Free Rad. Res., Vol. 21, No. 6, pp. 409-416
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ANTIOXIDANT PROPERTIES OF PLASTOQUINOL AND OTHER BIOLOGICAL PRENYLQUINOLS IN LIPOSOMES AND SOLUTION

JERZY KRUK[1], GEORG H. SCHMID[2] and KAZIMIERZ STRZAŁKA[1]*

[1]*Department of Plant Physiology and Biochemistry, The Jan Zurzycki Institute of Molecular Biology, Jagiellonian University, Al. Mickiewicza 3, 31-120 Kraków, Poland* [2]*Lehrstuhl für Zellphysiologie, Fakultät für Biologie, Universität Bielefeld, Postfach 100131, D-33501, Bielefeld, Germany*

(Received April 22, 1994; in final form June 6, 1994)

Oxidation of biological prenylquinols, like plastoquinol-9 (PQH_2-9), ubiquinol-10 (UQH_2-10), reduced vitamins K_1(VK_1H_2) and K_2(VK_2H_2), α-tocopherol quinol (α-TQH_2) and α-tocopherol (α-T) was followed by their fluorescence during sonication of egg yolk lecithin/prenylquinol liposomes. The order of magnitude of oxidation of the prenylquinols by free radicals generated during sonication was UQH_2-10› VK_2H_2 › VK_1H_2 › α-TQH_2 › PQH_2-9› α-T. It was shown that egg yolk lecithin undergoes degradation even when sonicated briefly under atmosphere of nitrogen and at 0°C. A kinetic study of free radical scavenging action of the prenylquinols in solvents of different polarity was performed. The pseudo-first-order rate constants, k, for the reaction of the prenylquinols with 1,1-diphenyl-2-picryl-hydrazyl (DPPH) in hexane showed that their scavenging activity changes in the order VK_2H_2 › VK_1H_2 › α-TQH_2 › PQH_2-9› α-T› UQH_2-10, being the highest in hexane and methanol, whereas in acetone and ethyl acetate the scavenging activity appeared much lower. The reaction rate constants, k, were apparently not dependent on the solvent polarity. The antioxidant activity of the prenylquinols in natural membranes is discussed.

KEY WORDS: Antioxidant activity; Plastoquinol; Prenylquinol; Free radical; Sonication; Liposome.

ABBREVIATIONS: EYL, egg yolk lecithin; DPPH, 1,1-diphenyl-2-picrylhydrazyl; PQ-9, plastoquinone-9; PQH_2-9, plastoquinol-9; UQ-10, ubiquinone-10; UQH_2-10, ubiquinol-10; VK_1 and VK_2, vitamins K_1 and K_2; VK_1H_2 and VK_2H_2, reduced vitamins K_1 and K_2; α-TQ, α-tocopherol quinone; α-TQH_2, α-tocopherol quinol; α-T, α-tocopherol; TCL, thin-layer chromatography.

INTRODUCTION

Prenylquinones are essential constituents of respiratory and photosynthetic electron transport chains since they play an important role in coupling electron flow to proton translocation across mitochondrial, bacterial, and chloroplast membranes.[1] They occur usually in an equilibrium of oxidized and reduced (quinol) forms. Plastoquinone-9 (PQ-9) and ubiquinone-10 (UQ-10) form redox systems in chloroplast and mitochondrial membranes, respectively. α-Tocopherol quinone (α-TQ), which always occurs with PQ-9 but in minor amounts,[2] is probably also a hydrogen

*Correspondence to: K. Strzałka, Department of Plant Physiology and Biochemistry, The Jan Zurzycki Institute of Molecular Biology, Jagiellonian University, Al. Mickiewicza 3, 31-120 Kraków, Poland. Tel: (48-12)-341305 ext. 237 Fax: (4812)-336907 e-mail: strzalka@mol.uj.edu.pl

carrier.[3,4] The α-TQ – αTQH_2 redox system was also identified in animal membranes[4,5,6] and bacteria.[7,8,9] Another prenylquinone essential in the photosynthesis of higher plants is vitamin K_1 (phylloquinone, VK_1), which is an electron acceptor in photosystem I.[10] Menaquinones (vitamin K_2, VK_2) are involved in electron transport systems of some bacteria.[11] They were also identified in human plasma.[12]

