Differential Scanning Calorimetric Studies of Aqueous Dispersions of Mixtures of Cholesterol with Some Mixed-Acid and Single-Acid Phosphatidylcholines†

P. J. Davis‡ and K. M. W. Keough*

ABSTRACT: Differential scanning calorimetric studies have been performed on aqueous dispersions of mixtures of cholesterol with either 1,2-distearoyl-*sn*-glycero-3-phosphocholine, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine, 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine, or 1-oleoyl-2-stearoyl-*sn*-glycero-3-phosphocholine. At low concentrations of cholesterol, the endotherms obtained on heating dispersions of lecithin–cholesterol mixtures had different shapes for each lecithin at a fixed cholesterol concentration. The endotherms could be resolved into broad and narrow components. The enthalpy changes (ΔH) and the temperatures of maximum excess heat capacity (T_{max}) for both narrow and broad components of each type of mixture responded in different ways to increasing cholesterol concentration. At high cholesterol concentrations, all mixtures gave one broad endotherm, but the concentration of cholesterol at which the endotherms could not be resolved from base lines was different for each lipid. The results were consistent with there being at least a quantitative difference in the interaction between cholesterol and different lecithins in the gel phase.

Cholesterol occurs in a wide range of concentrations in a large number of biological membranes [for a review, see Green (1977)], and its interactions with phospholipids in natural and model membranes have been studied by using a variety of physical techniques [for a review, see Demel & de Kruyff (1976)]. It has been known for some time that cholesterol may be important in the modulation of motion in membrane lipid chains (Ladbrooke et al., 1968). Differential scanning calorimetry of some model membrane systems containing phospholipid mixtures has indicated that cholesterol may exhibit preferential interaction with different lipid types, this preference being influenced by both the head groups and the transition temperatures of the individual lipids (de Kruyff et al., 1973; van Dijck et al., 1976). Differential interactions have not been observed, however, in all mixtures of lipids with cholesterol (Calhoun & Shipley, 1979).

The predominant forms of phospho- and glycolipids in membranes are those with saturated fatty acyl chains at the *sn*-1 position of glycerol and unsaturated, branched, cyclopropane or short saturated chains at the *sn*-2 position. It has been reported that some *Mycoplasmas* which require and incorporate large amounts of cholesterol during growth have a large amount of lipids with the more unusual 1-unsaturated–2-saturated distribution (Rottem & Markowitz, 1979a,b). In comparison, the related species, *Acholeplasma laidlawii B*, which requires little or no cholesterol for growth has the more common 1-saturated–2-unsaturated positional distribution in its lipids (McElhaney & Tourtellotte, 1970). It has been reported that the amount of cholesterol in rat hepatomas is higher than that in normal rat liver (van Hoeven & Emmelot, 1972; Dyatlovitskaya et al., 1975), and Dyatlovitskaya et al. (1974) have found that there is a loss of positional specificity in the lecithins from rat hepatomas.

These observations suggest that there may be some differential interactions between cholesterol and positional isomers of lipids. Such a potential difference was discussed by Huang (1977) for the interactions between cholesterol and lipids containing a saturated or an unsaturated chain in the 1-position. We have recently observed that the positional isomers of lecithins containing a saturated and an unsaturated chain exhibit different thermotropic properties in the bilayer (Davis et al., 1981). We have used one such pair of isomers containing stearate and oleate to investigate the possibility of differential interactions between cholesterol and positional isomers of lecithins. de Kruyff et al. (1972) have studied monolayers of mixtures of cholesterol and these two positional isomers of lecithins. They did not find any significant difference between the monolayer properties of the two mixtures at low surface pressures, but those studies were carried out at a temperature above the gel to liquid-crystalline transition where the acyl chains would be fluid (Davis et al., 1981; Hawco et al., 1981). Here, we report the effect of cholesterol on the gel to liquid-crystalline phase transitions of a pair of positional isomers of lecithin, 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (SOPC) and 1-oleoyl-2-stearoyl-*sn*-glycero-3-phosphocholine (OSPC), and on the thermotropic properties of mixtures of cholesterol with the lecithins containing only a single type of acyl chain, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC).

Materials and Methods

Materials. DSPC, cholesterol, stearic and oleic acids, and (dimethylamino)pyridine were obtained from Sigma Chemical Co., St. Louis, MO. The fatty acids were analyzed by gas–liquid chromatography, were found to contain less than 1% of other components, and were used without further purification. Other reagents and solvents and Rexyn I-300 resin were from Fisher Scientific Co., Dartmouth, Nova Scotia. Absolute and 95% ethanol were products of Consolidated Alcohol, Toronto, Ontario. ACS-grade chloroform and methanol were distilled before use. Preswollen Whatman microgranular CM52 carboxymethylcellulose was obtained from Whatman Inc., Clifton, NJ.

† From the Department of Biochemistry, Memorial University of Newfoundland, St. John's, Newfoundland, Canada A1B 3X9. *Received May 13, 1983; revised manuscript received August 16, 1983.* This work was supported by the Medical Research Council of Canada and the Canadian Lung Association. The work formed part of a Ph.D. thesis submitted to Memorial University of Newfoundland by P.J.D.

‡ Present address: Department of Membrane and Ultrastructure Research, The Hebrew University—Hadassah Medical School, Jerusalem, Israel.

0006-2960/83/0422-6334$01.50/0

Lipids. (*A*) *DSPC.* On thin-layer chromatography of 500–750 μg of DSPC in a 2 cm wide lane on silica gel G using chloroform–methanol–water (65:25:4 by volume) (Wagner et al., 1961), a major spot containing ≥99% of the total lipid phosphorus was found along with a minor spot (≤1%) attributable to the 1,3-isomer. DSPC was used without further purification.

