Calorimetric Studies on Saturated Mixed-Chain Lecithin–Water Systems. Nonequivalence of Acyl Chains in the Thermotropic Phase Transition†

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ABSTRACT: Aqueous dispersions of synthetic lecithins with different fatty acids in positions 1 and 2 of the glycerol molecule were studied by calorimetry. The data show that variation of the acyl chains in different positions of the glycerol backbone either have no influence upon or contribute 0.5 kcal/mol per CH_2 segment to the phase transition enthalpy. Different molecular ordering of the mixed acyl chain lecithins in the bilayer is discussed in light of the results.

Phospholipid–water systems have been intensively studied in recent years as models for biological membranes. These model systems show a thermally triggered phase transition which is manifested as a transition of the acyl chains from a more ordered to a disordered state and is coupled to a sudden change in membrane fluidity (Chapman et al., 1967).

This transition can be calorimetrically followed by measuring the change in enthalpy, ΔH_m.[1] Calorimetric investigations to date on phospholipid–water systems have shown that the phase transition behavior is dependent upon the structure of the phospholipid molecule as well as the properties of the aqueous phase (Blume, 1976).

The influence of the aqueous phase upon the phase transition temperature, T_m, of single-charged phospholipids can be understood in terms of electrostatic considerations (Jähnig, 1976; Träuble et al., 1976). Phospholipid–water systems cannot, however, be unambiguously described in their phase transition behavior by just giving the enthalpy change, ΔH_m, and the transition temperature, T_m, of the so-called main transition. In addition to the transition from the ordered to the disordered state (main transition) in lecithin–water systems, there appears a second, so-called pretransition, which is observed at a temperature lower than the main transition temperature. This pretransition has been demonstrated in other phospholipid–water systems through variation of the pH and ionic strength of the aqueous phase (Stümpel et al., 1980; Harlos et al., 1979; Watts et al., 1978).

From measurements of homologous lecithins with two identical acyl chains in the 1- and 2-positions of the glycerol backbone (Hinz & Sturtevant, 1972; Phillips et al., 1969) it is known that the enthalpy change, ΔH_m, and the phase transition temperature, T_m, vary linearly with the length of the acyl chains. In this paper the influence of varying the acyl chain length and its position on the glycerol backbone upon the phase transition behavior of mixed-chain lecithins has been investigated.

A comparison with available data for lecithins with identical acyl chains allows conclusions as to how an acyl chain is ordered within the lecithin molecule. The special structure of these lipids was until now, with one exception (Keough & Davis, 1979), not available for physiochemical investigation in membrane model systems.

Materials and Methods

The synthesis of mixed-chain phospholipids will be published separately (H. Eibl, in preparation). The purity of the compounds was established by thin-layer chromatography and elemental analysis to be better than 99%. The positional purity of the compounds was confirmed by determination of the phosphate/vicinal diol ratio and by gas chromatographic analysis of the fatty acids as methyl esters after hydrolysis with phospholipase A_2 (van Deenen & de Haas, 1963). Phosphate was determined according to Eibl & Lands (1969) and vicinal diol after fatty acid hydrolysis as described (Eibl & Lands, 1970). The ratio of phosphate to vicinal diol of 1:1 indicated the absence of 1,3-diacyl isomers. The positional purity of the mixed-chain lecithins was better than 98%.

The calorimetric measurements were performed with a Perkin-Elmer "DSC 2" (with "Ultracooler I") calorimeter. Weighed amounts of lipid (3–4 mg) and 50 μL of water were sealed in stainless steel pans and equilibrated for 1 h at a temperature of 5 °C above T_m. The reference pan contained 50 μL of water. For each sample at least three scans were performed between 0 and 10 °C above T_m, with heating rates of 1.25 °C/min in the sensitivity range of 1 mcal/s. Repetitive scans did not show significant differences in T_m or in ΔH_m values. The T_m values given are taken as the temperature of the peak inversion, and enthalpies are calculated from the peak areas. The precision of the ΔH determination is ±5%. To be sure that the asymmetry between 1M-2S-PC and the 1S-2M-PC compound was no artifact, we did the calorimetric measurements also with heating rates of 0.31 °C/min. In all cases the same curves were reproduced.

Results

The calorimetric heating curves of the mixed-chain lecithins are shown in Figure 1. As seen, all three lecithins show a sharp transition which occurs in a temperature interval of less than 4 °C.