Apart from the function as redox components, prenylquinols are potential antioxidants, similar to α-tocopherol (α-T). Among them, the antioxidant properties of ubiquinol have been most extensively investigated in recent years.[13–19] α-TQH_2 was shown to inhibit lipid peroxidation in liposomes and mitochondria.[6] Antioxidant activity of TQH_2 isomers and some other biological prenylquinols in organic solvents has recently been examined.[20,21]

We extended these studies to other biological prenylquinols as well as some of their synthetic homologues, measuring their antioxidant (free radical scavenging) activities in liposomal membranes and in solvents of different polarity. This type of study is of great importance for understanding of the mechanism by which these structures may confer protection upon illuminated membranes. For comparison, vitamin E (α-T), which is known to be essential in animal[22] and plant[23] cells as an antioxidant, was included in these studies. In order to determine the reaction rate constant of the prenylquinols in organic solvents, we chose their reaction with 1,1-diphenyl-2-picrylhydrazyl (DPPH), a stable free radical which is soluble in all the solvents used. Prenylquinols incorporated into liposomal membranes were subjected to free radicals generated during water sonolysis.[24] The effect of ultrasonic irradiation on egg yolk lecithin (EYL) was also studied.

MATERIALS AND METHODS

PQ-9 and α-TQ were obtained and purified as described in reference.[25] UQ-10, UQ-4 and PQ-3 were a gift from Hoffmann-La Roche Co. (Basel, Switzerland). VK_1 and α-T were from Aldrich and Merck, respectively. VK_2 (menaquinone-4), DPPH and EYL were purchased from Sigma. PQ-3 was purified by TLC as described in.[26] All the other compounds were used without further purification. Solvents were purchased from Aldrich and Polskie Odczynniki Chemiczne and they were of analytical or spectral grade. The hydroquinone forms were prepared by reduction of the corresponding quinones with $NaBH_4$ in methanol. Solutions of hydroquinones in hexane were prepared in vials closed under nitrogen with gas-tight mininert valves (Chromatographic Specialities Ltd., Brockville, Ontario, Canada). All operations with hydroquinone solutions were performed under nitrogen. Organic solvents were flushed with nitrogen before use. The purity of quinone and hydroquinone forms was checked spectrophotometrically and chromatographically. The concentrations of prenylquinols was determined spectrophotometrically in ethanol using molar extinction obtained in reference[27] or presently determined for VK_1H_2 (ϵ_{245} = 34880 $M^{-1}cm^{-1}$) and VK_2H_2 (ϵ_{245} = 30760 $M^{-1}cm^{-1}$) in ethanol.

Determination of prenylquinol-DPPH reaction rate constants was performed under pseudo-first order conditions, and the observed rate constants were calculated using a standard least-square analysis. The initial concentrations of DPPH and prenylquinol in the reaction mixture were 76 μM and 7.6 μM, respectively. 20 μl of a quinol solution was rapidly injected into 2 ml of DPPH solution under continuous stirring at 25°C. Bleaching of DPPH was followed spectrophotometrically by measuring absorbance changes at 520 nm. DW-2000 (SLM AMINCO, U.S.A.) and

Specord M40 (Carl Zeiss, Germany) were used for spectroscopic measurements. Pseudo-first-order rate constants (k) were determined from the expression

$$kt = \ln[\,(A_0 - A_\infty)/(A_t - A_\infty)\,]$$

where A_t is the DPPH absorbance at time t, A_0 the initial absorbance, and A_∞ the absorbance at reaction completion.[28]

Samples for sonolysis experiments were prepared by mixing EYL and quinol solutions (EYL to quinol molar ratio of 50) in a 40 ml plastic tube, evaporation of the solvent under stream of nitrogen and then under vacuum. The dried mixture was dispersed by vortexing in 5 ml of water bubbled with nitrogen, giving final concentration of 4 mM for EYL. The suspensions were then sonicated up to 16 min under continuous flow of high purity nitrogen with a probe type ultrasonic disintegrator UD-11 (Techpan, Poland) at a power level of 20 W. Sonication was carried out in an ice bath, intermittently for 30 s, followed by a 30 s cooling period. Throughout sonication, aliquots of 300 μl were taken, mixed with 1.7 ml ethanol in a cuvette and the quinol concentration was determined by a fluorescence method.[29] The excitation and emission wavelengths were: 290 and 370 nm for UQH_2-10, 245 and 430 nm for VK_1H_2 and VK_2H_2, 290 and 330 nm for other prenylquinols. In the case of UQH_2-10, its fluorescence was determined in suspension/hexane mixture (200 μl/1.8 ml) because of poor fluorescence efficiency of UQH_2-10.[29] Fluorescence spectra were recorded on Perkin-Elmer Luminescence Spectrometer LS-50.

