J. Membrane Biol. 58, 67–74 (1981)

The Journal of
Membrane Biology

Transmembrane Distribution of α-Tocopherol in Single-Lamellar Mixed Lipid Vesicles

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Summary. A study of the molar ratio dependence of the incorporation of α-tocopherol into single-lamellar vesicles showed that the number of molecules which the bilayers can accommodate increased linearly with increasing α-tocopherol/phosphatidylcholine initial molar ratios till about 0.05, then approached a saturation limit. At 5 mol%, one α-tocopherol molecule per 60 phospholipids can be incorporated into the membranes. Up to this limit the distribution of α-tocopherol in the bilayers is uniform, while at initial molar ratios higher than 0.05 a disproportionation toward the inner monolayer of the vesicles is observed. The average outer/total ratio is found to be 0.27 ± 0.03 at α-tocopherol/phosphatidylcholine molar ratios above 0.07 and is similar to asymmetrical distributions that have been reported in vesicles containing other one-chain amphiphiles (e.g., cholesterol). This large disproportionation is in contrast with the packing distribution of certain two-chain amphiphiles, and indicates that one of the driving forces for asymmetry formation in lipid bilayers might be dependent on the number of hydrocarbon chains per amphiphile molecule. A possible reason for the disproportionation effect observed in our experiments is the displacement of unsaturated phospholipids to the outer monolayer of the single-lamellar vesicles, by the more rigid isoprene units of α-tocopherol.

Recent investigations of biological membranes and model phospholipid lamellae have suggested that certain permeability properties of these systems are related to the presence of α-tocopherol in the lipid bilayers (Diplock et al., 1977; Fukuzawa et al., 1979). Available experimental data show that these properties may result from a structural relationship between the isoprenic units of α-tocopherol and the polyunsaturated fatty acid residues of the membrane lipids (Diplock & Lucy, 1973; Lucy, 1978). This hypothesis has been also suggested to explain the membrane stabilizing activity of α-tocopherol (Lucy, 1972). Regulation of *trans-gauche* transitions of aliphatic fatty acids and interactions of isoprenic methyl groups with *cis* pockets of unsaturated hydrocarbon chains are thus among the possible mechanisms of tocopherol-controlled molecular mobility and order in the membrane. It is emphasized, nevertheless, that the regulatory activity of the hydrophobic portion of α-tocopherol is not unique. When the OH group of the chroman ring is substituted for amino or N-methylamino groups, the stabilizing effects of the isoprenic chain are lost (Fukuzawa, Hayashi & Suzuki, 1977).

One of the ways to investigate the validity of the foregoing assumptions is to determine the geometric arrangement of α-tocopherol in the lamellar system in relation to molecular diffusion across the bilayer. As a prerequisite for such studies it is important to ascertain the α-tocopherol distribution in the outer and inner surfaces of the vesicles walls on account of possible transmembrane lipid asymmetries – e.g., asymmetrical distribution of double bonds and *gauche* configurations (Huang et al., 1974; Thompson, Huang & Litman, 1974; Yeagle et al., 1976) – which may influence distinctively the fluidity and permeability properties of the two monolayers of the vesicle wall. To this end, the present paper describes first a procedure for the direct chemical determination of the α-tocopherol incorporated in single-lamellar vesicles. With this method one measures the kinetics of the reaction of the free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) with the hydroxy function of the chromanol moiety of α-tocopherol (*see* Bellemare & Fragata, 1980). The variations in membrane composition investigated concern the concentration of α-tocopher-

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0022-2631/81/0058-0067 $01.60

ol incorporated in the lamellae as a function of the initial mole fraction of binary mixtures of α-tocopherol and phosphatidylcholine dispersed in buffer, and the symmetries of distribution of α-tocopherol in the vesicle's wall.

The structure-function relationships discussed here let us believe (*see* Conclusion) that the characterization of bilayers formed with lipids containing isoprenic chains constitutes a valuable tool for model membrane studies of molecules involved in biological oxidation-reduction, in particular the chlorophylls, the carotenes, or the quinones (*see*, e.g., Rosenberg, 1967; Oettmeier, Norris & Katz, 1976; Hauska, 1977; Fragata, 1978).

Materials and Methods

Materials

Phosphatidylcholine was isolated from egg yolk and purified according to the procedure of Singleton et al. (1965). The resultant product gave a single orange spot with the Dragendorf reagent (Waldi, 1965) when assayed by thin-layer chromatography (chloroform/methanol/water, 65:25:4) on silica gel G (Sigma Chemical Company, Lot 93C-1390). D-α-Tocopherol was obtained from Eastman Kodak Company (Lot B8), and its purity was verified by thin-layer chromatography (benzene/ethanol, 99:1; cyclohexane/chloroform, 2:1) on silica gel G; a single blue spot was observed upon reaction with K_3FeCN_6 (3%) followed by $FeCl_3$ (3%). All other chemicals were reagent grade.