(*B*) *DOPC.* DOPC was prepared by acylation of the $CdCl_2$ complex of L-α-glycerophosphocholine (Brockerhoff & Yurkowski, 1965; Chakrabarti & Khorana, 1975) with oleoyl anhydride (Selinger & Lapidot, 1966) by using the method of Gupta et al. (1977). A small modification of this method was introduced in that the eluant from the Rexyn I-300 column which is a mixture of chloroform–methanol–water (4:5:1 by volume) was adjusted to yield a chloroform:methanol:water ratio of 2:1:0.6 (Folch et al., 1957). The material from the lower phase was taken up in a small volume of chloroform and chromatographed on Whatman CM52 carboxymethylcellulose by using a modification of the procedure of Comfurius & Zwaal (1977). The fractions containing only 1,2-lecithin were pooled, taken to dryness under vacuum, redissolved in a small amount of chloroform, and stored at −15 to −20 °C until used. When this material was analyzed by gas–liquid chromatography, essentially as described by Thompson (1969), it was found to contain greater than 99% oleic acid. When approximately 650 μg of this lipid was analyzed by thin-layer chromatography as described above, only a single spot corresponding to 1,2-diacyllecithin could be observed by using I_2 vapor or a phosphate stain (Dittmer & Lester, 1964; Ryu & MacCoss, 1979).

(*C*) *Cholesterol.* Cholesterol was recrystallized twice from 95% ethanol at 4 °C, and the crystals were dried under vacuum over P_2O_5 at 45 °C for at least 16 h. The recrystallized material was stored dry at −20 °C.

(*D*) *SOPC and OSPC.* Lysooleoyl- and lysostearoyllecithins were prepared by the digestion of DOPC or DSPC by phospholipase A_2 using the methods of Chakrabarti & Khorana (1975) or Keough & Davis (1979) and dried in vacuo over P_2O_5. The mixed-acid lecithins were prepared by acylation of the free lysolecithins with appropriate fatty acyl anhydrides in the presence of (dimethylamino)pyridine (Gupta et al., 1977) using a mole ratio of anhydride to lysolecithin of 2.3:1. The products of the acylation were passed through Rexyn I-300 columns, dried, redissolved in chloroform, and purified on CM52 carboxymethylcellulose columns. The purified SOPC and OSPC were precipitated from small volumes of chloroform by the addition of 4 volumes of acetone and cooling to 4 °C. The lecithins were redissolved in hexane:methanol 98:2 (v/v) to make a 2% (w/v) solution. Permanganate-distilled water was added to the solution in the amount of 1 mole of water per mole of lipid phosphorus. This solution was allowed to stand at −20 °C for periods between 2 and 6 weeks over which time microcrystals of the materials appeared. The crystals were collected by centrifugation at $1900g_{av}$ for 10 min at 4 °C. The crystals were dried in a stream of N_2 gas at 35 °C and left under vacuum for 16 h. The crystals were taken up in chloroform, and the phosphorus content was determined by small modifications of published procedures (Bartlett, 1959; Dawson, 1960; Fiske & Subbarow, 1925). Analysis of the materials by thin-layer chromatography showed that each presented a single major spot of 1,2-lecithin containing ≥99% of the total phosphorus and a minor spot corresponding to the 1,3-lecithin. Fatty acid analyses and positional distributions were determined as described previously (Keough & Davis, 1979).

Differential Scanning Calorimetry. Lipids and cholesterol were mixed in chloroform solution, the solvents removed in a stream of N_2 gas at 35 °C, and the residues evacuated over P_2O_5 for 16 h. The mixtures, or the pure lipids which had been dried from chloroform solution, were dispersed in permanganate-distilled water at concentrations of 33% (w/w) by vortexing at 35–40 °C except for samples containing DSPC which were vortexed at 65 °C. Differential scanning calorimetry was performed with a DSC-2 (Perkin-Elmer Corp., Norwalk, CT). The samples were analyzed by using heating and cooling rates of 5 °C/min, sensitivities of 0.5–2.0 mcal/s full-scale, and water in the reference pans. Each sample was heated and cooled through the lipid phase transition region at least twice to ensure reproducibility of the observed behavior. All the samples were checked for the presence of excess water by carrying the thermograms through the ice–water melt at least once. After the calorimetric analyses, the sample pans were opened and the contents extracted 3 times with 1.5–2.0 mL of 1:1 chloroform:methanol (v/v). The pooled extracts were made up to 10 mL, and the phosphorus content was determined.

Areas under the endotherms and exotherms were determined by using a MOP-III digital analyzer from Carl Zeiss (Don Mills, Ontario). The calorimeter traces were normalized in the following way. The displacement of the trace from the extrapolated base line was measured at 0.25 °C intervals by using the MOP-III. These displacements were then normalized to calories per mole of lipid phosphorus and replotted vs. the temperature. The normalized scans are shown in Figures 1 and 2. Endotherms were resolved into components by using a computer program kindly provided by Dr. C. H. Spink. The program determined through an iterative procedure the contributing components to an excess heat capacity curve, assuming that the overlapping components are represented by equations for two-state transitions (Privalov & Khechinashvili, 1974). Estimates of T_{max}, the maximum excess heat capacity, and half-height widths (the latter used in the program to compute an initial value of ΔH) made by visual inspection of the curves provided the input parameters. The calorimetric and van't Hoff enthalpies together with the T_{max} for each component were varied until the standard deviation of the sum of the calculated component curves from the observed calorimetric curve was minimal (Spink et al., 1982). In Figures 1 and 2, only single normalized endotherms are shown, but the variation between runs was very small. Analyses were performed on two heating scans for each sample, and the individual data points in Figures 4–6 represent an average of these two scans, the scan to scan variability for the values being within the size of the symbols. Different sets of samples were made at the various lipid:cholesterol ratios. These separate samples are indicated as separate data points of the same shape, each in turn being the average of two heating runs. For the mixed-acid lecithins, an additional check on variability was introduced by making dispersions for each lecithin–cholesterol with two totally separate synthetic preparations, that is, two syntheses of OSPC and two of SOPC.