The effect of sonolysis on EYL was performed at a lecithin concentration of 1.27 mM (1 mg/ml) under nitrogen or air. Sonication time was 60 s with a 30 s cooling interval. After sonication the suspension was dried under vacuum and the absorption spectrum of hexane solution of EYL was recorded.

RESULTS AND DISCUSSION

Pseudo-first-order rate constants, k, for reaction of the investigated prenylquinols with DPPH in different solvents in relation to k of α-T – DPPH reaction in acetone, which was found to be $6.64 \times 10^{-3}\ s^{-1}$, are given in Table I. As can be seen, VK_1H_2 and VK_2H_2 show the highest reaction rate constant with DPPH among the investigated prenylquinols in all the solvents used. Also α-TQH_2 and both PQH_2 homologues in hexane and methanol are more active in scavenging DPPH than α-T.

TABLE I

Pseudo-first order rate constants (k) for the reaction of biological prenylquinols with DPPH in different solvents in relation to the k of α-T in acetone, which is $6.64 \cdot 10^{-3}\ s^{-1}$. ε is dielectric constant

	hexane	methanol	acetone	ethyl acetate
ε	1.89	32.6	20.7	6.02
α-T	11.4	6.2	1.0	1.0
UQH_2-4	6.6	2.4	0.8	0.9
UQH_2-10	8.7	7.5	0.8	0.9
PQH_2-3	13.8	6.7	0.8	1.1
PQH_2-9	13.2	13.1	0.8	1.1
α-TQH_2	14.7	8.4	1.4	1.7
VK_1H_2	20.1	15.2	10.7	9.3
VK_2H_2	27.9	14.8	6.6	8.7

The k value for PQH_2 and UQH_2 short-chain homologues is considerably lower in methanol compared to the corresponding long-chain homologues, whereas in the other solvents there are no significant differences in k. For all the prenylquinols k is highest in hexane and much lower in acetone and ethyl acetate. There is apparently no polarity relation of k for the investigated reaction, as the reaction rate is similar in both apolar (hexane) and strongly polar (methanol) media, whereas k is the lowest in solvents of moderate polarity (acetone, ethyl acetate). It seems that k is strongly influenced by the hydrogen bonding power of a solvent and the protic or aprotic character of the medium. A similar relationship was observed for α-T - DPPH reaction in a variety of organic solvents,[28] where k was the highest in strongly hydrogen-bonded (protic) solvents and the lowest in moderately hydrogen-bonded (aprotic) solvents. Our data indicate that this effect also applies to other prenylquinols. The values of k for the reaction of α-T with DPPH in the investigated solvents agree well with those obtained by Fragata *et al.*[28] apart from k in hexane which was considerably lower in comparison to our results.

There was no measurable reaction of the corresponding quinone forms with DPPH in any of the solvent.

The prenylquinols concentrations, monitored by their fluorescence intensity, during sonication of prenylquinol - EYL liposomes are shown in Figure 1. The oxidation rate of α-T is the lowest among the investigated compounds, followed by PQH_2-9 and α-TQH_2. The most sensitive to degradation are UQH_2-10 and reduced vitamins K which were completely oxidized within the first 2 min of sonication (Figure 1). Apart from UQH_2-10, the reactivity order of the prenylquinols is similar to that in hexane.