Preparation of Small, Single-Lamellar Vesicles

For membrane formation, phosphatidylcholine-α-tocopherol mixtures with various molar ratios of the two lipids were used. We followed the preparative procedure of Huang (1969) with some modifications. About 150 mg of phosphatidylcholine were diluted in diethylether containing α-tocopherol. The binary mixture was evaporated to dryness under argon, and the dried lipids were dispersed in 5 ml of 0.1 M NaCl, 0.01 M Tris-HCl buffer (pH 8.0). The aqueous mixture of the lipids was subjected to 8–10 min sonication at 30 °C in a sonifier cell disrupter (Heat Systems-Ultrasonics) set at about 60 W output, with argon bubbling into the mixture. α-Tocopherol oxidation was checked before and after the sonication by its reaction with DPPH. The lipid dispersion was then subjected to centrifugation at $100{,}000 \times g$ for 1 hr, followed by concentration in a Amicon cell to about 1.5 ml and fractionation in a Sepharose 4B (Pharmacia Fine Chemicals) column (2.5× 60 cm). Only the homogeneous fractions (*see* Fig. 2*A*) were retained for reactions of DPPH with α-tocopherol.

Determination of Lipid Phosphorus and α-Tocopherol Contents

The concentration of phosphatidylcholine in the different fractions eluted from the Sepharose 4B column is expressed in terms of lipid phosphorus as determined by a modified method of Bartlett (1959). Upon color development the absorbance of the samples was read at 660 nm and compared to a KH_2PO_4 standardization curve whose angular coefficient was calculated to be 0.625 ml·μmole^{-1}.

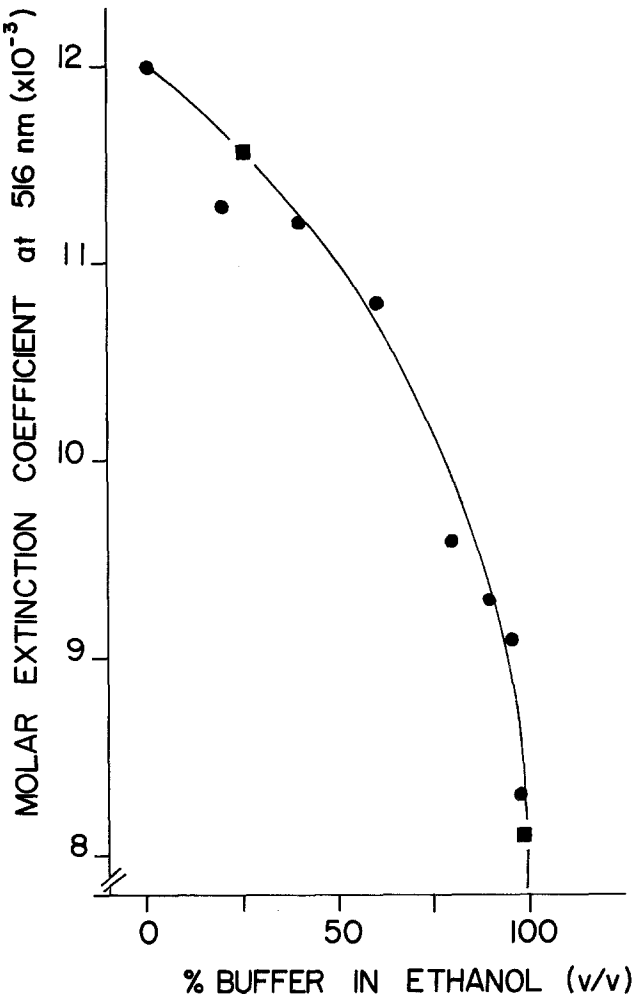

Fig. 1. Molar extinction coefficients at 516 nm for 1,1-diphenyl-2-picrylhydrazyl in various binary mixtures of buffer (0.1 M NaCl-0.01 M Tris-HCl, pH 8.0) and ethanol. The symbol ■ specifies the % buffer in ethanol used for the determination of total (low%) and outer (high%) α-tocopherol in the vesicles