Results

Figures 1 and 2 show typical normalized data points from endotherms obtained on heating dispersions of mixtures of SOPC, OSPC, DOPC, and DSPC with varying amounts of cholesterol. Also shown are the best-fit composite curves obtained by using the curve-fitting program described above, along with the two components contributing to those curves. Pure SOPC showed a slight high-temperature tailing observed previously for such mixed-acid lipids (Davis et al., 1981). The

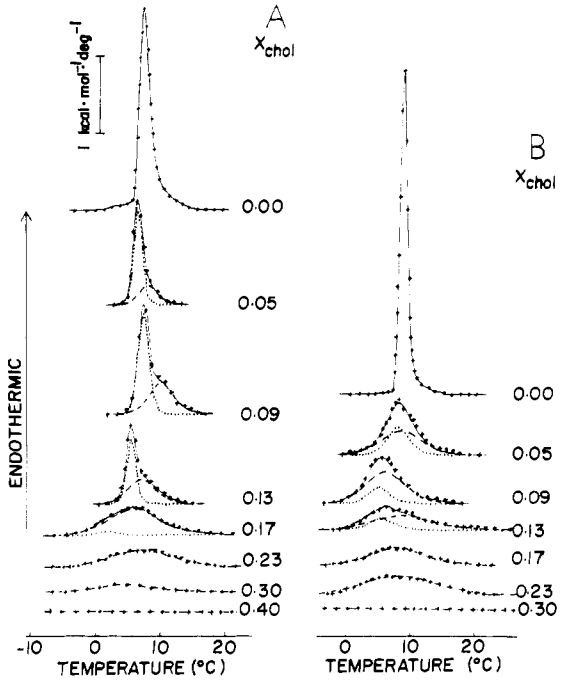

FIGURE 1: DSC heating thermograms of SOPC (A) and OSPC (B). (+) Points from normalized experimental endotherms; (—) composite curves from the computer analysis described in the text; (- - -) broad and (···) narrow components from computer analysis.

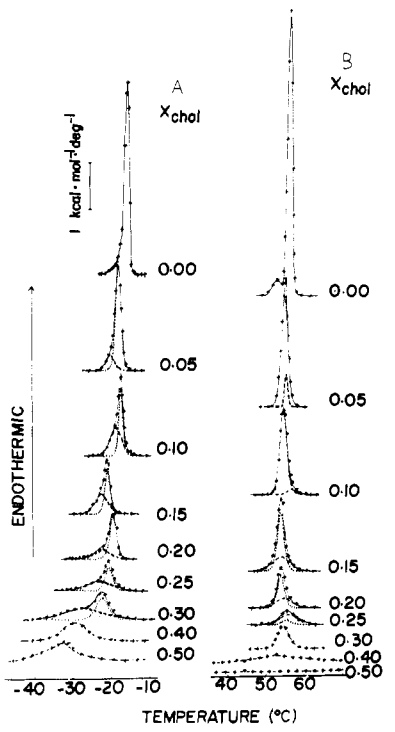

FIGURE 2: DSC heating thermograms of DOPC (A) and DSPC (B). (+) Points from normalized experimental endotherms; (—) composite curves from the computer analysis described in the text; (- - -) broad and (···) narrow components from computer analysis.

transition endotherms for the mixed-acid lipids were compared with those of DOPC, DSPC, dipalmitoylphosphatidylcholine, (dipalmitoyl-PC), hexadecane, and indium by using, for our ΔH values, the equal-area version of the Hill test (Hill, 1963) described by Lumry et al. (1966). Within our precision, and with consideration of the variability between all the compounds, the transitions of the mixed-acid lipids were found to be no different than those of the other compounds for which two-state transitions have generally been assumed (the ratios of upper to lower areas found by applying the Hill test were found to be the following: $SOPC_1$, 1.22; $SOPC_2$, 1.46; $OSPC_1$, 0.80; $OSPC_2$, 1.04; DSPC, 0.82; DOPC, 0.54; dipalmitoyl-PC, 0.98; indium, 1.14; hexadecane, 1.37). When we carried out curve deconvolution by using two two-state transitions for pure mixed-acid PC, the fit of the calculated endotherms was not as good (standard deviation of residuals was 85–150 cal·deg^{-1}·mol^{-1}) as when the model of one two-state transition was used (SD = 8–84 cal·deg^{-1}·mol^{-1}). When the endotherms for 13 mol% cholesterol in the mixed-acid PC were fit by using a total of three two-state transitions (assuming the lipid itself was represented by two), the same difference between the effect of cholesterol on SOPC and OSPC was observed as seen in Figures 1 and 4 (indeed the difference was slightly exaggerated). A set of SOPC and OSPC each with 9% cholesterol was also analyzed for only one component and for two components. The one-component fit for SOPC–cholesterol was very poor. The two-component fit for OSPC was slightly better than the one-component fit. The endotherms of lecithin–cholesterol mixtures were thus analyzed in terms of two components.

