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Spectrophotometric investigation of the binding of vitamin E to water-containing reversed micelles

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Abstract

The distribution constants of vitamin E partitioned between apolar organic phase and water-containing reversed micelles of sodium bis (2-ethylhexyl) sulfosuccinate (AOT), didodecyldimethylammonium bromide (DDAB), soybean phosphatidylcholine (lecithin) and tetraethylene glycol monododecyl ether ($C_{12}E_4$) have been evaluated by a spectrophotometric method. The results suggest that in the presence of domains from apolar organic solvent to surfactant and to water, vitamin E is partitioned between the micellar palisade layer and the organic solvent and also that its binding strength to reversed micelles depends mainly by specific interactions between the head group of vitamin E and that of the surfactant. Moreover, in addition to the advantageous interactions between vitamin E and water, the dependence of the distribution constants upon the molar ratio R ($R = [\text{water}]/[\text{surfactant}]$) indicates a competition between water and vitamin E for the binding sites at the water/surfactant interface. The biological implications of the preferential location and confinement of vitamin E in water-containing reversed micelles are discussed.

Keywords: Vitamin E; Reversed micelles; Membrane models; UV–vis spectroscopy

1. Introduction

Vitamin E (α-tocopherol; Fig. 1) is a fat-soluble compound mainly located in biomembranes where, by donating hydrogen atoms, acts as a membrane stabilizing agent and free radical scavenger preventing the peroxidation of the polyunsaturated fatty acyl chains of membrane lipids. Its molecule is constituted by two spatially distinct domains: a chromanol nucleus terminated with a polar OH group and a long hydrophobic phytyl side chain (Fig. 1).

It has been demonstrated that, in some highly polar environments, the phenolic hydroxyl group of vitamin E acts as chain-breaking of peroxidation reactions by donating an electron to free radicals, mainly peroxyl radicals (Burton and Ingold, 1986; Niki, 1989), whereas, the long hydrophobic phytyl side chain assures that vitamin E is preferentially located in the membrane bilayer

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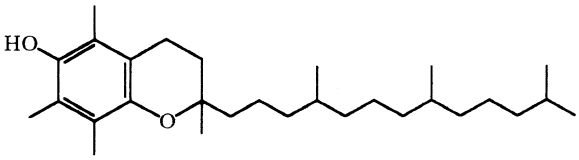

Fig. 1. Schematic representation of the molecular structure of vitamin E.

leading to an increase of its local concentration and consequently an enhancement of its antioxidant action (Fukuzawa et al., 1981; Urano et al., 1987; Wassall et al., 1991).

Recently, it has been found that a combination of melatonin and α-tocopherol resulted in a synergistic antioxidant effect (Livrea et al., 1997). In order to give an explanation to this finding the solubilization of melatonin in solutions of water-containing reversed micelles of sodium bis (2-ethylhexyl) sulfosuccinate (AOT) and soybean phosphatidylcholine (lecithin) has been studied by UV–vis spectrophotometry (Ceraulo et al., 1999). It has been demonstrated that melatonin is preferentially located in the surfactant polar head group domain independently of the nature of the surfactant and the amount of water encapsulated into the micellar core. This behavior was attributed to the coexistence in its molecule of hydrophilic and lipophilic moieties and allowed to suggest that also in biological systems the melatonin, a potent scavenger of active oxygen species, is mainly confined within the polar head groups of membrane phospholipids where it is ready to play a role in the antioxidant protection of lipids scavenging aqueous as well as lipophilic radicals. Taking into account the structural peculiarities of the vitamin E molecule, it follows that the synergistic antioxidant effect of the combination of melatonin and α-tocopherol could be consistently rationalized by hypothesizing that both molecules act as free radical scavengers inside the biomembranes but being concentrated in spatially distinct solubilization sites. In order to confirm or disprove this hypothesis, it seemed very interesting to us to extend the previous investigation also to the partitioning of vitamin E. In particular, solutions of water-containing reversed micelles formed by AOT, didodecyldimethylammonium bromide (DDAB), lecithin and tetraethylene glycol monododecyl ether ($C_{12}E_4$) in apolar organic solvents were selected as model membranes to investigate the partitioning of vitamin E between the various microdomains characterizing these systems (organic solvent phase, surfactant alkyl chains, surfactant polar or ionic head groups and water) (Turco Liveri, 1997; Fendler, 1987). This set of surfactants (anionic, cationic, zwitterionic and polar) was chosen in order to study the influence of structurally different micellar head group regions on the binding strength of vitamin E.