Total α-tocopherol content in single- and multilamellar vesicles was determined by titration with DPPH (a product of Eastman Organic Chemicals, Lot 11A, and BDH, Lot 1876240). 1.0 ml of vesicles dispersions in 0.1 M NaCl, 0.01 M Tris-HCl buffer (pH 8.0) were mixed with 3.0 ml of a 0.1 mM solution of DPPH in ethanol. The final ethanol concentration (75%, vol/vol) is sufficient to disintegrate the vesicles (Huang et al., 1970). DPPH bleaching was followed at 516 nm with a Perkin-Elmer/Coleman 124, or a Cary 17D spectrophotometer. The number of moles of α-tocopherol that reacted with DPPH were determined from the Beer-Lambert expression for the absorbance. Molar extinction coefficients for DPPH in various binary mixtures of buffer and ethanol are given in Fig. 1.

For the determination of α-tocopherol at the outer surface of single-lamellar vesicles, 1.0-ml samples of the vesicles solutions were mixed with 3.0 ml of diluted ethanolic solutions of DPPH. The final ethanol concentration in the reaction mixture was not more than 1% (vol/vol). This ethanol concentration does not disrupt the vesicles as shown by Sepharose 4B gel filtration (*unpublished results*), and does not interfere with the course of the reaction. Experiments using methanol and propanol as the diluting solvent

of DPPH instead of ethanol, showed that the pseudo-first-order rate constants k' of the reactions differ by about 3–4%. The extent of these variations assure that solvent effects must not be involved at the ethanol concentration used.

Results and Discussion

Vesicle Content of α-Tocopherol and Phosphatidylcholine

Typical elution diagrams of dispersions containing single- and multilamellar phosphatidylcholine-α-tocopherol vesicles were obtained upon sonication of binary mixtures of the lipids followed by centrifugation and gel filtration in a Sepharose 4B column. The diagram of Fig. 2*A* consists of a fraction *I* (large multilamellar vesicles), a fraction *II* (single-lamellar vesicles about 200–250 Å in diameter), and an intermediate region where fractions *I* and *II* may overlap. Figure 2*B* gives the vesicles content of lipid phosphorus (P_i) and α-tocopherol (αT) in each tube eluted from the column. It should be noted that the similitude of the elution patterns of the two lipids is the result of a relation between the concentrations of phosphatidylcholine and α-tocopherol which can be incorporated in the vesicles. This is best seen in Fig. 3.

Plots of absorbance *vs.* P_i and αT contents are given in Fig. 3*A*. The numbers adjacent to data points correspond to the tube numbers plotted in Fig. 2*A*. The graph shows that the absorbance of fraction *II* vesicles is proportional to P_i and αT concentrations. Regression lines covering samples 36 to 42 were obtained from linear least-squares curve fitting program. The corresponding correlation coefficients were calculated to be 0.999 for phosphatidylcholine and α-tocopherol. The linearity of such plots was shown to be characteristic of samples constituted of homogeneous vesicles (Huang, 1969, Litman, 1973). This is corroborated in the present work by a plot of α-tocopherol *vs.* lipid phosphorus concentrations for samples 36–42 (Fig. 3*B*). The linear regression line passes almost through the origin, and the regression correlation coefficient is 0.999.

From the straight line represented in Fig. 3*B* it can be calculated that in the particular example represented in the figure (initial $\alpha T/P_i$ molar ratio of 0.11) about one α-tocopherol molecule per 39 phosphatidylcholine molecules were included in small homogeneous vesicles. This question was clarified for a wide range of initial molar fractions. Figure 4 shows that the number of α-tocopherol molecules which the membrane can accommodate increases with the relative concentrations of the lipids in the binary mixtures dispersed in buffer up to $\alpha T/P_i$ molar ratios of about 0.048. This value corresponds to a $\alpha T/P_i$ ratio in the vesicles of 1:60. For initial compositions in the sonication vessel larger than 0.048, the regression line representing the vesicles content of α-tocopherol bends drastically to what appears to be a saturation limit. These observations bear an impressive ressemblance to the results obtained by Ritt and Walz (1976). These authors prepared single-walled vesicles containing chlorophyll *a* and egg lecithin according to experimental procedures basically identical to ours (Huang's method modified; *see* Huang, 1969). They showed that up to one chlorophyll *a* (Chl *a*) molecule per 55 phosphatidylcholine molecules can be incorporated in the bilayers. Moreover, the limiting Chl a/P_i initial molar ratio was found to be nearly 0.03.