We have attempted in two other ways to determine if the heating rate we employed could have in some way artifactually affected the transitions. First, we have analyzed DPPC–cholesterol dispersions under our operating conditions. We observed endotherms which could be analyzed in terms of two components by using the deconvolution. We have compared our data for the enthalpy of the narrow components of the endotherms with the data of Estep et al. (1978) and those of Mabrey et al. (1978). We obtained excellent agreement between our enthalpies of the narrow components and those of authors who had carried out the calorimetry on high-sensitivity instruments using programming rates between 15 and 60 °C/h. Our data were slightly closer to those of Mabrey et al. (1978) (average difference = 0.3 kcal·mol^{-1}), who used a deconvolution analysis like ours, than to those of Estep el al. (1978) (average difference = 0.5 kcal·mol^{-1}), who used a different method of deconvolution. Second, we have analyzed dispersions of SOPC and OSPC, each with 9% cholesterol at programming rates of 5 and 0.62 °C/min (300 and 37 °C/h) (Figure 3). The signal to noise ratios in the instrumental outputs are not as good at the slower scan rate, but the endotherms obtained for each dispersion were essentially the same at both scanning rates. The distinct difference between SOPC–cholesterol and OSPC–cholesterol in the shape of the endotherms was present at both scanning rates (Figure 3). Also, there was little difference between the normalized scans or deconvoluted curves for a given mixture at either programming rate. Analysis of the sample of OSPC used in this test indicated that it contained about 3% excess oleate (from DOPC). The OSPC–cholesterol endotherms at 0.62 and 5 °C/min were more symmetric than the SOPC–cholesterol endotherms, and the normalized data for OSPC could be fitted almost as well by one-component fits as opposed to two-component fits while those for SOPC gave very poor fits with one component only. The goodness of fit, for this OSPC sample, and the distribution of deconvoluted components were influenced a bit by the choice of base lines. The one shown gave the best total fit.

The inclusion of increasing amounts of cholesterol in the bilayer dispersions of SOPC (containing 6% of the reversed isomer) resulted in a progressive decrease in the heights of the maximum excess heat capacity, a broadening of the transitions, and the appearance of high-temperature shoulders on the endotherms (Figure 1A). At 17 mol % cholesterol with SOPC,

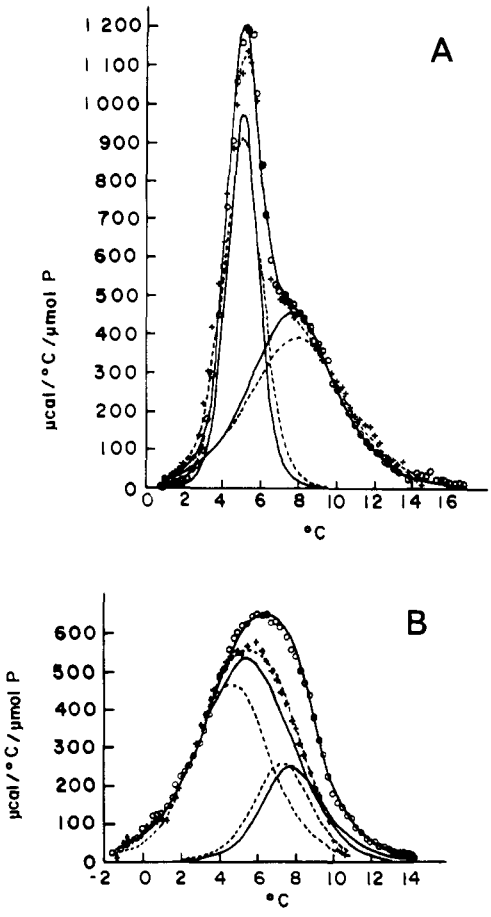

FIGURE 3: Normalized and deconvoluted endotherms obtained from dispersions of (A) SOPC–cholesterol (91:9) and (B) OSPC–cholesterol (91:9) scanned at 5 °C/min (○, —) and 0.62 °C/min (+, ---). This sample of OSPC contained approximately 3% excess oleate (from DOPC).

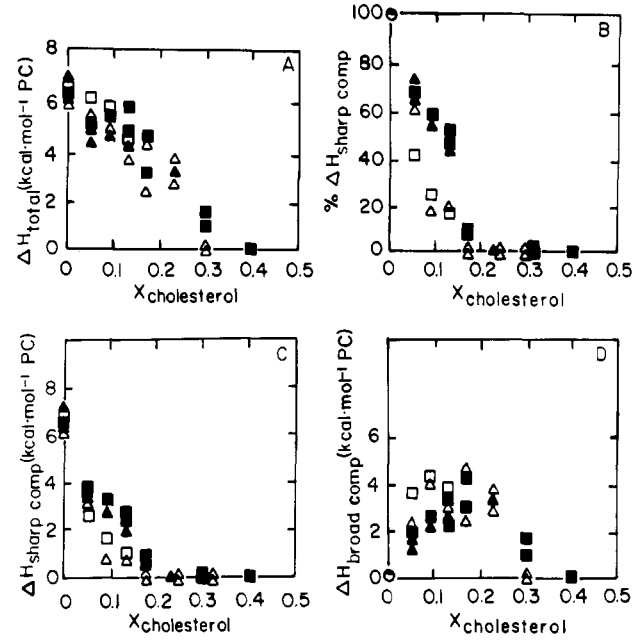

FIGURE 4: Enthalpies associated with the transition of dispersions of mixtures of cholesterol with two samples of OSPC [(▲) 4% acyl migrated and (□) <1% acyl migrated] and two samples of SOPC [(▲) 6% acyl migrated and (■) 11% acyl migrated]. (A) Total transition enthalpies; (B) percentages of total enthalpies contributed by resolved narrow components; (C) enthalpies of resolved narrow components; and (D) enthalpies of resolved broad components. In this and subsequent figures, each point represents the average of two thermograms run on each separate sample. For example, in panel A, there were two different dispersions of OSPC (▲ and □), each run twice on the calorimeter and averaged. There were three different dispersions [two (■) and one (▲)], each run on the calorimeter and averaged. Scan to scan variability was within the size of the data points. In cases where the separate dispersions gave coincidental values, these are shown with symbols which are slightly displaced from one another. (◓) Values for pure lecithins.