2. Materials and methods

Vitamin E (95%), sodium bis (2-ethylhexyl) sulfosuccinate (AOT 98%), didodecyldimethylammonium bromide (DDAB 98%), tetraethylene glycol monododecyl ether (C12E4 98%), *n*-heptane (99%), cyclohexane (99%), (Sigma–Aldrich Co, Gillingham–Dorset, UK), soybean phosphatidylcholine (lecithin, Epikuron 200, 98%, Lucas Meyer, Hamburg, Germany), were used as received.

The micellar solutions were prepared by weight dissolving the surfactants in the organic solvents. Vitamin E was added to these solutions in order to obtain a solubilizate molal concentration of about 1.4×10^{-4} mol kg^{-1}. Different R values were obtained by adding appropriate amounts of water to the vitamin E/surfactant/organic solvent systems. UV-spectra of the samples were recorded at 25 °C in the wavelength range 200–360 nm with a frequency resolution of 0.5 nm by a Shimadzu Spectrophotometer UV 1601. As a background it was used the water/surfactant/organic solvent systems at the same composition.

3. Results and discussion

The interactions between vitamin E and water-containing reversed micelles were studied by varying the micellar concentrations at various molar ratio R ($R = [\text{water}]/[\text{surfactant}]$), which represents the main parameter controlling both the size of the water domain encapsulated within the re-

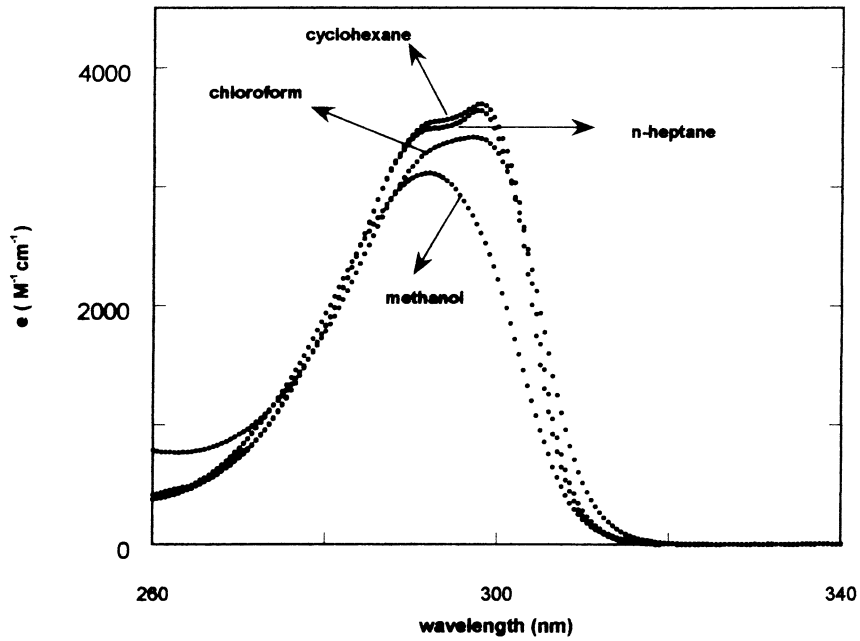

Fig. 2. UV spectra of solutions of vitamin E in methanol, chloroform, *n*-heptane and in cyclohexane.

versed micelles and the hydration degree of the surfactant head groups. UV absorption spectroscopy is considered a suitable technique to provide direct information on the location of vitamin E in these systems, since in a spectral region quite free from surfactant and organic solvent contributions, vitamin E displays an intense band centered at a wavelength of 298 nm, which is sensitive to its conformation and molecular environment.

Preliminary experiments were carried out to ascertain to what extent the UV spectra of vitamin E were affected by the nature of the surrounding molecules. In Fig. 2 the spectra of dilute solution of vitamin E in methanol, chloroform, *n*-heptane and in cyclohexane are reported.