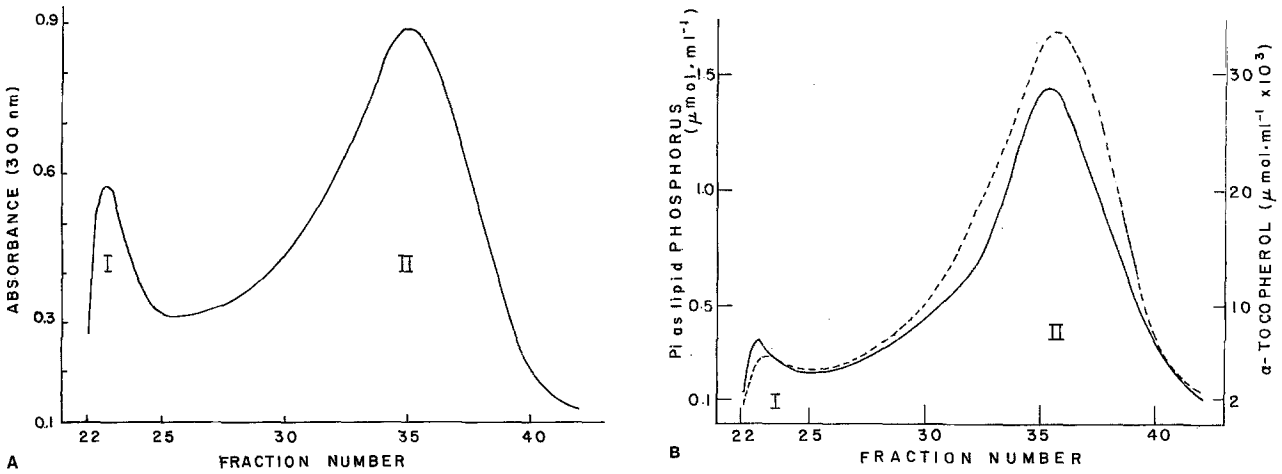

Fig. 2. Elution profiles of phosphatidylcholine-α-tocopherol vesicles prepared by ultrasonic dispersion in 0.1 M NaCl-0.01 M Tris-HCl at pH 8.0. (*A*): Absorbance at 300 nm of the tubes eluted from the Sepharose 4B column. (*B*): Lipid phosphorus (full line) and α-tocopherol (broken line) contents of each of the tubes