the component with the lower T_{max} was not discernible, leaving only a broad component which, in turn, disappeared between 30 and 40 mol % cholesterol. Similar curves were obtained with a different preparation of SOPC containing 11% of the reversed isomer, OSPC. A third preparation of SOPC which contained 18 mol % of the reversed isomer produced endotherms like those shown, but the endotherms appeared by eye to contain somewhat more of the broad-component band and proportionally less of the narrow component for any given amount of cholesterol.

Figure 1B shows normalized endotherms for a series of mixtures made with a preparation of OSPC which contained less than 1% SOPC. (These were similar to thermograms obtained when mixtures were made with a different preparation of OSPC which contained 4% of the reversed isomer.) At low cholesterol concentrations, the shapes of the endotherms for OSPC–cholesterol (Figure 1B) were different from those of SOPC–cholesterol (Figure 1A). In the case of thermograms of OSPC–cholesterol, there was less of a contribution from the narrow component at any given proportion of cholesterol in comparison to the thermograms obtained for SOPC–cholesterol. Endotherms of OSPC plus 9% or 13% cholesterol had less asymmetry than the endotherms of the corresponding mixtures with SOPC. Endotherms for OSPC–cholesterol could not be distinguished from base lines at 30 mol % cholesterol, whereas endotherms for SOPC–cholesterol were still visible at that concentration but not at 40 mol % cholesterol.

Endotherms for the mixtures of cholesterol with DOPC (Figure 2A) had shapes different from those observed with any of the other lipids. The endotherms were distinctly skewed to low temperature and could be resolved into a narrow component at high temperatures and a low-temperature broad component. For DOPC–cholesterol, the sharp component did not disappear until about 30 mol % cholesterol, and there was a transition of substantial enthalpy for a mixture containing 50% cholesterol.

Mixtures of cholesterol with DSPC yielded endotherms which are illustrated in Figure 2B. With up to 25 mol % cholesterol, these endotherms were nearly symmetrical with only slight skewing to higher temperatures. The asymmetry found in the endotherms for the other types of lipid–cholesterol mixture (Figures 1 and 2A) was not present. While the individual endotherms of DSPC–cholesterol could be resolved into two components in the range of 5–25 mol % cholesterol, they could also be considered as one component which broadens and disappears.

Figure 4A illustrates that the total transition enthalpies for SOPC and OSPC decreased with the addition of cholesterol. Under our operating conditions, transitions could not be resolved from base lines at cholesterol concentrations of 30% and 40% in OSPC and SOPC, respectively. The enthalpies associated with the sharp components (Figure 4C) decreased rapidly with the addition of cholesterol, such that by the addition of 17 mol % cholesterol there were little or no contributions from sharp components in mixtures with either isomeric lecithin. In the range of 5–13%, the absolute enthalpies associated with the sharp components of endotherms were smaller for mixtures with OSPC than for mixtures with SOPC (Figure 4C). The proportional contributions of the narrow components to the enthalpies of the observed endotherms at

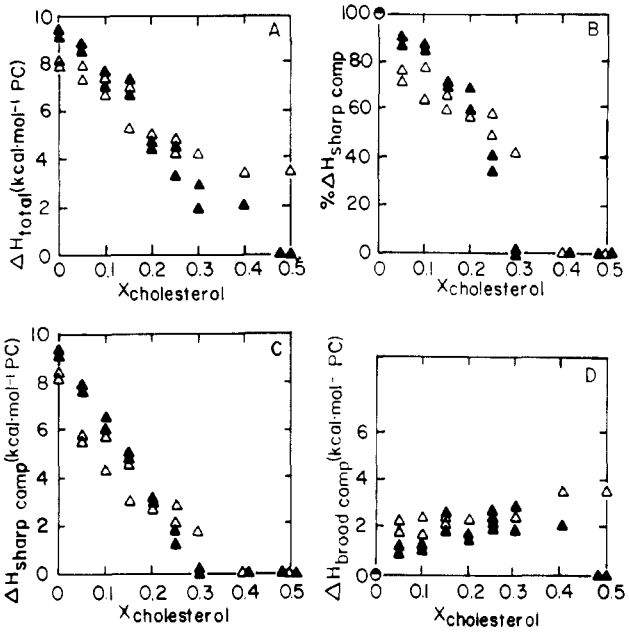

FIGURE 5: Enthalpies associated with transitions of dispersions of a mixture of DOPC (△) and DSPC (▲) with cholesterol. (A) Total transition enthalpies; (B) percentages of total enthalpies contributed by narrow components; (C) enthalpies of resolved narrow components; and (D) enthalpies of resolved broad components. Each point represents the average of two thermograms run on the same sample. (⊖) Values for pure lecithins.

