this work the X-ray diffraction study of an Iva-containing 3_{10}-*helical* peptide is reported. These findings agree well with the results of our conformational energy computations of the Iva monopeptide.[9]

However, on the basis of the present crystallographic analysis we have been unable to correlate Iva configuration with helix handedness. Surprisingly, it appears that a single *chiral* Iva residue is *not* sufficient to induce a preferential handedness in a 3_{10}-helical, Aib-rich pentapeptide. As a result, both right- and left-handed helices concomitantly occur in the same crystal, as already observed for fully blocked, *achiral* $(Aib)_5$ homopeptides.[61-63] This result is in contrast with that obtained in an X-ray diffraction analysis of *p*BrBz-$(Aib)_n$-*S*-Leu-$(Aib)_2$-OMe (n = 4, 5), which have been shown to adopt the expected *right*-handed 3_{10}-helical structure in the crystal state,[64] and, to our knowledge, represents the *first observation* of a helical peptide containing a chiral residue *not* showing a discrimination between screw senses under these conditions. Work is in progress to determine (*i*) the minimum length of the R side chain in the chiral $-NH-C(CH_3)R-CO-$ X residue of Ac-$(Aib)_2$-X-$(Aib)_2$-OMe and/or (ii) the minimum number and main-chain position of Iva residues in an N-acetylated, C-methoxylated Aib/Iva pentapeptide required to induce a preferential screw sense.

Acknowledgment. We thank the Deutsche Forschungsgemeinschaft for a grant (Ri 146/17-2). R.B. thanks the Land Baden-Württemberg for a graduation scholarship. We also thank Priv.-Doz. Dr. H. Karfunkel and Dr. W. Koch, Tübingen, for many helpful discussions and their assistance in our computational work. For the determination of the optical purity, we are obliged to Priv.-Doz. Dr. H. Brückner, Hohenheim.

Registry No. PChd-Aib-OH, 74763-76-1; H-Aib-Ome·HCl, 15028-41-8; PChd-Aib-Aib-OMe, 121141-82-0; H-Aib-Aib-OMe·TFA, 121141-83-1; Ac-Aib-OH, 5362-00-5; Ac-Aib-Aib-OMe, 121141-84-2; Ac-Aib-Aib·OH, 118724-99-5; H-*S*-Iva-OMe·HCl, 92760-72-0; Ac-Aib-Aib-*S*-Iva-OMe, 121141-85-3; Ac-Aib-Aib-*S*-Iva-OH, 121141-86-4; Ac-Aib-Aib-*S*-Iva-Aib-Aib-OMe, 121141-87-5.

Supplementary Material Available: Tables of angles and bond lengths for molecules A and B of Ac-$(Aib)_2$-(*S*)-Iva-$(Aib)_2$-OMe (2 pages). Ordering information is given on any current masthead page.

(61) Shamala, N.; Nagaraj, R.; Balaram, P. *J. Chem. Soc., Chem. Commun.* **1978**, 996-997.
(62) Pulla Rao, C.; Shamala, N.; Nagaraj, R.; Rao, C. N. R.; Balaram, P. *Biochem. Biophys. Res. Commun.* **1981**, *103*, 898-904.
(63) Benedetti, E.; Bavoso, A.; Di Blasio, B.; Pavone, V.; Pedone, C.; Crisma, M.; Bonora, G. M.; Toniolo, C. *J. Am. Chem. Soc.* **1982**, *104*, 2437-2444.
(64) Bavoso, A.; Benedetti, E.; Di Blasio, B.; Pavone, V.; Pedone, C.; Toniolo, C.; Bonora, G. M.; Formaggio, F.; Crisma, M. *J. Biomol. Struct. Dyn.* **1983**, *5*, 803-817.

Tetraalkylammonium Salts and Phospholipid Polymorphism

Richard M. Epand,* Robert J. B. Chen, and Kelli S. Robinson

Contribution from the Departments of Biochemistry and Chemistry, McMaster University Health Sciences Centre, Hamilton, Ontario L8N 3Z5, Canada. *Received January 23, 1989*

Abstract: A series of tetraalkylammonium salts were tested for their ability to change the L_α-H_{II} phase transition temperature of dielaidoylphosphatidylethanolamine. Tetrapropylammonium bromide has little effect on this transition while tetrabutyl- and tetrapentylammonium bromides increase the transition temperature by 5 and 15 K/mol fraction, respectively. The longer chain tetrahexyl- and tetraoctylammonium bromides cause a rate of change of this transition temperature of −85 and −650 K/mol fraction of additive. This decrease in transition temperature was independent of the concentration or nature of the anion. It is concluded that long-chain tetraalkylammonium salts partition into the hydrophobic region of phospholipids as dissociated cations and they are potent promoters of the H_{II} phase. We estimate that approximately 23 kcal/mol of hydrophobic and van der Waals interactions is required to compensate for the unfavorable free energy change of bringing a charged group into the membrane.