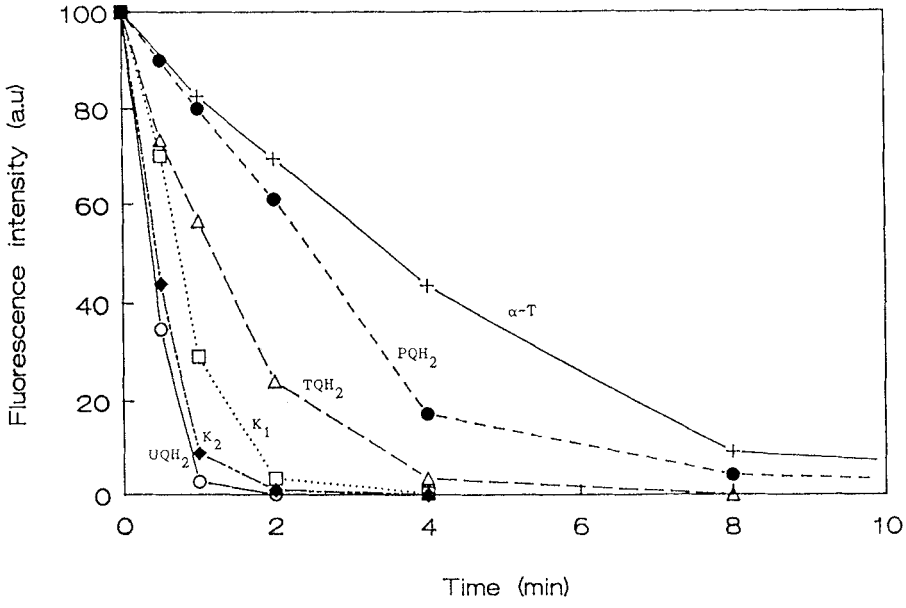

FIGURE 1 Fluorescence intensity changes of prenylquinols in EYL liposome membranes during sonication (see Materials and Methods for details). The symbols K_1 and K_2 in this case denote reduced forms of both vitamins; UQH_2 and PQH_2 correspond to UQH_2-10 and PQH_2-9, respectively.

The oxidation products of the prenylquinols were identified spectrophotometrically as the corresponding quinones (data not shown).

During sonolysis of water, the main reactive species are the hydrated electron (e_{aq}^-), the hydrogen atom (H·), and the hydroxyl radical (OH·).[24] The first two radicals are reducing species, whereas OH· is one of the most oxidizing radicals known.[30] In the presence of oxygen, different oxygen radicals may also be formed. Although sonication was performed under nitrogen and at a low temperature, fast oxidation of prenylquinols as well as of EYL takes place even at short sonolysis times (Figure 2). The absorption spectra of unsonicated EYL, EYL irradiated under nitrogen or air (Figure 2), show that nitrogen atmosphere does not significantly protect against degradation of EYL during sonication. This indicates that the main oxidizing factor during sonication is the hydroxyl radical.

As shown in organic solvents, free radical scavenging activity of different prenylquinols in liposomes and natural membranes may also depend on the polarity and hydrogen-bonding activity of the local environment. Hence, different localizations of the head group of the antioxidant molecule within the membrane would determine its apparent antioxidant activity.

Data from Table I indicate that if the head group of α-T would be hydrogen bonded to carbonyl groups of membrane phospholipids, as suggested,[31,32] its reactivity as a free radical scavenger would be much lower than that of other prenylquinols, like PQH_2-9 or UQH_2-10, whose head groups are assumed to reside closer to the hydrophobic midplane region of lipid membranes. This could possibly be the reason for high free radical scavenging activity of UQH_2-10 in sonicated liposomes. However, it has been reported[15] that in biomembranes short-chain ubiquinol

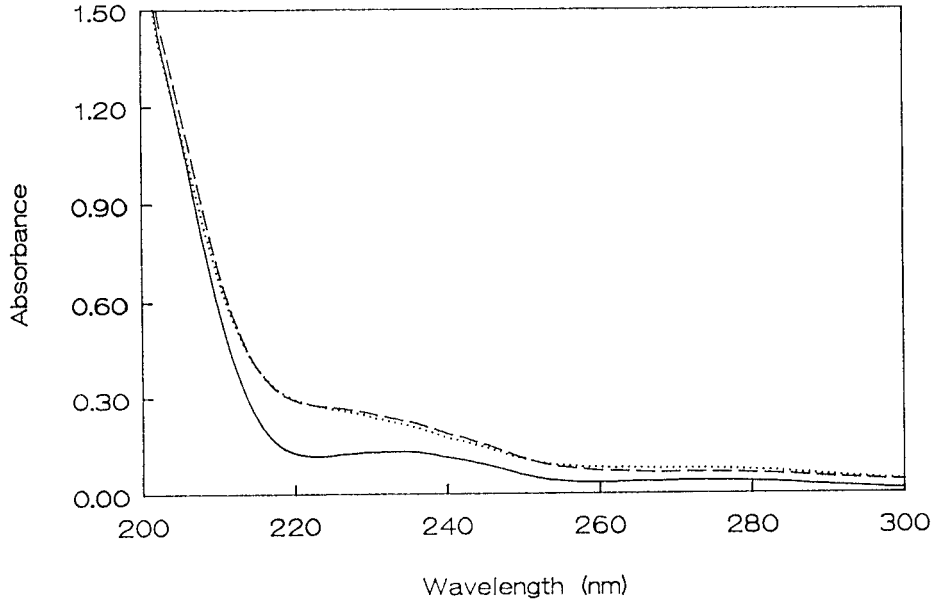

FIGURE 2 Absorption spectra of 0.169 mM EYL in hexane before (———) and after sonication under nitrogen (· · · · · ·) and air (- - - - - -) atmosphere.