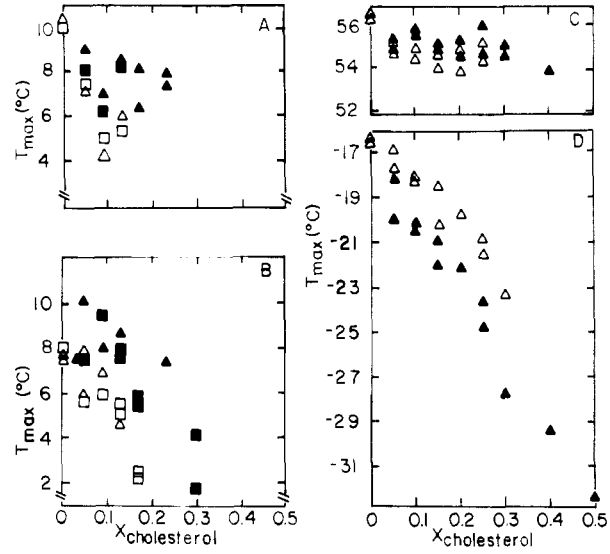

FIGURE 6: T_{max} of components of resolved endotherms. (A) Narrow components for mixtures of cholesterol with two samples of OSPC [(△) 4% acyl migrated and (□) <1% acyl migrated] and broad components for the same mixtures [(▲) 4% acyl migrated and (■) <1% acyl migrated]; (B) narrow components for mixtures of cholesterol with two samples of SOPC [(△) 6% acyl migrated and (□) 11% acyl migrated] and broad components for the same mixtures [(▲) 6% acyl migrated and (■) 11% acyl migrated]; (C) narrow (△) and broad (▲) components for mixtures of cholesterol with DSPC; (D) narrow (△) and broad (▲) components for mixtures of cholesterol with DOPC. Each point represents the average of two thermograms run on the same sample.

each cholesterol concentration were also less for OSPC mixtures than for SOPC mixtures (Figure 4B). For mixtures with SOPC or OSPC, the enthalpies of the broad components increased up to 17 mol % and then decreased (Figure 4D). Although not shown, because it is implied by Figure 4B,C, in the range of 5–13 mol % cholesterol, the broad components contributed substantially more to the total enthalpies of the mixtures with OSPC than of the mixtures with SOPC. Thus, in comparison to SOPC, it took less cholesterol with OSPC to decrease and remove the total transitional endotherm, to reduce the absolute sizes of the narrow components, and to reduce the proportion of the total endotherm attributable to the narrow component.

The endotherms of DSPC–cholesterol decreased in size with increasing cholesterol concentrations until they became undetectable at $X_{chol} = 0.48$ (Figures 2B and 5A). There was a progressive decrease in the enthalpy of DOPC–cholesterol mixtures up to $X_{chol} = 0.3$, at which point the enthalpy remained constant at 3.5 kcal·mol^{-1} up to $X_{chol} = 0.5$ (Figures 2A and 5A). For mixtures with either DSPC or DOPC, the enthalpies attributable to the narrow components and their proportional contributions to each endotherm decreased in a nearly linear and similar fashion with increasing cholesterol concentration for both lipids (Figure 5B,C). The percentage contributions to the individual endotherms from narrow components were generally greater at any given concentration of cholesterol for the mixtures of single-acid lecithins with cholesterol (Figure 5B) than they were in the endotherms of mixtures with the mixed-acid lecithins (Figure 4B). For mixtures with the single-acid lecithins, narrow components were resolvable at higher concentrations of cholesterol (>25 mol %) than those for the mixed-acid lecithins (<20 mol %). For the endotherms of mixtures with DOPC or DSPC, the values of the enthalpies attributable to the broad components of the endotherms did not show strong dependence on cholesterol concentration (Figure 5D). This was also different from the pattern for the mixed-acid lecithins where the broad components had maxima near 17 mol % cholesterol (Figure 4D).

For mixtures of cholesterol with OSPC (Figure 6A), the T_{max} values for both broad and narrow components of the endotherms were lower than those for the pure OSPC, the T_{max} values of the narrow components being displaced by greater amounts than those of the broad components. The T_{max} for the broad component in the SOPC–cholesterol mixtures (Figure 6B), while variable, showed a tendency to decrease when cholesterol concentration was above 13 mol %. For the narrow components, T_{max} values were always lower than the T_{max} value of pure SOPC, and they decreased with increasing amounts of cholesterol. The T_{max} values of the two components of DSPC–cholesterol endotherms were slightly lower than that of the pure DSPC (Figure 6C), and they were essentially independent of cholesterol concentration. With DOPC–cholesterol mixtures (Figure 6D), the T_{max} values of both narrow and broad components were shifted progressively downward from that of the pure lipid with increasing cholesterol concentration. In the endotherms for the DOPC–cholesterol mixtures, the T_{max} values for the broad components were lower than those for the sharp components.

The widths at half maximum excess heat capacity for the component parts of the endotherms for each of the four types of mixtures showed similar dependencies on cholesterol concentration. For all four, the widths of the narrow components were essentially unaffected by cholesterol, but the widths of the broad components increased with rising cholesterol concentration.

Cooling curves for all these mixtures have not been analyzed in the detail that has been applied to the heating curves. However, the cooling curves had shapes which were commensurate with two components and had T_{max} values for those components which were consistent with those observed for the ones seen in the heating curves, allowing for slight supercooling and thermal lags.

Discussion

There were some subtle, but significant, differences between the shapes of the endotherms of the four lecithin–cholesterol mixtures studied here and also between some of these endotherms and those seen by other workers for other lipid–cholesterol mixtures (Estep et al., 1978, 1979, 1981; Mabrey et al., 1978; Calhoun & Shipley, 1979; Blume, 1980; Melchoir et al., 1980). These were seen in the shapes of the endotherms at a given cholesterol concentration, in the concentrations of cholesterol at which the total enthalpy disappeared, in the contributions of broad and narrow components to the total endotherm at any given concentration of cholesterol, and in the effect of cholesterol on the T_{max} of the narrow and broad components. Our analysis of the effects of the scan rate indicates that our data can reasonably be compared with those of other workers and that differences between our observed shapes and theirs should not result only from differences in analytical techniques and protocols. Certainly, our own technique is self-consistent, and there are distinct reproducible differences between each type of lecithin–cholesterol mixture analyzed here.

