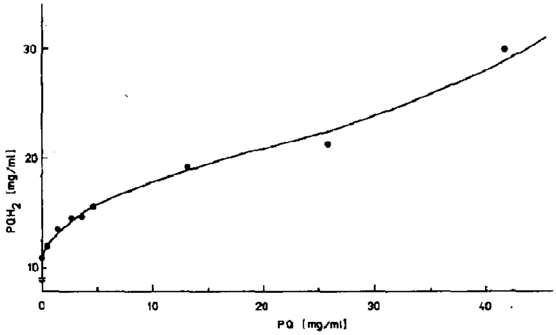


Fig. 1. Effect of PQ on the solubility of PQH_2 in hexane (22 °C).

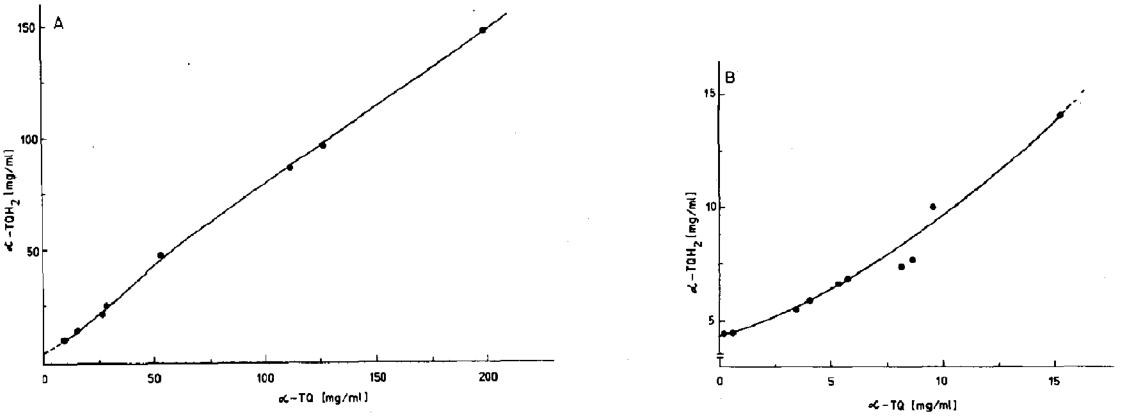


Fig. 2. Effect of α-TQ on the solubility of α-TQH$_2$ in hexane in the range: (A) 15–200 mg/ml α-TQ, (B) 0–15 mg/ml α-TQ (29° C).

molar extinction coefficients used for the estimation of α-TQ and α-TQH$_2$ equilibrium concentrations were $\epsilon_{260} = 18\,880$ M^{-1} cm^{-1} for α-TQ at λ_{max}, $\epsilon_{293} = 4020$ M^{-1} cm^{-1} for α-TQH$_2$ at λ_{max}, $\epsilon_{293} = 752$ M^{-1} cm^{-1} for α-TQ and $\epsilon_{260} = 914$ M^{-1} cm^{-1} for α-TQH$_2$. Fig. 2 shows that the increase in α-TQ concentration results in an increase in solubility of α-TQH$_2$, especially in the case of α-TQ concentrations above 10 mg/ml. In the range examined, the solubility of α-TQH$_2$ increased as much as 33-fold, e.g., from 4.44 to 147 mg/ml. This fact also indicates the interaction of α-TQ and α-TQH$_2$ molecules in the hexane solution which in turn implies the formation of charge-transfer complexes.

The solubility of I_2 in hexane at room temperature was 5.37 mg/ml ($\epsilon_{522} = 1600$ M^{-1} cm^{-1}) and 64.9 mg/ml in benzene ($\epsilon_{501} = 1870$ M^{-1} cm^{-1}). These results show that iodine in the charge-transfer complex with benzene is much more soluble (12-times) than in an uncomplexed form in hexane.

The solubility of the compounds investigated in hexane and acetone is detailed in table 1.

α-TQ, which is a viscous and oily liquid at both low (−30° C) and room temperature, shows unlimited miscibility with the solvents used. PQ obtained by crystallization from ethanol or acetone is a solid substance (m.p. 46° C), but after drying the hexane solution of PQ, it remained as an oily yellow liquid which froze slowly when cooled to a lower temperature. α-TQH$_2$ at −30 and 29° C, and PQH$_2$ at room temperature behaved in the reverse fashion. Both quinols crystallized from hexane solutions, while condensing and evaporating the acetone solutions left α-TQH$_2$ and PQH$_2$ in the form of colourless oily liquids. In all cases these compounds behaved like supercooled liquids.