As it can be seen, a quite marked dependence of the band shape and intensity on the nature of the solvent molecules is observed. It must be pointed out that the absorption spectrum of vitamin E in water was not carried out since it is practically insoluble in this medium: this involves that the amount of vitamin E solubilized in the aqueous micellar core can be neglected and consequently it can be expected that it is, in principle, only partitioned between the micellar palisade layer and the organic continuum.

Subsequently, we recorded the spectra of vitamin E dissolved in water/AOT/*n*-heptane, water/DDAB/cyclohexane, water/lecithin/cyclohexane and water/$CE_{12}E_4$/*n*-heptane microemulsions as a function of the surfactant concentration at some selected R values. As an example, in Fig. 3 are shown the spectra obtained for the system water/AOT/*n*-heptane at a fixed R value ($R = 10$) and various surfactant concentrations.

As it can be seen, there is a progressive evolution of the band with the increase of the AOT concentration. A similar behavior was observed for all the other investigated systems. This finding can be reasonably attributed to the progressive increase of the fraction (X_m) of vitamin E solubilized in reversed micelles with the increase of the surfactant concentration (Magid et al., 1981).

In order to describe quantitatively the partitioning of vitamin E between water containing reversed micelles and the organic solvent we have chosen to use the absorbance ratio (S) at two appropriately selected wavelengths (292 and 298 nm). These wavelengths were chosen in order to

maximize the sensitivity of the S parameter to the band evolution with the increase of the surfactant concentration (Magid et al., 1981). Moreover, since the experimental S value results from the contribution of the fraction of vitamin E solubilized in micelles (X_m) and in the organic solvent ($1 - X_m$), it follows that:

$$S = X_m S_m + (1 - X_m) S_o \quad (1)$$

where S_m and S_o are the S values when vitamin E is totally solubilized in the reversed micelles and in the pure organic solvent, respectively. Besides, in order to analyze the dependence of S on the surfactant concentration, it is necessary to develop a model for the partitioning of vitamin E between reversed micelles and apolar organic solvent.

Let us consider a system consisting of water-containing reversed micelles dispersed in an organic solvent. Adding a non-ionic solute, in our case vitamin E, it will be distributed between the organic and micellar pseudo-phases according to the distribution law:

$$K = \frac{m_m}{m_o} \quad (2)$$

assuming, as a first order approximation, an ideal behavior of the solute in both phases. In Eq. (2) K represents the Nernstian distribution constant, m_m and m_o represent the equilibrium molal concentrations of the solubilizate in the micellar and in the organic domain, respectively. Taking into account that the fraction of vitamin E solubilized in micelle is surely located in the micellar palizade layer, m_m must be expressed with respect to the micellized surfactant and considering 1 kg of the organic solvent, from Eq. (2) we obtain:

$$K = \frac{n_m}{n_o m_s P_A} \quad (3)$$

In Eq. (3) n_m and n_o are the moles of the solubilizate in the micellar and in the organic phases, m_s is the molal concentration of the surfactant and P_A represents the surfactant molecular weight (expressed in kg). Eq. (3) can be put in the form:

$$K' = \frac{n_m}{n_o m_s} \quad (4)$$

formally identical to that used by Magid et al. to analyze UV–vis spectra of phenols in water/AOT/isooctane system (Magid et al. 1981; D'Aprano et al., 1989). Note that the value of K of the above equation is $K' = KP_A$.

Eq. (4) can be rearranged as:

$$X_m = \frac{K' m_s}{1 + K' m_s} \quad (5)$$

Finally combining Eq. (1) and Eq. (5), we obtain:

$$S = S_o + \frac{(S_m - S_o) K' m_s}{1 + K' m_s} \quad (6)$$

It must be pointed out that Eq. (6) holds under the assumption that S_m is independent of the surfactant concentration at fixed R. As can be seen from Fig. 4, a non linear regression analysis of Eq. (6) reproduces, within the experimental