homologues (UQH_2-1 – UQH_2-4), located near membrane surface, were more active as antioxidants than long-chain homologues (UQH_2-5 – UQH_2-10), located closer to the membrane interior. On the other hand UQH_2-3 and UQH_2-7 exhibited similar antioxidant activity in model membranes.[18] A reason for this apparent discrepancy could be different site and method of lipid peroxidation initiation; at the membrane surface in the first case (by Fe^{2+} salts) and in the lipid phase (by an azo-initiator) in the other system.

The location of a prenylquinol within the hydrophobic membrane interior should facilitate direct contact of its head group with peroxyl radicals which are formed there during lipid peroxidation. The lipid peroxyl radicals, because of relatively high polarity of peroxyl group,[30,33] are supposed to move away from the membrane interior towards the membrane surface, where they could be scavenged near the water/lipid interface by more polar antioxidants. However, lipid peroxyl radicals may not approach membrane surface directly exposed to the water phase, since they are scavenged by α-tocopherol but not by ascorbate, when peroxidation is generated by a lipid soluble initiator.[33] Therefore, both the site (water or lipid phase) and mechanism of lipid peroxidation initiation in a membrane may influence an apparent prenylquinol antioxidant activity.

Unlike in our studies, the second-order rate constants for the reaction of some biological prenylquinols with substituted phenoxy radicals in different solvents,[21] correlated well with the polarity change and absence of an influence of hydrogen-bonding was observed. This indicates that correlation of the antioxidant activity of biological prenylquinols in organic solvents with their corresponding activity in natural membranes must be done with care as the dependence of k on polarity is influenced by the radical species investigated. Nevertheless, in both cases it was shown that the activity of vitamins K in all the investigated solvents was the highest of all the prenylquinols, although the relative activities of vitamins K obtained in our study with DPPH were considerably lower.

Our results show that most of the investigated prenylquinols have higher free radical scavenging activity than α-T both in solution and liposome membranes. It is also evident that the prenylquinols incorporated into liposome membranes by sonication, as well as EYL, undergo unavoidable oxidation even at short sonication times. Therefore in studies dealing with antioxidant activity, other methods of liposome preparation should be applied, like preparation of multilamellar liposomes or liposomes obtained by injection of ethanol solution of lipids into water.[34]

Antioxidant activity of PQH_2-9 and UQH_2-10 would be an additional function of these prenylquinols apart from their role as electron and proton carriers. Both PQH_2-9 and UQH_2-10 could be easily regenerated in the photosynthetic and mitochondrial electron transport chains, respectively. The prenylquinols are also efficient singlet oxygen quenchers,[35] and there was found a correlation between rates of singlet oxygen quenching and free radical scavenging properties of tocopherol derivatives.[36]

A special attention among these derivatives deserves α-TQ which commonly occurs in photosynthetic systems and which physiological function is still not thoroughly understood. It has been shown that in chloroplast membranes of spinach[37] and tomato[38] sacrificial chemical scavenging activity of α-T leads to formation of α-TQ. α-TQ after being reduced to α-TQH_2 by the photosynthetic electron transport chain becomes a new potent antioxidant exhibiting even higher free radical scavenging activity than its parent compound α-T.

Acknowledgements

This work was sponsored by Deutsche Forschungsgemeinschaft - Polish Academy of Sciences exchange program No 436 POL-113/57/1 and Committee for Scientific Research (KBN) grant No. 4 1660 91 01.