The *a*/*h* ratio provides an indication of the affinity of a given substance for the hydrophobic

Table 1

Solubility of PQ, PQH$_2$, α-TQH$_2$ in hexane and acetone at −30 and 22 or 29° C (in mg/ml) (∞, unlimited miscibility)

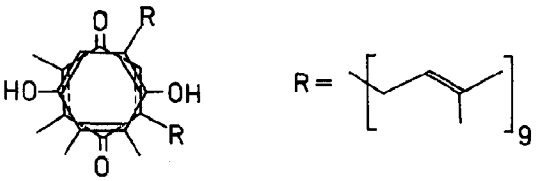
Solvent	PQ		PQH$_2$		α-TQH$_2$	
	−30° C	22° C	−30° C	22° C	−30° C	29° C
Hexane (*h*)	9.77	∞	0.24	10.8	0.05	4.44
Acetone (*a*)	7.14	105.5	72.8	∞	∞	∞
a/*h*	0.73	<1	303	high	very high	high

phase or for a phase of intermediate polarity (acetone). As shown in table 1, both PQ and α-TQ have approximately the same affinity for both phases, however, α-TQH_2 and PQH_2 have a much higher preference for the acetone phase than for that of hexane, at both temperatures. It was found that with the increase in PQ concentration in acetone at $-30\,^{\circ}C$ the solubility of PQH_2 did not increase but even decreased slightly (data not shown). A similar effect probably takes place at room temperature.

4. Discussion

The increase in solubility of PQH_2 and α-TQH_2 in hexane under the influence of PQ and α-TQ indicates an interaction of quinone and quinol molecules in a solution, whose consequence is the formation of charge-transfer complexes by these molecules. The above situation is similar to the action of a latent solvent which solubilises or enhances the solubility of a given substance only in the presence of an appropriate solution mediator, e.g., such a substance which is soluble in a latent solvent and can interact with a solute [23]. During this process polar or hydrophobic parts of a solute molecule and of a solution mediator molecule overlap, while the remaining parts of these molecules are exposed to contact with the solvent. The fragment of a solute molecule that has no affinity for the solvent is covered by a solution mediator molecule and the resulting complex shows a greater solubility. A relatively polar hydroquinone ring of a PQH_2 molecule limits its solubility in hexane. The addition of PQ (solution mediator) to the solution causes interference from the PQH_2 ring with the ring of PQ which is relatively hydrophobic; as a consequence partial screening of -OH groups in PQH_2 from hexane takes place, which in turn brings about an increase in its solubility. The overlapping of PQ and PQH_2 rings also enhances the formation of the charge-transfer complex. A similar process may concern α-TQH_2.

The conformation of plastoquinhydrone (PQ-PQH_2 complex), in which the hydroxyl groups of PQH_2 are partially screened from hexane by substituents of the PQ ring, is shown below. An analogous case may be that of the conformation of tocopherol quinhydrone in hexane, in which -OH groups of the α-TQH_2 ring are screened from the solvent by methyl groups of the quinone ring of α-TQ. The above conformation of plastoquinhydrone may be responsible for the long-wavelength band (430–440 nm) in the absorption spectrum of the charge-transfer complex [19]; its occurrence is probably due to the conformation with the alternate arrangement of substituents.



The influence of the charge-transfer interaction on the increase in solubility of one complex component is also evident in the case of the iodine-benzene complex, in spite of the fact that the dipole moment of this complex is 0.38–0.7 [24], while those of the two complex components equal zero.

The results concerning the solubilities of PQ, α-TQ and the corresponding quinols may provide approximate information as to their location in the thylakoid membrane. With respect to polarity the middle layer of the membrane, composed of hydrocarbon chains of fatty acids (mainly of linolenic acid), approximates hexane. On the other hand, the polarity of acetone corresponds to the subsurface layer of the membrane, e.g. the ester bond region of galactolipids. The solubility of the compounds investigated in hexane and acetone would approximately correspond to their solubility in the middle and subsurface layer, respectively. The a/h ratio would be the partition coefficient of the compounds investigated between these two layers. The a/h coefficients (table 1) show that both PQ and α-TQ may be located mainly in the hydrophobic part of the membrane but they can also penetrate the subsurface layer of the membrane. These assumptions are consistent with results of other studies [15,16,18]. On the other hand, PQH_2 and α-TQH_2 molecules would be almost entirely located in the subsurface layer.

However, in order to perform the function of transferring hydrogen atoms across the membrane, PQH_2 has to overcome the highly hydrophobic middle layer of the membrane to reach its inner side. The lateral diffusion of PQH_2 would also be effective only in the fluid midplane region of the membrane [6,7]. Thus, there must exist some mechanism enabling the sinking of PQH_2 molecules into the hydrophobic layer. A mechanism which could partially explain this process would be charge-transfer complex formation between PQ and PQH_2 molecules that would facilitate PQH_2 diffusion into the hydrophobic layer of the membrane and its traversal.