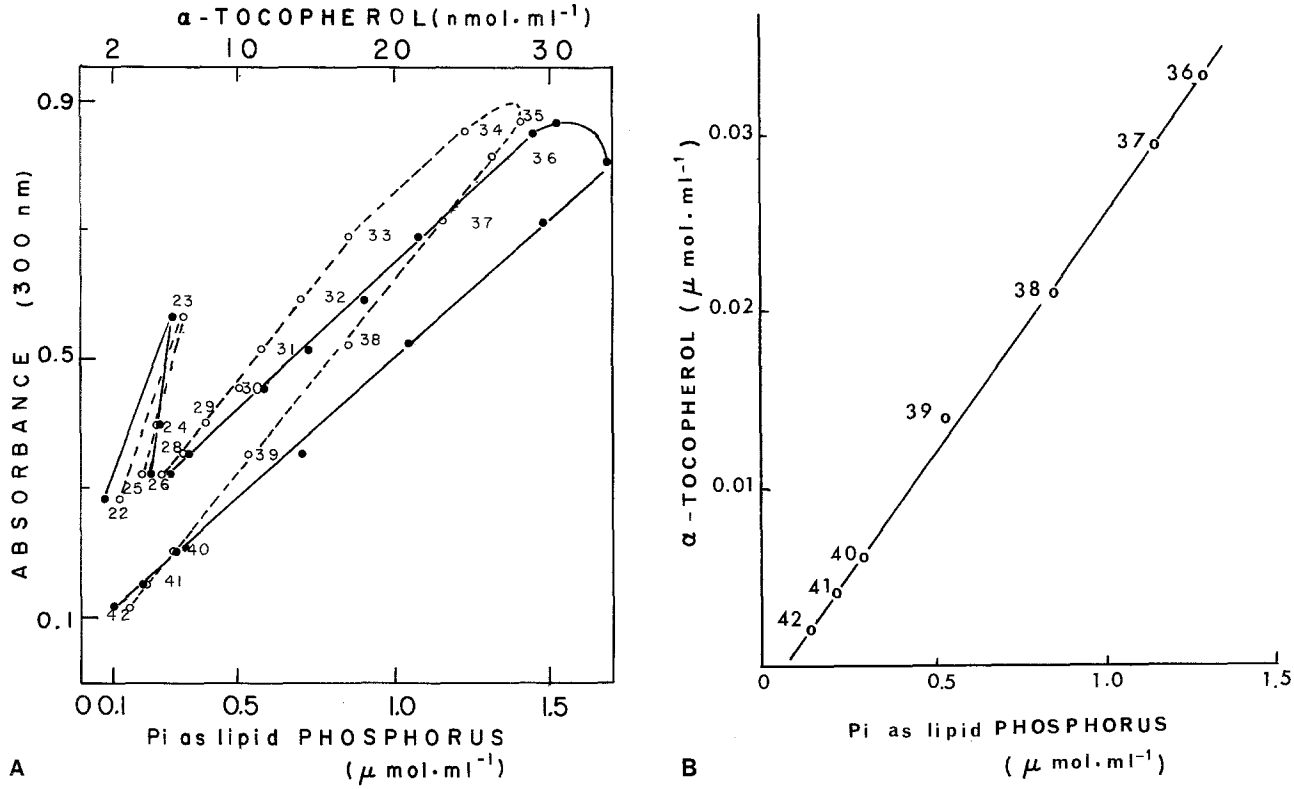

Fig. 3. (*A*): Absorbance of phosphatidylcholine-α-tocopherol vesicles at 300 nm *vs.* lipid phosphorus (open dots) and α-tocopherol (solid dots) contents. The number adjacent to each data point indicates the tube number of the solutions eluted from the Sepharose 4B column. Regression lines for tubes *36–42* were obtained from linear least-squares curve fitting program. The correlation coefficients for lipid phosphorus and α-tocopherol were calculated to be 0.999. (*B*): Plot of α-tocopherol *vs.* lipid phosphorus concentrations for tubes *36–42*. The straight line is represented by the equation $y = -0.00144 + 0.0268\,x$. The linear regression correlation coefficient is 0.999

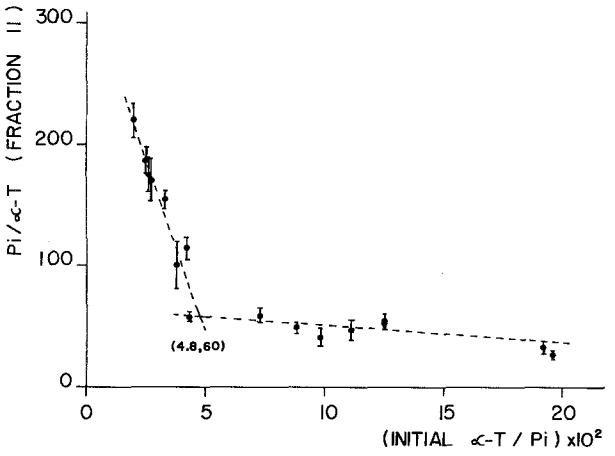

Fig. 4. The dependence of the incorporated α-tocopherol in small, single-lamellar vesicles on the initial α-tocopherol/phosphatidylcholine molar ratio. Data are given as mean ± SD. αT, α-tocopherol. P_i, phosphatidylcholine as lipid phosphorus

The relevance of this comparison is straightforward if one takes into account that the chlorophyll *a* and α-tocopherol molecules have in common a saturated isoprenic chain of comparable size and differ only in the chemical structures of their chroman and tetrapyrrole cycles, as well as by the presence of a double bond near the hydroxy function of the chlorophyll phytyl. Nonetheless, emphasis is laid on a work of Dijkmans et al. (1979) with chlorophyll *a*-phosphatidylcholine vesicles prepared without sonication by the method of Batzri and Korn (1973), where it is reported an incorporated Chl *a*/P_i molar ratio of 1:11 when the initial chlorophyll concentration in the membrane-forming solution is 0.09%. As yet, we have no satisfactory explanation for these discrepancies, although it may be presumed reasonably that major differences in the preparative procedures of single-walled vesicles – the sonication toward the alcohol injection method – could be one of the possible causes. A comparative study of these methods is under investigation in our laboratory.

It is worthy of mention in this same connection that saturation of lipid incorporation was also observed in binary mixtures of thiocholesterol (SH) and egg phosphatidylcholine (Huang et al., 1970). It was found that at SH/P_i equal to one, i.e., at 50% cholesterol in the sonication vessel, about one thiocholesterol molecule per 33 phosphatidylcholine molecules are included in the homogeneous vesicles (*cf.* Fig. 4 in

Huang et al., 1970). Also, limiting molar ratios of egg yolk phosphatidylcholine/cholesteryl linolenate of 50:1 to 22:1 were found to predominate in lamellar liquid-crystalline structures formed with binary mixtures of the lipids in water (Janiak et al., 1974). The aforediscussed experiments raise the interesting point that low lipid incorporation would seem to be expected with one-chain amphiphiles. However, this has been demonstrated not to be the case with certain two-chain amphiphiles. For example, it is clearly established (Litman, 1973) that the molar ratio PE/P_i of incorporated phosphatidylethanolamine (PE) in single-lamellar vesicles prepared with phosphatidylcholine is similar to the PE/P_i molar ratio in the initial binary mixture of the phospholipids.