References

1. P. Mitchell (1976) Possible molecular mechanisms of the protonmotive function of cytochrome systems. *Journal of Theoretical Biology*, **62**, 327–367.
2. R. Barr and F.L. Crane (1979) Quinones in algae and higher plants. *Methods in Enzymology*, **23A**, 372–408.
3. H.K. Lichtenthaler (1980) Prenylquinones in plant leaves. In Biogenesis and Function of Plant Lipids (eds. P. Mazliak, P. Benveniste, C. Costes and R. Douce), Elsevier/North-Holland, Amsterdam, pp. 299–310.
4. R.A. Dilley and F.L. Crane (1964) Light-dependent conversion of endogenous α-tocopherylquinone and plastoquinone-D in Spinacia oleracea chloroplasts. *Plant Physiology*, **39**, 33–36.
5. P.E. Hughes and S.B. Tove (1980) Synthesis of α-tocopherolquinone by the rat and its reduction by mitochondria. *Journal of Biological Chemistry*, **225**, 7095–7097.
6. A. Bindoli, M. Valente and L. Cavallini (1985) Inhibition of lipid peroxidation by α-tocopherolquinone and α-tocopherolhydroquinone. *Biochemistry International*, **10**, 753–761.
7. T. Hayashi, A. Kanetoshi, M. Nakamura, M. Tamura and H. Shirahama (1992) Reduction of α-tocopherolquinone to α-tocopherolhydroquinone in rat hepatocytes. *Biochemical Pharmacology*, **44**, 489–493.
8. P.E. Hughes and S.B. Tove (1980) Identification of an endogenous electron donor for biohydrogenation as α-tocopherolquinol. *Journal of Biological Chemistry*, **255**, 4447–4452.
9. P.E. Hughes and S.B. Tove (1982) Occurence of α-tocopherolquinone and α-tocopherolquinol in microorganisms. *Journal of Bacteriology*, **151**, 1397–1402.
10. H.U. Schoeder and W. Lockau (1986) Phylloquinone copurifies with the large subunit of photosystem I. *FEBS Letters*, **199**, 23–27.
11. F.L. Crane (1977) Hydroquinone dehydrogenases. *Annual Review of Biochemistry*, **46**, 439–469.
12. K. Hirauchi, T. Sakano and A. Morimoto (1986) Measurement of K vitamins in human and animal plasma by high-performance liquid chromatography with fluorometric detection. *Chemical and Pharmaceutical Bulletin*, **34**, 845–849.
13. B. Frei, M.C. Kim and B.N. Ames (1990) Ubiquinol-10 is an effective lipid-soluble antioxidant at physiological concentrations. *Proceedings of National Academy of Sciences USA*, **87**, 4879–4883.
14. Y. Yamamoto, E. Komuro and E. Niki (1990) Antioxidant activity of ubiquinol in solution and phosphatidylcholine liposome. *Journal of Nutrition Science and Vitaminology*, **36**, 505–511.
15. V.E. Kagan, E.A. Serbinova, G.M. Koynova, S.A. Kitanova, V.A. Tuyrin, T.S. Stoytchev, P.J. Quinn and L. Packer (1990) Antioxidant action of ubiquinol homologues with different isoprenoid chain length in biomembranes. *Free Radicals in Biology and Medicine*, **9**, 117–126.
16. L. Cabrini, C. Stefanelli, D. Fiorentini and L. Landi (1991) Ubiquinol prevents α-tocopherol consumption during liposome peroxidation. *Biochemistry International*, **23**, 743–749.
17. U.K. Ingold, V.W. Bowry, R. Stocker and C. Walling (1993) Autoxidation of lipids and antioxidation by α-tocopherol and ubiquinol in homogeneous solution and in aqueous dispersions of lipids: Unrecognized consequences of lipid particle size as exemplified by oxidation of human low density lipoprotein. *Proceedings of the National Academy of Sciences USA*, **90**, 45–49.
18. D. Fiorentini, L. Cabrini and L. Landi (1993) Ubiquinol-3 and ubiquinol-7 exhibit similar antioxidant activity in model membranes. *Free Radical Research Communications*, **18**, 201–209.
19. L. Landi, L. Cabrini, D. Fiorentini, C. Stefanelli and G.F. Pedulli (1992) The antioxidant activity of ubiquinol-3 in homogenous solution and in liposomes. *Chemistry and Physics of Lipids*, **61**, 121–130.
20. K. Mukai, S. Itoh and H. Morimoto (1992) Stopped-flow kinetic study of vitamin E regeneration reaction with biological hydroquinones (reduced forms of ubiquinone, vitamin K, and tocopherolquinone) in solution. *Journal of Biological Chemistry*, **267**, 22277–22281.
21. K. Mukai, H. Morimoto, S. Kikuchi and S. Nagaoka (1993) Kinetic study of free-radical-scavenging action of biological hydroquinones (reduced forms of ubiquinone, vitamin K and tocopherol quinone) in solution. *Biochimica et Biophysica Acta*, **1157**, 313–317.
22. M.J. Fryer (1992) The antioxidant effects of thylakoid Vitamin E (α-tocopherol). *Plant, Cell and Environment*, **15**, 381–392.