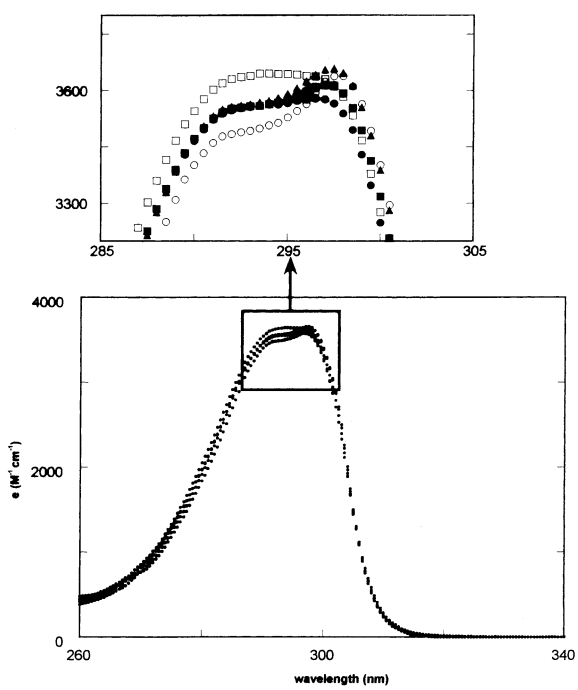

Fig. 3. UV spectra of water/AOT/n-heptane microemulsions at $R = 10$ at various AOT concentrations (○, $m_{AOT} = 0$ mol kg^{-1}; ▲, $m_{AOT} = 0.106$ mol kg^{-1}; ■, $m_{AOT} = 0.227$ mol kg^{-1}; ●, $m_{AOT} = 0.530$ mol kg^{-1}; □, $m_{AOT} = 0.956$ mol kg^{-1}).

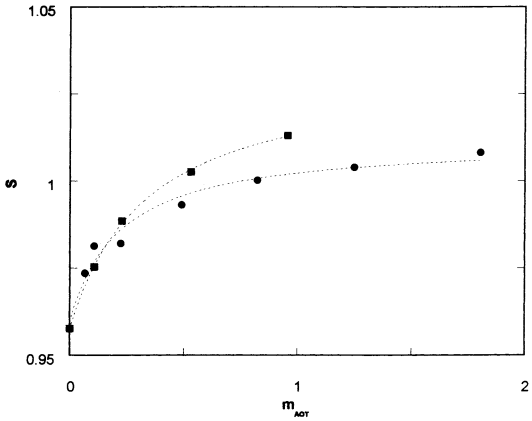

Fig. 4. Typical spectrophotometric data (S, m_s) and fitting curves of vitamin E in water/AOT/n-heptane at two selected R values. (●, $R=0$; ■, $R=10$).

errors, the spectrophotometric data (S, m_s) for vitamin E in water/AOT/n-heptane at two selected R values. To save space, similar curve fittings for the other systems analyzed in the present work are not reported.

The derived quantities K ($K=K'/P_A$) and S_m are summarized in Table 1.

In Fig. 5 the K values for vitamin E in the different microemulsions examined are reported as a function of R.

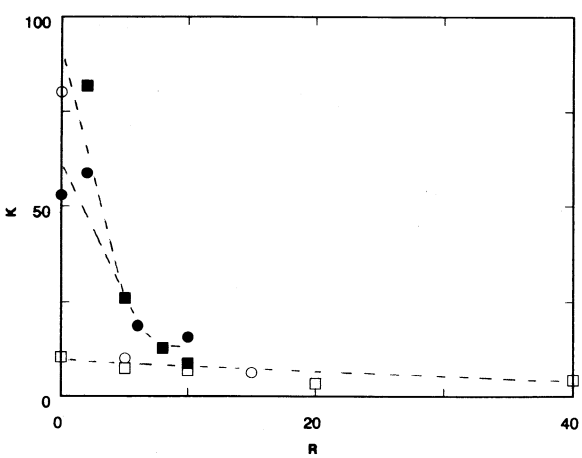

Fig. 5. Distribution constants (K) of vitamin E to water-containing reversed micelles as a function of R. The dashed lines are only guides for the eye. (□, AOT; ■, DDAB; ○, lecithin; ●, $C_{12}E_4$).