Detectable transitions disappeared at different cholesterol concentrations for each lecithin. The effects of cholesterol concentration on the reduction of the total enthalpies from mixtures with either DSPC, SOPC, or OSPC were similar to those observed for mixtures of cholesterol with other lecithins and sphingomyelins (Estep et al., 1978, 1979; Mabrey et al., 1978; Calhoun & Shipley, 1979). The total enthalpies of DOPC–cholesterol mixtures decreased in an approximately linear fashion only up to $X_{chol} = 0.30$ and then remained nearly constant at least up to $X_{chol} = 0.50$, in agreement with the results of Gershfeld (1978). The distribution of the enthalpy between the narrow and the broad components of the various mixtures was not identical. The decrease in enthalpy of the narrow components was nearly linear with cholesterol concentration for DSPC, DOPC, and SOPC, and thus these endotherms were like those observed with cholesterol plus either DMPC, DPPC, or lignoceroylsphingomyelin (Estep et al., 1978, 1979; Mabrey et al., 1978). The reduction in the enthalpy of the narrow components was nonlinear with cholesterol concentration for OSPC, a property also shown by palmitoylsphingomyelin–cholesterol dispersions (Estep et al., 1979). With the mixed-acid lipids, the narrow components disappeared at $X_{chol} < 0.23$, and in this way, the mixtures were similar to many of the mixtures with other lipids seen before (Estep et al., 1978, 1979, 1981; Mabrey et al., 1978). In mixtures of cholesterol with DOPC and DSPC, narrow components were resolvable up to about 30 mol % cholesterol, so these mixtures were like those with lignoceroylsphingomyelin (Estep et al., 1979).

The T_{max} for the components of endotherms for each of the four lipids responded differently to increasing cholesterol concentration (Figure 6). Endotherms of the broad components of DOPC–cholesterol had lower T_{max} values than those of the narrow components. This is different from the endotherms seen with other cholesterol–lecithin mixtures by us and by others (Mabrey et al., 1978; Estep et al., 1978, 1979). The shapes of the endotherms of mixtures of cholesterol with some saturated phosphatidylethanolamines (Blume, 1980) suggest these might have a broad component with the T_{max} value lower than that for the narrow component. These observations and those on other mixtures (Mabrey et al., 1978; Estep et al., 1978, 1979) indicate that there are at least quantitative differences in the interactions between cholesterol and phospholipids which are determined by the phospholipid structures, including the nature and location of acyl chains.

Various models have been proposed to describe the effects of cholesterol on phospholipids in bilayers [e.g., see Engelman & Rothman (1972), Martin & Yeagle (1978), Huang (1977), Cornell et al. (1979), Pink & Carroll (1978), Pink & Chapman (1979), Snyder & Freire (1980), Slater & Caillé (1981), and Presti et al. (1982)]. The narrow components have been ascribed to transitions occurring in almost pure lipid domains, and the broad components have been ascribed to transitions of either cholesterol-rich domains or interfacial regions between cholesterol-rich domains and domains of nearly pure lipid (Estep et al., 1978, 1979; Mabrey et al., 1978; Snyder & Freire, 1980; Slater & Caillé, 1981). In the low range of cholesterol concentration, and for any given amount of cholesterol, the enthalpies of the narrow components in OSPC–cholesterol were less than those in SOPC–cholesterol. If the narrow components arose from the melting of a pure lipid domain, this observation suggested that in the bilayers more OSPC than SOPC was associated with cholesterol at a given molar ratio. Viewed in light of the model of Snyder & Freire (1980), the data suggested that OSPC was more miscible with cholesterol than SOPC. Whatever the molecular explanation, OSPC was more affected by cholesterol than SOPC. Both OSPC and SOPC were more affected by a given amount of cholesterol than either of the two single-acid lecithins, DOPC and DSPC.

The data presented above suggest that there may be quantitatively, if not qualitatively, different interactions between cholesterol and various phospholipids including positional isomers, at least in the gel state of lipid bilayers. If such differences in lipid–cholesterol interactions were to persist in the liquid–crystalline state more commonly found in membranes, they may have important consequences for membrane-associated processes. For the majority of membranes where detailed examination of acyl chain distribution has been carried out, there is a preponderance of phospholipid species with a saturated chain in the *sn*-1 position and an unsaturated (or short) chain in the *sn*-2 position. The consequences of this arrangement for lipid and protein arrangements in membranes are essentially unknown at the present time since so little information is yet available about the properties of positional isomers. It is interesting to speculate whether the unique distribution of positional isomers is an accident in the evolution of the synthetic enzymes of the particular organism or if special constraints (biophysical or biochemical or both) may dictate the occurrence of a preponderance of one type of isomer. Rottem & Markowitz (1979a,b) observed that a number of cholesterol-requiring *Mycoplasmas*, which can incorporate large amounts of cholesterol into their membranes, had a preponderance of unusual 1-unsaturated–2-saturated fatty acid positional distribution in their principal lipid, phosphatidylglycerol. Dyatlovitskaya et al. (1974) reported that the lecithins from rat hepatoma contained large amounts of 1,2-diunsaturated and 1-unsaturated–2-saturated compounds. There was also an elevated cholesterol content in hepatoma membranes compared to those of normal liver cells (van Hoeven & Emmelot, 1972; Dyatlovitskaya et al., 1975). The occurrence of high cholesterol together with lipids with unusual positional specificity, together with the fact that the phase behaviors of bilayers composed of cholesterol plus positional isomers are different from one another, would suggest that the distribution of lipid components may be under very sensitive control.