Phosphatidylethanolamines readily interconvert between a planar bilayer (L_α) structure and an aggregate of water-filled cylinders, the inverted hexagonal phase (H_{II}).[1] The temperature at which this phase transition occurs is very sensitive to the presence of low concentrations of hydrophobic substances.[2] Only substances that partition mainly into the hydrophobic region of the membrane are good hexagonal-phase promoters. Inorganic salts, at high concentration, also modulate the L_α-H_{II} transition through their effects on lipid solvation.[3] In this work we wish to determine how a series of homologous tetraalkylammonium salts can modify the L_α-H_{II} phase transition temperature of dielaidoylphosphatidylethanolamine (DEPE). Tetraalkylammonium salts with short alkyl chain lengths will not penetrate deeply into the lipid structure. Hence, these salts will cause the membrane surface to expand, and they will introduce electrostatic repulsion, especially in the H_{II} phase. These factors would raise the L_α-H_{II} transition temperature. Increasing the alkyl chain length initially should favor increased partitioning from water into the membrane phase as well as cause a greater increase in headgroup area. Both factors would lead to an increase in the L_α-H_{II} transition temperature. We also wished to determine if the tetraalkylammonium salts would eventually become sufficiently hydrophobic to penetrate into the bilayer and promote H_{II}-phase formation. This would indicate the degree of hydrophobicity which is required to bring a charged group into a membrane.

Experimental Section

Materials. DEPE was purchased from Avanti Polar Lipids, Birmingham, AL. Its purity was ascertained by its phase transition characteristics as determined by differential scanning calorimetry (DSC). Tetraalkylammonium bromides were purchased from Aldrich Chemical Co., Milwaukee, WI.

Sample Preparation. DEPE was codissolved with varying amounts of a tetraalkylammonium bromide in $CHCl_3$/methanol (2/1 v/v). The

(1) Cullis, P. R.; de Kruijff, B. *Biochim. Biophys. Acta* **1979**, *559*, 399-420.
(2) Epand, R. M. *Biochemistry* **1985**, *24*, 7092-7095.
(3) Epand, R. M.; Bryszewska, M. *Biochemistry* **1988**, *27*, 8776-8779.

0002-7863/89/1511-6833$01.50/0

Table I. Effect of Tetraalkylammonium Chlorides on the L_α–H_{II} Phase Transition Temperature of DEPE

alkyl chain length	slope (K/mol fraction)	linear regression coeff
propyl	−4 ± 10	0.6
butyl	4.7 ± 0.6	0.99
pentyl	14 ± 5	0.8
hexyl	−86 ± 12	0.96
octyl	−650 ± 100[a]	0.96

[a] Plot of mole fraction of tetraoctylammonium chloride vs transition temperature is nonlinear above a mole fraction of 0.03. The slope presented is the initial slope.

Table II. Salt Effect on the L_α–H_{II} Phase Transition Temperature of DEPE and DEPE with Quaternary Ammonium Salt

	L_α–H_{II} transition temp (°C)		
added salt[a]	DEPE	DEPE + THAB[b]	DEPE + TOAB[c]
10 mM NaCl	65.3	61.3	59.1
50 mM NaCl	64.8	61.2	58.8
150 mM NaCl	65.2	60.0	58.2
500 mM NaCl	62.6	58.8	56.7
150 mM NaBr	65.2	59.9	59.7
150 mM NaSCN	69.5	64.1	66.9
75 mM Na_2SO_4	65.2	59.3	58.3

[a] All buffers are 20 mM Pipes, 1 mM EDTA, and 20 mg/L NaN_3, pH 7.40, plus the indicated added salt. [b] DEPE with 0.08 mol fraction of tetrahexylammonium bromide admixed. [c] DEPE with 0.01 mol fraction of tetraoctylammonium bromide admixed.

solvent was evaporated under a stream of nitrogen so as to deposit the lipid as a film on the wall of a glass test tube. Last traces of solvent were removed to a cold trap under high vacuum for 90 min. The films were hydrated with 20 mM Pipes buffer, pH 7.4, 1 mM EDTA, and 20 mg/L NaN_3, containing 150 mM NaCl, NaBr, or NaSCN or 75 mM Na_2SO_4. The samples were suspended at 10 mg of DEPE/mL by heating to 45 °C and vortexing vigorously for 30 s.

DSC. Samples and buffer were degassed prior to being loaded into a Microcal MC-2 scanning calorimeter. Heating scans were generally performed at a scan rate of 45 K/h. Data points were stored in an IBM PC using software provided by Microcal. The L_α–H_{II} transition was fitted to a single van't Hoff component. The enthalpy of the transition was unaffected by the presence of the tetraalkylammonium salts within the experimental error of ±25% caused by the difficulty in accurately transferring an inhomogeneous suspension to the calorimeter.