It can be noted that at $R=0$, the K values are in the order $K_{lecithin} \approx K_{DDAB} > K_{Cl2E4} > K_{AOT}$. This order obviously reflects the strength of the binding between the polar head of vitamin E and that of the surfactant. Contributions to the binding due to the entrapment of the oriented vitamin E molecules between the alkyl chains of the surfactant can also be postulated. Besides, as a general trend, K decreases with R tending at a constant value at the higher R values. In particular, a dramatic K decrease is determined by the initial addition of water in the case of reversed micelles of DDAB, lecithin and $C_{12}E_4$. This finding indicates a marked competition between water and vitamin E for the binding sites at the water/surfactant interface involving a reduction of the binding strength with R and consequently a displacement of the vitamin E towards a more external part of the micellar palisade layer and to an increase of its motional dynamics within this domain. These considerations are also emphasized by the S_m values and the comparison of these values with those observed in pure n-heptane ($S_o=0.96$), cyclohexane ($S_o=0.96$), chloroform ($S_o=0.97$) and methanol ($S_o=1.19$). It can be noted, in fact, that only small variations of S_m occur by changing R or the nature of the surfactant and also that the S_m values are intermediate between the value in chloroform and that in methanol. This means that, despite the strong structural and dynamical variations of the head group regions induced by the water addition, the environment probed by the chromophore group of the vitamin E is characterized by a relatively low polarity independently of the R value and the surfactant nature. In other words, experimental data suggest that the fraction of vitamin E solubilized in water-containing reversed micelles is preferentially located opportunally oriented between the alkyl chains of the surfactant with the OH group protruding towards the hydrated micellar head group region (Takahashi et al., 1989).

4. Conclusions

Our results are consistent with the hypothesis that vitamin E is confined in the apolar domain of

Table 1
Fitting parameters for the partitioning of vitamin E between water-containing reversed micelles and apolar organic solvent at various R values and at 25 °C

AOT			DDAB			lecithin			$C_{12}E_4$		
R	K	S_m	R	K	S_m	R	K	S_m	R	K	S_m
0	10.6 ± 2.9	1.011 ± 0.003	2	81.8 ± 4.8	0.987 ± 0.001	0	80.2 ± 6.5	0.990 ± 0.001	0	53.0 ± 6.9	1.014 ± 0.001
5	7.4 ± 1.6	1.025 ± 0.004	5	26.0 ± 0.6	1.039 ± 0.001	5	10.1 ± 6.8	1.04 ± 0.04	2	58.8 ± 13.8	1.010 ± 0.002
10	6.8 ± 0.7	1.032 ± 0.003	8	12.8 ± 1.3	1.030 ± 0.002	15	6.3 ± 4.0	1.04 ± 0.04	6	18.8 ± 3.6	1.019 ± 0.003
20	3.4 ± 0.9	1.055 ± 0.007	10	8.7 ± 0.6	1.039 ± 0.002				10	15.7 ± 3.9	1.020 ± 0.004
40	4.3 ± 1.3	1.041 ± 0.008									

reversed micelle, with the OH group of the chromanol nucleus protruding in the hydrated polar or ionic head region of the surfactant. This is in agreement with the finding that vitamin E is located in the hydrophobic chain of the phospholipidic bilayer of cell membranes obtained by other techniques (Fukuzawa et al., 1981; Urano et al., 1987; Wassall et al., 1991).

A competition between water and vitamin E for the binding sites at the water/surfactant interface leading to a decrease of the binding constant and consequently to an increase of its motional dynamics within the micellar palisade layer has been pointed out (Urano et al., 1993). This could involve effects, both on the fluidity of this domain and on the rate of peroxidation reaction (Lefevre and Picquart, 1996).

Finally, the different preferential location of vitamin E and melatonin (Ceraulo et al., 1999) in the reversed micelles implies that while melatonin could act as chain-breaking of peroxidation reactions scavenging free radicals arising from aqueous regions, vitamin E could act more promptly with those residing in the apolar domain. Extending the validity of these considerations to the cell membrane, the combined action of both scavangers could improve their effectiveness in preserving the membrane damage, so explaining their synergistic antioxidant effect (Livrea et al., 1997).

Acknowledgements

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