A comparison of the phase transition temperatures of 1M-2P-PC and 1M-2S-PC shows that an elongation of the chain in the 2-position by two CH_2 segments leads to an increase in T_m of 5 °C. It is interesting to note that in the case of 1M-2S-PC no calorimetrically demonstrable pretransition is observable, in contrast to the case of 1S-2M-PC where it is

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[1] Abbreviations used: ΔH_p, free enthalpy change of pretransition; T_p, pretransition temperature; ΔH_m, free enthalpy change of main transition; T_m, main transition temperature; 1M-2M-PC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; 1M-2P-PC, 1-myristoyl-2-palmitoyl-*sn*-glycero-3-phosphocholine; 1M-2S-PC, 1-myristoyl-2-stearoyl-*sn*-glycero-3-phosphocholine; 1P-2M-PC, 1-palmitoyl-2-myristoyl-*sn*-glycero-3-phosphocholine; 1S-2M-PC, 1-stearoyl-2-myristoyl-*sn*-glycero-3-phosphocholine; 1P-2P-PC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine.

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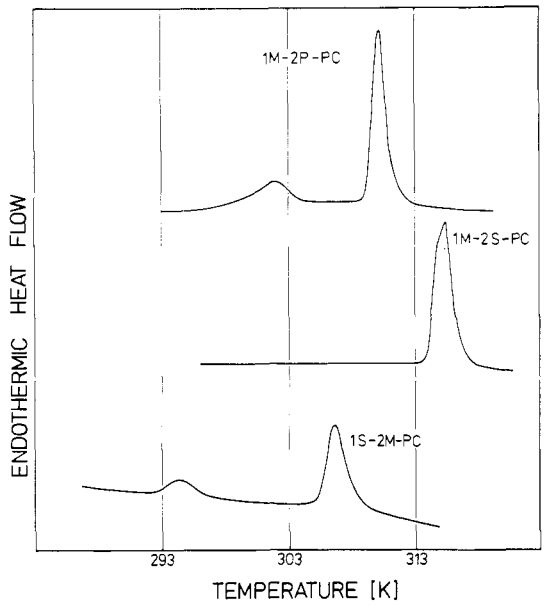

FIGURE 1: Calorimetric heating curves of saturated mixed-chain lecithins in excess water. Heating rate 1.25 °C/min; sensitivity range 1 mcal/s.

Table I: Transition Enthalpies and Transition Temperatures of Different Lecithins

substance	ΔH_p (kcal/mol)	T_p (°C)	ΔH_m (kcal/mol)	T_m (°C)
A. Increasing Chain Length in the 1-Position and Constant Chain Length in the 2-Position of *sn*-Glycerol				
1M-2M-PC[a]	1.0	13.5	6.1	23.9
1P-2M-PC[b]	*c*		6.5	27.2
1S-2M-PC	1.1	22	6.0	34
B. Increasing Chain Length in the 2-Position and Constant Chain Length in the 1-Position				
1M-2P-PC	0.9	28.5	7.3	37
1M-2S-PC	*d*		8.2	42

[a] There is no coincidence of the ΔH values obtained from different groups for 1M-2M-PC: (Hinz & Sturtevant, 1972) 6.26 kcal/mol; (Mabrey & Sturtevant, 1976) 5.4 kcal/mol; (Blume, 1976) 6 kcal/mol; (Janiak et al., 1979) 6.3 kcal/mol; (our value) 6.1 kcal/mol. [b] Data taken from Keough & Davis (1979). [c] A pretransition is observable. [d] No pretransition present (see Figure 1).

clearly separated from the main transition.

In Table IA is shown the effect of variation in the chain length in the 1-position by constant chain length in the 2-position. The other case of variation in the chain length in the 2-position by constant chain length in the 1-position is shown in Table IB.

As can be seen (Table IA), variation in chain length in the 1-position has only a slight effect on the ΔH_m values whereas variation in the chain length in the 2-position (Table IB) results in an increase of ΔH_m by 0.5 kcal/CH_2. The increase in ΔH_m due to elongation of the acyl chain in the 2-position coincides with the value found for lecithins with identical acyl chains.

The effect on the transition temperature is small. Starting with 1M-2M-PC, an elongation of the acyl chain by two CH_2 segments in the 1-position increases T_m only slightly from 23.9 to 27.2 °C. When the acyl chain in the 1-position is elongated by four CH_2 segments, the main transition temperature changes from 23.9 to 34 °C (Table IA).