Results

The ability of various tetraalkylammonium bromides to modify the L_α–H_{II} transition temperature of DEPE was measured by DSC in 20 mM Pipes, 1 mM EDTA, 20 mg/mL NaN_3, and 150 mM NaCl. For each chain length, five to eight different samples containing between 0 and up to 0.3 mol fraction of tetraalkylammonium bromide were used. The L_α–H_{II} transition temperature was plotted against the mole fraction of tetraalkylammonium bromide added. These plots were linear up to the highest mole fraction of added quaternary ammonium salt used except for the tetraoctylammonium bromide, which showed a decrease in slope above a mole fraction of about 0.03. Only the initial slope, over the linear portion of the plot, is reported (Table I).

The dependence of the L_α–H_{II} transition temperature on the nature and concentration of the salt used was determined (Table II). The NaSCN was the only salt to affect the transition temperature. The slope of a plot of L_α–H_{II} transition temperature vs mole fraction of tetraoctylammonium bromide was −590 ± 100 K/mol fraction in 150 mM NaSCN, which is indistinguishable from the value of −650 ± 100 K/mol fraction in 150 mM NaCl (Table I).

Discussion

None of the quaternary ammonium salts tested are good bilayer stabilizers. When the alkyl chain is too short, the salts partition mostly into water and have little effect on the L_α–H_{II} transition temperature as is the case for the tetrapropylammonium salt (Table I). Hydrophobic tetraalkylammonium salts partition into DEPE but promote the H_{II} phase such as the tetrahexyl or tetraoctyl salts (Table I). The intermediate cases of the tetrabutyl and tetrapentyl salts are weak bilayer stabilizers (Table I).

We wished to determine whether the hydrophobic tetrahexyl- and tetraoctylammonium salts were partitioning into the membrane as hydrophobic cations or as ion pairs. The transition temperature of mixtures of DEPE with either tetrahexyl- or tetraoctylammonium bromide are only weakly dependent on the chloride concentration (Table II). A similar dependence on salt concentration is seen with pure DEPE (Table II). Since the effect of the quaternary ammonium salt on the L_α–H_{II} transition temperature is independent of anion concentration, the hydrophobic cation is probably entering the membrane as a charged species rather than as an ion pair. Further support for this conclusion comes from studies with different sodium salts. The L_α–H_{II} transition temperature of DEPE or DEPE with 0.08 mol fraction of tetrahexylammonium bromide or 0.01 mol fraction of tetraoctylammonium bromide is independent of the nature of the anion for Cl^-, Br^-, or SO_4^{2-} (Table II). However, SCN^- raises the L_α–H_{II} transition temperature about 5 K compared with the transition temperature observed with the other salts (Table II). The effect of NaSCN was further evaluated by measuring the dependence of the L_α–H_{II} transition temperature of DEPE on the mole fraction of tetraoctylammonium bromide in 150 mM NaSCN vs 150 mM NaCl. For buffers containing either salt, the tetraoctylammonium reduced the L_α–H_{II} transition temperature by 600 K/mol fraction of tetraoctylammonium. It is known that SCN^- has a marked effect in stabilizing the bilayer phase,[3,4] but this effect is independent of the shift caused by the quaternary ammonium salt. We can thus conclude that the promotion of the hexagonal phase by tetrahexyl- and tetraoctylammonium bromide is independent of the nature or the concentration of the anion. The quaternary ammonium salt is therefore entering the membrane as a cation, rather than as an ion pair.

If one considers the incorporation of substances into membranes as analogous to the partitioning of these substances between organic and aqueous phases, then it seems unusual for cations to partition into the membrane as a charged species. Tetraalkylammonium halides as neutral salts are not soluble in nonpolar solvents. The free energy of transfer of tetraethylammonium iodide, as an ion pair, from water to hexane is 14.4 kcal/mol, and the decrement per CH_2 group in each of the four alkyl chains is 1.4 kcal/mol.[5] Therefore, the partitioning of tetrahexylammonium iodide from water to hexane would be unfavorable by 8.8 kcal/mol. As separated ions, this transfer would be even more unfavorable. The transfer of ion pairs is also sensitive to the nature of the anion, contrary to their partitioning into membranes. The transfer free energy increases to 20 and 24 kcal/mol for tetraethylammonium bromide and chloride, respectively.[5] The transfer from water to organic solvents that are somewhat more polar than hexane is a bit more favorable. For example, transfer from water to ethyl acetate is estimated to have an unfavorable free energy change of 2.9 kcal/mol for tetrahexylammonium iodide. These transfer energies are in agreement with our finding that the tetrahexyl- and tetraoctylammonium salts do not partition into the membrane as ion pairs. Dissociation of ion pairs of tetrabutylammonium bromide in methanol is markedly promoted by low concentrations of benzene.[6] For example, the dissociation constant for this salt changes from 0.055 in methanol to 0.31 in 98.32 mol % methanol–1.68 mol % benzene. This sensitivity of the dissociation constant to a low concentration of hydrophobic solvent has been interpreted as being caused by a change in the solvation of the cation in the mixed-solvent system.[7] It was suggested that the solvation of a large organic ion with a low density of surface charge is very much like that of a corresponding uncharged molecule.[7] Thus, the tetrahexyl- and tetraoctylammonium salts will preferentially be solvated by nonpolar