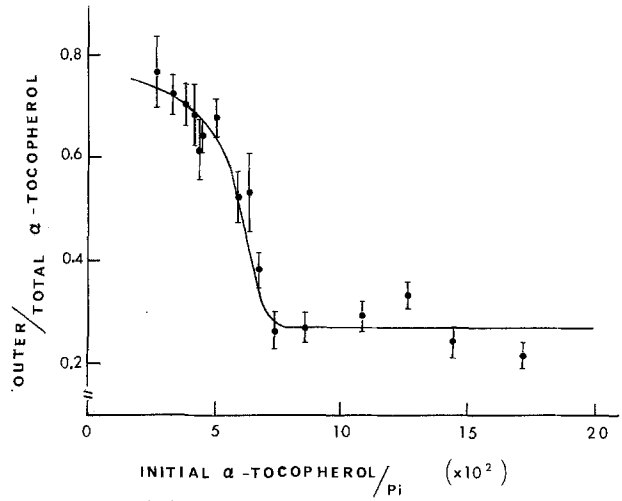

Fig. 5. Ratio of the outer monolayer α-tocopherol to the total α-tocopherol as a function of the composition of the small, single-lamellar mixed lipid vesicles. The results are given as mean ± SD. Statistical analysis of the data showed that the ratio outer α-tocopherol/total α-tocopherol is independent (linear correlation coefficient = 0.126) of initial α-tocopherol/phosphatidylcholine (P_i) molar ratios higher than 0.07. Its average value was found to be 0.27 ± 0.03

Transmembrane Distribution of α-Tocopherol

The partitioning of α-tocopherol between the two monolayers of single-lamellar vesicles was studied from determinations of outer and total α-tocopherol contents. It was not possible to obtain directly the concentration of α-tocopherol in the inner monolayer. The difference total minus outer α-tocopherol was thus used to estimate the inner concentration of the lipid. The variation of the ratio outer αT/total αT as a function of the composition of initial binary mixtures of α-tocopherol and phosphatidylcholine is plotted in Fig. 5. The ratio is approximately 0.67 at 5 mol% α-tocopherol (*see*, in this respect, Fig. 4 and related discussion in the text). This ratio corresponds to a distribution of 67% of the α-tocopherol molecules in the outer monolayer and 33% in the inner monolayer. These values are in close agreement with average surface areas and molecular packings determined for the outer and inner walls of small, single-lamellar vesicles prepared with egg phosphatidylcholine (Huang & Mason, 1978). We conclude therefore that at initial molar ratios smaller than 0.05, the α-tocopherol molecules appear to be uniformly solubilized in the bilayer membrane.

A disproportionation of α-tocopherol toward the inner monolayer was found at αT/P_i initial molar ratios greater than about 0.05. The curve represented in Fig. 5 decreased sharply and approached a outer/total ratio of 0.27 ± 0.03 (mean ± SD), at 7 mol% α-tocopherol. Statistical analysis of the data showed that this value is independent of initial αT/P_i molar ratios higher than 0.07 (*see* caption of Fig. 5).

The asymmetric distribution of α-tocopherol discussed above can be usefully compared to a ratio of 0.31 calculated for cholesterol included in vesicles prepared with 1,2-dioleoyl-*sn*-glycero-3-phosphorylcholine[1] (*cf.* Table II in de Kruijff, Cullis & Radda, 1975, and Cullis et al., 1977). In contrast with this, disproportionation studies with two-chain amphiphiles revealed that the outer/total ratios can reach values of about 0.5 (Litman, 1973; Lentz & Litman, 1978; Massari, Pascolini & Gradenigo, 1978; Low & Zilversmit, 1980) which are significantly higher than the values obtained with one-chain amphiphiles. These ratios of lipids distribution in lamellar systems may imply that we are dealing with selective self-association properties of one-chain and two-chain amphiphiles as has been hypothesized in the preceding section. The significance of these differences remains uncertain, but they suggest that, in addition to the headgroup packing requirements (Litman, 1975), the driving force for asymmetry formation in the bilayers is intimately connected with the number of hydrocarbon chains per lipid molecule. The work of Yeagle et al. (1976) on transmembrane asymmetrical distribution of double bonds will eventually provide an explanation of these effects if it can be proved that the more rigid α-tocopherol and cholesterol molecules enhance the displacement of unsaturated phospholipids to the outer monolayer of the unilamellar vesicles.