The other case (Table IB) shows the following picture: Starting from 1M-2M-PC, an elongation of the acyl chain in the 2-position by two CH_2 segments leads to an increase in the transition temperature, T_m, from 23.9 to 37 °C. An increase of the acyl chain in the 2-position by four CH_2 segments gives an increase in the transition temperature from 23.9 to 42 °C.

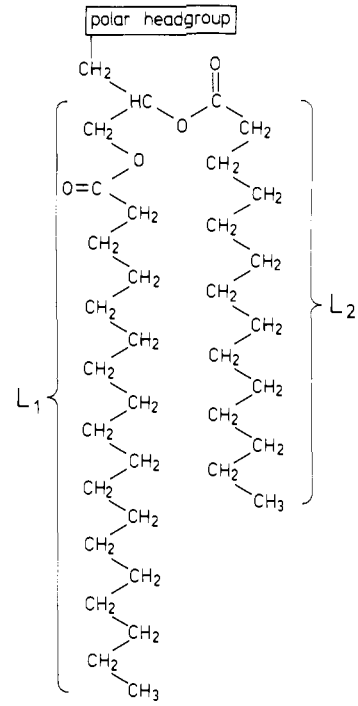

FIGURE 2: Fatty acid chain arrangement in 1-myristoyl-2-stearoyl-*sn*-glycero-3-phosphocholine. L_1 and L_2 are the effective lengths of the single chains in a bilayer [according to Seelig & Seelig (1974)].

Discussion

The positional purity of the mixed-chain lecithins used in this study is better than 98% with respect to acyl chain and phosphocholine distribution over the three positions of *sn*-glycerol. This is a significant improvement over the earlier preparations described by Keough & Davis (1979) who noted 5–22% isomerization during synthesis. Therefore, a reinvestigation of the effect of fatty acid position in the glycerol molecule on the physical properties of phospholipid–water systems was undertaken.

Calorimetric studies on phospholipid–water systems using lecithins with identical acyl chains lead to the observation that an increase in the acyl chain length by one CH_2 segment results in an increase in ΔH_m by about 0.5 kcal/mol. In comparison, our studies on mixed-chain lecithins give the following results: (1) Acyl chain elongation in the 1-position (Table IA) has no influence upon ΔH_p and ΔH_m. An influence is only seen in T_p and T_m, an increase in chain length giving rise to an increase in both transition temperatures. (2) An increase in the acyl chain length in the 2-position (Table IB) leads to an increase in the transition enthalpy, ΔH_m, by 0.5 kcal/mol per CH_2 segment. This value agrees well with the ΔH_m per CH_2 segment seen in lecithins with identical fatty acid chains (Phillips et al., 1969).

Order parameter measurements on selectively deuterated 1P-2P-PC above T_m (Seelig & Seelig, 1975) have shown that the acyl chains in the 1- and 2-positions have different conformations. The proposed conformation of the fatty acid chains in a lipid molecule is schematically shown in Figure 2 and was taken by us to be a general conformation principle for the acyl chains in the lipid molecule. From Figure 2 it is obvious that in lecithins with identical acyl chains these two chains have effectively different lengths (L_1, L_2); i.e., the chain

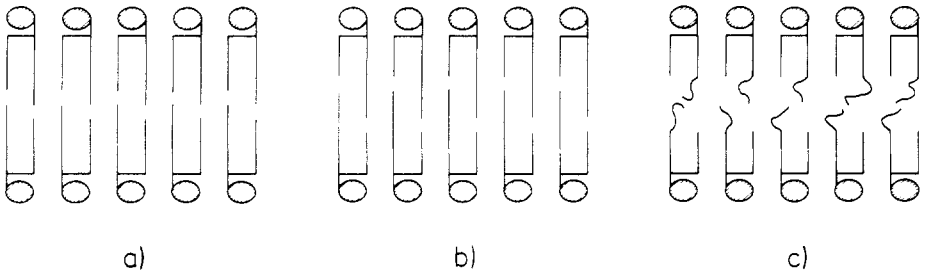

FIGURE 3: Molecular arrangement of phospholipid molecules in a bilayer of (a) lecithins with identical fatty acid residues; (b) lecithins with different fatty acid chains in the 1- and 2-positions of the glycerol backbone as proposed by Keough & Davis (1979), and (c) lecithins with different fatty acid chains under consideration of our calorimetric data.

in the 2-position is effectively shorter than that in the 1-position. This difference is found to be about 1.5 C–C bond lengths in 1P-2P-PC; i.e., effectively the chain in the 2-position is about 1.9 Å shorter (Büldt et al., 1978). A similar arrangement has been reported for lecithin single crystals, established by X-ray analysis (Pearson & Pascher, 1979).