(4) Yeagle, P. L.; Sen, A. *Biochemistry* **1986**, *25*, 7518–7522.
(5) Abraham, M. H. *J. Chem. Soc., Perkins Trans. 2* **1972**, 1343–1357.
(6) Miller, R. C.; Fuoss, R. M. *J. Am. Chem. Soc.* **1953**, *75*, 3076–3080.
(7) Grunwald, E.; Baughman, G.; Kohnstam, G. *J. Am. Chem. Soc.* **1960**, *82*, 5801–5811.

molecules. This explains why these cations can enter the membrane as charged species.

Our results can be used as a measure of how much hydrophobicity is required to transfer a charged group, such as a charged amino acid side chain in a transmembrane segment of a protein, from water to the interior of a membrane. Studies of the thermodynamics of the transfer of alkanes from water to dodecyl sulfate micelles[8] indicate that for four hexyl groups the free energy of transfer is −22.8 kcal/mol. The transfer free energy is similar to that for the transfer of alkanes between water and a number of organic solvents and is insensitive to the polarity of the organic solvent. The 22.8 kcal/mol is the approximate value of the favorable energy, resulting from bringing four alkyl chains from water into a micelle, which must compensate the unfavorable energy change for bringing a charged group into the membrane.

The partitioning of the hydrophobic cation into the membrane does not indicate that we can separate anion and cation into two different phases. Clearly the anion must still be associated with the surface of the membrane and be separable from the aqueous phase. A similar situation occurs when anionic phospholipids, such as phosphatidylserine, are incorporated into a membrane. The exact location in the membrane of the anion and cation in the case of the quaternary ammonium salts cannot be accurately ascertained from the results presented. However, it is clear that the anion must stay at the surface of the membrane since its nature and concentration have little influence on the transition temperature. In contrast, the hydrophobic tetrahexyl- and tetraoctylammonium cations must expand the hydrocarbon interior of the membrane since they promote the hexagonal-phase arrangement although the positive charge does not have to be very deeply imbedded in the membrane. One possible location for these amphiphilic cations is on the surface of an aggregate of hexagonal-phase cylinders. Each hexagonal-phase cylinder has a hydrophobic exterior. Contact between this hydrophobic surface and water is prevented by aggregation of these cylinders. This aggregate of cylinders, however, has finite dimensions and must eventually have a hydrophobic surface in contact with water. Since these H_{II} aggregates are large, the surface to volume ratio is small, and hence, the destabilization caused by contact between hydrocarbon and water is also small and can be counterbalanced by energetically favorable interactions. However, this destabilization at the interface between lipid and water can be relieved by the hydrophobic quaternary ammonium salts. This would explain the apparent limited solubility of the tetraoctylammonium cation in DEPE. Regardless of its location, we can definitely state that the tetrahexyl- and tetraoctylammonium cations are interacting with the hydrophobic portion of the phospholipid and they are affecting the phase transition of the entire sample.

(8) Wishnia, A. *J. Phys. Chem.* **1963**, *67*, 2079–2082.

Conclusions

Quaternary ammonium salts containing butyl or pentyl alkyl chains do not penetrate into the interior of DEPE membranes. They are situated at the surface of this phospholipid and thereby weakly stabilize a bilayer arrangement. More hydrophobic quaternary ammonium salts with hexyl or octyl chains do enter the hydrophobic region of DEPE. They do so as cations rather than as ion pairs. Their effect is to promote the rearrangement of bilayers to the inverted hexagonal phase. The tetraoctylammonium salt is particularly potent in this regard. It is estimated that it requires about 23 kcal/mol to promote the partitioning of a cation into a membrane.

Acknowledgment. The work was supported by a grant from the Medical Research Council of Canada.

Registry No. DEPE, 16777-83-6; SCN^-, 302-04-5; tetrapropylammonium bromide, 1941-30-6; tetrabutylammonium bromide, 1643-19-2; tetrapentylammonium bromide, 866-97-7; tetrahexylammonium bromide, 4328-13-6; tetraoctylammonium bromide, 14866-33-2.