23. M.J. Fryer (1993) Evidence for the photoprotective effects of Vitamin E. *Photochemistry and Photobiology*, **58**, 304–312.
24. J.P. Lorimer and T.J. Mason (1987) Sonochemistry. I. The physical aspects. *Chemical Society Reviews*, **16**, 239–274.
25. J. Kruk (1988) Charge-transfer complexes of plastoquinone and α-tocopherol quinone *in vitro*. *Biophysical Chemistry*, **30**, 143–149.
26. D. Guay and R.M. Leblanc (1987) Excess free energies of interaction of chlorophyll a with α-tocopherylquinone and plastoquinone 3 and 9. A mixed-monolayer study. *Langmuir*, **3**, 575–580.
27. J. Kruk, K. Strzałka and R.M. Leblanc (1992) Monolayer study of plastoquinones, α-tocopherol quinone, their hydroquinone forms and their interaction with monogalactosyldiacylglycerol. Charge-transfer complexes in a mixed monolayer. *Biochimica et Biophysica Acta*, **1112**, 19–26.
28. M. Fragata and F. Bellemare (1985) Micropolarities of lipid bilayers and micelles. II. Application limits of dielectric constant determinations with the polarity probe α-tocopherol. *Journal of Colloid and Interface Sciences*, **107**, 553–559.
29. J. Kruk, K. Strzałka and R.M. Leblanc (1993) Fluorescence properties of plastoquinol, ubiquinol and α-tocopherol quinol in solution and liposome membranes. *Journal of Photochemistry and Photobiology B: Biology*, **19**, 33–38.
30. G.R. Buettner (1993) The pecking order of free radicals and antioxidants: lipid peroxidation, α-tocopherol and ascorbate. *Archives of Biochemistry and Biophysics*, **300**, 535–543.
31. A.N. Erin, V.V. Skrypin and V.E. Kagan (1985) Formation of α-tocopherol complexes with fatty acids. Nature of complexes. *Biochimica et Biophysica Acta*, **815**, 209–214.
32. S. Urano, M. Kitahara, Y. Kato, Y. Hasegawa and M. Matsuo (1990) Membrane stabilizing effect of vitamin E: Existence of a hydrogen bond between α-tocopherol and phospholipids in bilayer liposomes. *Journal of Nutrition Science and Vitaminology*, **36**, 513–519.
33. G.W. Burton and K.U. Ingold (1986) Vitamin E: application of the principles of physical organic chemistry to the exploration of its structure and function. *Accounts of Chemical Research*, **19**, 194–201.
34. S. Batzri and E.D. Korn (1973) Single bilayer liposomes prepared without sonication. *Biochimica et Biophysica Acta*, **298**, 1015–1019.
35. K. Mukai, S. Itoh, K. Daifuku, H. Morimoto and K. Inoue (1993) Kinetic study of the quenching reaction of singlet oxygen by biological hydroquinones and related compounds. *Biochimica et Biophysica Acta*, **1183**, 323–326.
36. K. Mukai, K. Daifuku, K. Okabe, T. Tanigaki and K. Inoue (1991) Structure-activity relationship in the quenching reaction of singlet oxygen by tocopherol (vitamin E) derivatives and related phenols. Finding of linear correlation between the rates of quenching of singlet oxygen and scavenging of peroxyl and phenoxyl radicals in solution. *Journal of Organic Chemistry*, **56**, 4188–4192.
37. R. Yamauchi and S. Matsushita (1979) Light-induced lipid peroxidation in isolated chloroplasts and the role of α-tocopherol. *Agricultural and Biological Chemistry*, **43**, 2157–2162.
38. W.P. Michalski and Z. Kaniuga (1981) Photosynthetic apparatus of chilling-sensitive plants. IX. The involvement of α-tocopherol in the electron transport chain and the anti-oxidising system in chloroplasts of tomato leaves. *Biochimica et Biophysica Acta*, **635**, 25–37.

Accepted by Professor H. Sies