Under the assumption that this conformation is preserved in the mixed-chain lecithins, the following interpretation of our data are possible:

Case A. Starting from 1M-2M-PC, our results show that replacement of the acyl chain in the 1-position by palmitic or stearic acid is of little, if any, influence upon the ΔH values of the pretransition and main transition. In consideration of the acyl chain arrangement shown in Figure 2, our data can be interpreted to mean that the acyl chain in the 2-position is responsible for the magnitude of the transition enthalpy. In the case of elongation of the acyl chain in the 1-position, the CH_2 groups at the end of the elongated chain ($(CH_2)_n$, $n \geq 14$) no longer interact with the acyl chain in the 2-position. These no-interacting methylene segments in the 1-position would then have a higher conformational mobility and exist in the "fluid" state. As a result, they do not contribute to the enthalpy of the phase transition. From the constancy of the ΔH values, it may be concluded that the bent conformation of the acyl chain in the 2-position is independent of its length.

The possible conformation of the acyl chains in a bilayer may be schematically depicted as in Figure 3. For lecithins with identical chains, the accepted acyl chain conformation is shown in Figure 3a, while Figure 3b,c shows the possible conformation of the mixed-chain lecithins. Considering the proposed model of Keough & Davies (1979), our calorimetric data for 1P-2M-PC and 1S-2M-PC lead to the prediction that the ordering of these compounds in the bilayer is as shown in Figure 3c; i.e., in between the two halves of the bilayer, a "fluid region" exists which gives no contribution to the transition enthalpy. It cannot be predicted from our data whether a further difference in chain lengths would lead to the partially interdigitated ordering shown in Figure 3b. This points require further investigation.

Case B. We shall now consider the effect of variation in the chain length of the acyl chain in the 2-position. Once again starting from 1M-2M-PC, the replacement of the myristoyl chain in the 2-position by a palmitic or stearic acid residue leads to an increase in the transition enthalpy, ΔH_m, by about 0.5 kcal/mol per CH_2 segment. An increase in the acyl chain length in the 2-position by two CH_2 segments gives an effectively equal length to both acyl chains; i.e., it contributes to the ordered–disordered phase transition and leads to an increase in the transition enthalpy. An increase by four CH_2 segments still results in an interaction of the whole 2-positioned chain with the chain in the 1-position and contributes, analoguously to the case of 1P-2P-PC, to the transition enthalpy. A comparison of the transition enthalpies of 1M-2S-PC and 1P-2P-PC shows that they are comparable within limits of experimental error (ΔH_m = 8.7 kcal/mol for 1P-2P-PC and 8.2 kcal/mol for 1M-2S-PC). It is, however, noteworthy that 1M-2S-PC has no calorimetrically observable pretransition.

The pretransition disappears by variation of the acyl chain length and its positioning on the glycerol backbone. In order to confirm the interpretation of the calorimetric data here, we plan to do nuclear magnetic resonance studies on selectively deuterated mixed-chain lecithins. In the present discussion we have not considered the acyl chain tilt which is accepted to occur in lecithins with identical chains. Such consideration is not possible from the calorimetric data presented here.

In summary, it can be said that: (1) the effectively shorter chain in the mixed-chain lecithins determines, regardless of its position, the magnitude of the transition enthalpy ΔH_m; (2) the pretransition behavior is determined by the effective chain length and their position on the glycerol backbone. Thus, in order to understand the phase transition behavior of phospholipid–water systems, it is not enough to know only the number of CH_2 segments. Equally important are the effective chain lengths and the positions on the glycerol backbone.

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Copper(II)·Bleomycin, Iron(III)·Bleomycin, and Copper(II)·Phleomycin: Comparative Study of Deoxyribonucleic Acid Binding†

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ABSTRACT: The kinetics and mechanism of binding of Cu(II)·bleomycin, Fe(III)·bleomycin, and Cu(II)·phleomycin to DNA were studied by using fluorometry, equilibrium dialysis, electric dichroism, and temperature-jump and stopped-flow spectrophotometry. The affinity of Cu(II)·bleomycin for DNA was greater than that of metal-free bleomycin but less than that of Fe(III)·bleomycin. Cu(II)·bleomycin exhibited a two-step binding process, with the slow step indicating a lifetime of 0.1 s for the Cu(II)·bleomycin·DNA complex. Fe(III)·bleomycin binding kinetics indicated the presence of complexes having lifetimes of up to 22 s. DNA was lengthened by 4.6 Å/molecule of bound Cu(II)·bleomycin and by 3.2 Å/bound Fe(III)·bleomycin but not at all by Cu(II)·phleomycin, suggesting that both bleomycin complexes intercalate while the phleomycin complex does not. However, phleomycin exhibited nearly the same specificity of DNA base release as bleomycin. These results suggest that the coordinated metal ion plays a major role in the binding of metal–bleomycin complexes to DNA but that intercalation is neither essential for DNA binding and degradation nor primarily responsible for the specificity of DNA base release by these drugs.