It is of interest to note that a long-term stability of compositional asymmetries of single-lamellar vesicles cannot be taken for granted (*see* Table 1). We

[1] Dioleoylphosphatidylcholine and egg phosphatidylcholine molecules have in common a large percentage of *cis* double bonds in their fatty acyl residues. We can assume to a certain extent that the two lamellar systems have comparable unsaturated hydrophobic cores.

Table 1. Ratio of the outer monolayer α-tocopherol to the total α-tocopherol as a function of time and the composition of the binary mixture of lipids used to form the small, single-lamellar vesicles

Initial molar composition[a] ($\alpha T/P_i \times 10^2$)	Outer αT/Total αT				
	1	3	4	5	6 days
5.9	0.53 ±0.05	0.65 ±0.05	0.70 ±0.05		
12.5	0.33 ±0.03			0.58 ±0.02	
20.0	0.29 ±0.04	0.38 ±0.04			0.42 ±0.08

The experimental errors are represented as ±SD.

[a] αT, α-tocopherol; P_i, lipid phosphorus.

observed that some of the lowest outer αT/total αT ratios increased with time and eventually approached the equilibrium ratio, i.e. 0.67. For example, the table indicates that it took about six days for phosphatidylcholine vesicles prepared with 20 mol% α-tocopherol (initial ratio) to change their outer αT/total αT ratio from 0.29±0.04 to 0.42±0.08. To explain these results we postulated a slow migration of α-tocopherol at 2–3 °C (storage temperature of the vesicles) from the inner vesicle monolayer to the outer monolayer. It was not possible at this point to determine the half-time ($t_{1/2}$) for the α-tocopherol exchange through the bilayer; this requires further investigation of the kinetics involved. We presume, nevertheless, that an acceptable $t_{1/2}$ could be of the order of 3 to 6 days.

Transbilayer diffusions (flip-flop) of lipid components were not described very often. A recent review of Thompson and Huang (1978) summarizes current evidence for the occurence of these phenomena in single-bilayer vesicles and biological membranes. However, any comparison with our results should be done cautiously because the data available in the literature concern in general two-chain amphiphiles. Another complication that may hamper any reasonable interpretation of the results comes from the disagreement among the authors concerning the half-time for transbilayer migration. To give but an example, reported $t_{1/2}$ values for egg phosphatidylcholine extend from 6.5 hr to 11 days or more (Kornberg & McConnell, 1971; Rothman & Dawidowicz, 1975). These matters are of a fundamental nature and deserve a more detailed examination in future works as the effects described here may have their origin in thermodynamic instabilities that are probably dissipated by flip-flop mechanisms.

Conclusion

At first one may argue that the aforediscussed incorporation of α-tocopherol (2–3 mol%, *cf.* Fig. 4) in single-lamellar vesicles is surprisingly low. However, such low lipid incorporations were also reported to occur in model membranes prepared with chlorophyll *a* and thiocholesterol. Table 2 gives an account of the packing densities (molecules/cm^2) of these molecules in black lipid membranes (BLM) and single-lamellar vesicles, and of our results given heretofore. The table provides compelling evidence that the incorporation of α-tocopherol in single-lamellar vesicles is in the range of the packing densities that are to be expected with one-chain amphiphiles. As an example, at similar initial molar ratios of chlorophyll *a*/phosphatidylcholine (>1:33) and α-tocopherol/phosphatidylcholine (1:21) the total number of amphiphiles incorporated per cm^2 of vesicle surface area is 2.6×10^{12} and 2.4×10^{12}, respectively. In this connection, it should be mentioned that corrections to account for the disproportionation effect at high α-tocopherol concentrations revealed that the number of α-tocopherol molecules incorporated per cm^2 of inner wall surface equals approximately 11.6×10^{12} when the initial $\alpha T:P_i$ ratio of lipids dispersed in buffer is 1:5. This calculated value is quite higher than the global low lipid concentrations reported herein and is to be compared with 26×10^{12} chlorophyll *a* molecules/cm^2 incorporated in BLM prepared from binary mixtures of the pigment with dioleoyl-phosphatidylcholine at initial ratios of 2:3. In the conditions of our experiments it was not possible to obtain packing densities higher than 11.6×10^{12} molecules/cm^2 in small, single-lamellar vesicles. We suggest that this value might approach an upper limit in view of the difficulty to form aqueous dispersions of phosphatidylcholine and α-tocopherol at concentrations of the latter lipid above 20%.