The increase in PQH_2 solubility in the complexed form in the hydrophobic solvent would be tantamount to a decrease in the partition coefficient a/h for PQH_2 between the subsurface and the hydrophobic layers of the membrane, because PQ diffuses only slightly into the subsurface layer and in this layer PQ does not bring about an increase in PQH_2 solubility. However, the question arises of whether the physiological concentration of PQ in the thylakoid membrane corresponds to the concentrations which cause the increase in PQH_2 solubility (fig. 1). The ratio of PQ to thylakoid acyl lipids is about 1 : 140 (mol/mol) [25]. If we accept that PQ moves only in the region of acyl chains, then the length of each of the two acyl chains of the galactolipid molecule corresponds to three hexane molecules. Thus, there would be one PQ molecule for every 840 (6 × 140) hexane molecules. A simple calculation ($\rho_{PQ} = 0.88$ g/cm^3, $\rho_{hex} = 0.66$ g/cm^3) gives an estimate of the PQ concentration in the hydrophobic layer of the membrane as equal to 7 mg/ml (9.36 mM). As shown in fig. 1, in this range the PQH_2 solubility increased 1.5 fold. Taking into account the fact that the solubility of PQH_2 in the layer whose polarity corresponds to that of acetone does not increase but even slightly decreases, the partition coefficient a/h decrease about 1.5-fold, which is indicative of the facilitated PQH_2 diffusion into the hydrophobic layer of the membrane. Simultaneously the above PQ concentration in the membrane (9.36 mM) corresponds to the concentration at which the formation of charge-transfer complexes was observed [19].

The above considerations suggest that in the thylakoid membrane there are conditions for the formation of charge-transfer complexes between PQH_2 and PQ molecules. PQH_2 molecules in the complexed form would undergo sinking into the hydrophobic layer of the membrane that enables them to function efficiently in lateral and transverse diffusion. In the case of α-TQH_2 the possible formation of charge-transfer complexes in the membrane would rather have no influence upon its mobility in the membrane because of the lower α-TQ concentration as compared to PQ.

Acknowledgements

This study was carried out under project CPBP 05.02. coordinated by the Polish Academy of Sciences.

References

1 P.J. O'Malley, G.T. Babcock and R.C. Prince, Biochim. Biophys. Acta 766 (1984) 283.
2 A.W. Rutherford and I.L. Zimmerman, Biochim. Biophys. Acta 767 (1984) 168.
3 D.J. Kyle, Photochem. Photobiol. 41 (1985) 107.
4 E. Hurt and G. Hauska, Biochim. Biophys. Acta 682 (1982) 446.
5 R. Tiemann, G. Renger, P. Gräber and H.T. Witt, Biochim. Biophys. Acta 546 (1979) 498.
6 W. Haehnel, Annu. Rev. Plant Physiol. 35 (1984) 659.
7 P.A. Millner and J. Barber, FEBS Lett. 169 (1984) 1.
8 G. Hauska and E. Hurt, in: Function of quinones in energy conserving systems, ed. B.L. Trumpower (Academic Press, New York, 1982) p. 87.
9 A. Futami, E. Hurt and G. Hauska, Biochim. Biophys. Acta 547 (1979) 583.
10 C. Bucke, R.M. Leech, M. Hallaway and R.A. Morton, Biochim. Biophys. Acta 112 (1966) 583.
11 R. Barr and F.L. Crane, Methods Enzymol. 23A (1971) 372.
12 H.K. Lichtenthaler, in: Biogenesis and function of plant lipids, eds. P. Mazliak, P. Benveniste, C. Costes and R. Donce (Elsevier/North-Holland, Amsterdam, 1980) p. 299.
13 B. Maggio, A.T. Diplock and J.A. Lucy, Biochem. J. 161 (1977) 111.
14 P.J. Quinn and M.D. Esfahani, Biochem. J. 185 (1980) 715.
15 C. Liljenberg, G. Wachtmeister and G. Oquist, in: Proc. 5th Int. Cong. Photosynth., vol. 1, ed. G. Akoyunoglou (Balaban, Philadelphia, 1981) p. 235.
16 P.B. Kingsley and G.W. Feigenson, Biochim. Biophys. Acta 635 (1981) 602.

17 A. Spinsi, L. Masotti, L. Lenaz, E. Bertoli, G.F. Pedulli and C. Zanoni, Arch. Biochem. Biophys. 190 (1978) 454.

18 B. Chance, in: Biochemistry and biophysics of mitochondrial membranes, eds. G.F. Azzone, E. Carafoli, A.L. Lehninger, E. Quagliarello and N. Siliprandi (Academic Press, New York, 1972) p. 85.

19 J. Kruk, Biophys. Chem. 30 (1988) 143.

20 R.C. Hirt, F.T. King and R.G. Schmitt, Anal. Chem. 26 (1954) 1270.

21 A. Langemann and O. Isler, in: Biochemistry of quinones, ed. R.A. Morton (Academic Press, New York, 1965) p. 90.

22 R. Foster, Organic charge-transfer complexes (Academic Press, New York, 1969).

23 O.A. Neumüller, Römpps chemie lexikon (Franckh'sche Verlagshandlung, Stuttgart, 1973).

24 N. Kulevsky, in: Molecular associations, vol. 1, ed. R. Foster (Academic Press, London, 1975) p. 111.

25 D.J. Chapman and J. Barber, Biochim. Biophys. Acta 850 (1986) 170.