Degradation of DNA by bleomycin is thought to be caused by radicals resulting from oxidation of DNA-bound Fe(II)·bleomycin to Fe(III)·bleomycin (Sausville et al., 1978a,b). To understand better the details of this process, we have studied the interaction of DNA with Fe(III)·bleomycin, Cu(II)·bleomycin, and Cu(II)·phleomycin. Cu(II)·bleomycin was chosen as a model for Fe(II)·bleomycin, whose oxygen lability makes study extremely difficult. Phleomycin is identical with bleomycin, except that it lacks one double bond in the bithiazole moiety (Figure 1) (Takita et al., 1972), which in bleomycin intercalates between DNA base pairs (Povirk et al., 1979). Although both drugs have similar DNA strand-breaking activity (Stern et al., 1974; Suzuki et al., 1969), this structural difference makes phleomycin a less likely candidate for intercalation.

Materials and Methods

Drugs. Blenoxane, the clinical mixture containing primarily bleomycins A_2 and B_2, was used in most experiments. Fluorescence studies (not shown) indicated that purified bleomycins A_2 and B_2 had the same affinity for DNA in both high and low salt as blenoxane.

Metal–bleomycin complexes were formed by mixing equimolar quantities of bleomycin and either $CuCl_2$ or $Fe^{II}(NH_4)_2(SO_4)_2 \cdot 6H_2O$ (all at concentrations of at least 2 mM) in distilled water, pH 5. Fe(II)·bleomycin oxidized rapidly to Fe(III)·bleomycin (Povirk, 1979). Fe(III)·bleomycin was used within 3 h of its preparation because its ultraviolet–visible spectrum, although stable for several hours, showed measurable changes after incubation for 1 day at 25 °C. The clinical mixture of phleomycins (95% copper free) was used in base-release experiments. Chromatographically purified Cu(II)·phleomycin A_2, which was used in all binding studies, was a gift of Dr. T. Takita of the Institute of Microbial Chemistry, Tokyo. All drugs and drug solutions were stored at −20 °C.

DNA. Either high molecular weight repurified calf thymus DNA or sonicated, fractionated, 150 base pair long calf thymus DNA was used in binding studies. Details of these preparations have been described (Hogan et al., 1978; Povirk et al., 1979). Specifically labeled *Escherichia coli* DNA was isolated from cultures grown in the presence of [^{14}C]thymidine, [^{3}H]adenosine, or [^{3}H]cytidine, as previously described (Povirk et al., 1978). All DNA concentrations are expressed in moles of base pairs.

Equilibrium Dialysis. Sonicated, fractionated 2 mM calf thymus DNA (2 mL) was dialyzed against a known concentration of Cu(II)·bleomycin (50–100 mL). After 2 days of dialysis at 25 °C, 0.1 volume of 25% sodium dodecyl sulfate was added to solutions from inside and outside the dialysis bag, and the Cu(II)·bleomycin concentrations were determined from the A_{310}–A_{340} of the solutions. This procedure minimized the effect of small but variable amounts of ultraviolet-absorbing material which accumulated in DNA samples during dialysis. Dialysis was usually begun with equal Cu(II)·bleomycin concentrations inside and outside the dialysis bag; however, the same results were obtained with samples having an initial excess on either side.

Fluorescence Studies. Binding of bleomycin and phleomycin and their metal complexes to DNA was measured by fluorometry (Chien et al., 1977); the fluorescence of the bithiazole rings is quenched upon binding to DNA. For bleomycin studies in low-salt buffer, high molecular weight calf thymus DNA was used satisfactorily. however, for studies in high-salt buffer, where higher DNA concentrations were required, and for studies with Cu(II)·phleomycin, whose fluorescence was only $\sim 1/30$ as strong as Cu(II)·bleomycin, light scattering by DNA became a significant problem. Therefore, sonicated DNA (Hogan et al., 1978) was used instead. Emission was measured at 353 nm, and exitation was at either 300 or 310

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