In addition, it is pointed out that the special $\alpha T/P_i$ initial molar ratio of 0.05 represented in Fig. 4, is considered tentatively as a critical micellar concentration (cmc: *cf.* Fig. 7-1 in Tanford, 1973) because the ordinate axis of the figure is a measure of the number of α-tocopherol monomers included in the bilayer. We wish to remark that this assumption can be also applied to oxidation-reduction data reported by Futami, Hurt and Hauska (1979) (*cf.* Fig. 3 of their work). These authors prepared lipid vesicles (liposomes) constituted of binary mixtures of plastoquinone (PQ) or a ubiquinone containing nine isoprene units in the side chain (Q-9), and soy bean phosphatidylcholine. The liposomes had the electron acceptor ferricyanide trapped inside the aqueous compartment. It is found that the pseudo first-order rate constant (k) of electron transfer from an external electron donor (dithionite) to ferricyanide is dependent on the initial quinone/phospholipid molar ratio. Interesting enough, at initial molar ratios higher than 1:100 the k values

Table 2. Molar compositions of one-chain amphiphiles (Chla, SH, αT) per surface area of bilayer lipid membranes (BLM) and single-lamellar vesicles (SLV)

Lamellar system	Initial molar ratio[a]		Composition of the lamellar system[b]				References
			Molar ratio	One-chain amphiphile molecules $\times 10^{-12}/cm^2$			
				Total	Outer	Inner	
SLV: total bilayer	SH/PC	1:0.33	1:17	8.4			[e]
		1:1	1:33	4.3			[e]
Ibid.	Chla/PC	>1:33	1:55	2.6			[f]
Ibid.	αT/PC	1:21	1:60	2.4			This work
		1:14	1:58	2.5			This work
		1:5	1:32	4.5			This work
SLV/outer monolayer	αT/PC	1:21	1:60		2.2		This work
		1:14	1:58		1.0		This work
		1:5	1:32		1.7		This work
SLV/inner monolayer	αT/PC	1:21	1:60			2.9	This work
		1:14	1:58			6.6	This work
		1:5	1:32			11.4	This work
BLM/total bilayer	Chla/DOPC	1:50		0.65			[g]
		1:20		1.5			[g]
		1:10		4			[g]
Ibid.	Chla/DOPC	1:19		0.8[c]			[h]
		1:19		3.3[d]			[h]
		2:3		26			[h]
Ibid.	Chla/GMO	n.s.		1.4			[i]

[a] Chla, Chlorophyll *a*. DOPC, dioleoylphosphatidylcholine. GMO, glycerylmonooleate. n.s., not specified but lying above critical micellar concentration. PC, egg phosphatidylcholine. SH, thiocholesterol. αT, α-tocopherol.
[b] The number of molecules per cm^2 obtained from refs. e and f were calculated according to molecules packing determinations of Huang and Mason (1978).
[c] Fluorescence measurements.
[d] Photometric measurements.
[e] Huang et al. (1970).
[f] Ritt and Walz (1976).
[g] Alamuti and Läuger (1970).
[h] Steinemann et al. (1971).
[i] Trosper (1972).

decreased sharply to attain eventually an equilibrium rate constant. Figure 3 of Futami et al. and our Fig. 4 are to a great extent similar and suggest that the decrease of k arises as the result of saturation of the lipid bilayers at the quinone concentrations used. Taking into consideration the common isoprenoid character of PQ, Q-9 and α-tocopherol, it seems clear that the one-chain amphiphiles described here might have identical packing requirements in phospholipid-containing vesicles. Our investigations of the packing densities of α-tocopherol in the outer and the inner monolayers of mixed lipid vesicles may be thus instrumental to explain variations of rate constants of electron transfer in asymmetric bilayer membranes. This possibility is made attractive by the demonstration (Futami et al., 1979) that α-tocopherol is a good catalyst ($k=0.724\ s^{-1}$ compared to $0.293\ s^{-1}$ for PQ and $1.439\ s^{-1}$ for Q-9) for the transfer of electrons across the vesicles wall.

This work was supported by the Natural Sciences and Engineering Research Council Canada (Grant No. A6357), and the Research Committee (F.I.R.) and the Biophysics Research Group, University of Québec at Trois-Rivières.

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Received 12 May 1980, revised 13 October 1980