Acknowledgments

We acknowledge the excellent assistance of J. Moore and

N. Kariel, and we thank Dr. G. F. Bennett for making available to us the Zeiss MOP-3 digital analyzer. We are indebted to Dr. C. H. Spink for providing the computer program used for resolving the components of the calorimetric endotherms. P.J.D. thanks Memorial University of Newfoundland for financial assistance.

Registry No. Cholesterol, 57-88-5; DSPC, 816-94-4; DOPC, 4235-95-4; SOPC, 56421-10-4; OSPC, 7276-38-2.

References

Bartlett, G. R. (1959) *J. Biol. Chem. 234*, 466–478.
Blume, A. (1980) *Biochemistry 19*, 4908–4913.
Brockerhoff, H., & Yurkowski, M. (1965) *Can. J. Biochem. 43*, 1777.
Calhoun, W. I., & Shipley, G. G. (1979) *Biochemistry 18*, 1717–1722.
Chakrabarti, P., & Khorana, H. G. (1975) *Biochemistry 14*, 5021–5033.
Comfurius, P., & Zwaal, R. F. A. (1977) *Biochim. Biophys. Acta 488*, 36–42.
Cornell, B. A., Chapman, D., & Peel, W. E. (1979) *Chem. Phys. Lipids 23*, 223–237.
Davis, P. J., Fleming, B. D., Coolbear, K. P., & Keough, K. M. W. (1981) *Biochemistry 20*, 3633–3636.
Dawson, R. M. C. (1960) *Biochem. J. 75*, 45–53.
de Kruyff, B., Demel, R. A., & van Deenen, L. L. M. (1972) *Biochim. Biophys. Acta 255*, 331–347.
de Kruyff, B., Demel, R. A., Slotboom, A. J., van Deenen, L. L. M., & Rosenthal, A. F. (1973) *Biochim. Biophys. Acta 307*, 1–19.
Demel, R. A., & de Kruyff, B. (1976) *Biochim. Biophys. Acta 457*, 109–132.
Dittmer, J. C., & Lester, R. L. (1964) *J. Lipid Res. 5*, 126–127.
Dyatlovitskaya, E. V., Yanchevskaya, G. V., & Bergelson, L. D. (1974) *Chem. Phys. Lipids 12*, 132–149.
Dyatlovitskaya, E. V., Timofeeva, N. G., Gor'Kova, N. P., & Bergelson, L. D. (1975) *Biokhimiya (Moscow) 40*, 1315–1319.
Engelman, D. M., & Rothman, J. E. (1972) *J. Biol. Chem. 247*, 3694–3697.
Estep, T. N., Mountcastle, D. B., Biltonen, R. L., & Thompson, T. E. (1978) *Biochemistry 17*, 1984–1989.
Estep, T. N., Mountcastle, D. B., Barenholz, Y., Biltonen, R. L., & Thompson, T. E. (1979) *Biochemistry 18*, 2112–2117.
Estep, T. N., Freire, E., Anthony, F., Barenholz, Y., Biltonen, R. L., & Thompson, T. E. (1981) *Biochemistry 20*, 7115–7118.
Fiske, C. H., & Subbarow, Y. (1925) *J. Biol. Chem. 66*, 375–400.
Folch, J., Lees, M., & Sloane-Stanley, G. H. (1957) *J. Biol. Chem. 226*, 497–509.
Gershfeld, N. L. (1978) *Biophys. J. 22*, 469–488.
Green, C. (1977) *Int. Rev. Biochem. 14*, 101–152.
Gupta, C. M., Radhakrishnan, R., & Khorana, H. G. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 4315–4319.
Hawco, M. W., Davis, P. J., & Keough, K. M. W. (1981) *J. Appl. Physiol.: Respir., Environ. Exercise Physiol. 51*, 509–515.
Hill, T. (1963) *Thermodynamics of Small Systems*, Part 1, Chapter 5 et seq., W. A. Benjamin, New York.
Huang, C. (1977) *Lipids 12*, 348–356.
Keough, K. M. W., & Davis, P. J. (1979) *Biochemistry 18*, 1453–1459.
Ladbrooke, B. D., Williams, R. M., & Chapman, D. (1968) *Biochim. Biophys. Acta 150*, 333–340.
Lumry, R., Biltonen, R., & Brandts, J. F. (1966) *Biopolymers 4*, 917–944.
Mabrey, S., Mateo, P. L., & Sturtevant, J. M. (1978) *Biochemistry 17*, 2464–2468.
Martin, R. B., & Yeagle, P. L. (1978) *Lipids 13*, 594–597.
McElhaney, R. N., & Tourtellotte, M. E. (1970) *Biochim. Biophys. Acta 202*, 120–128.
Melchior, D. L., Scavitto, F. J., & Steim, J. M. (1980) *Biochemistry 19*, 4828–4834.
Pink, D. A., & Carroll, C. E. (1978) *Phys. Lett. A 66A*, 157–160.
Pink, D., & Chapman, D. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 1542–1546.
Presti, F. T., Pace, R. J., & Chan, S. I. (1982) *Biochemistry 21*, 3831–3835.
Privalov, P. L., & Khechinashvili, N. N. (1974) *J. Mol. Biol. 86*, 665–684.
Rottem, S., & Markowitz, O. (1979a) *Biochemistry 18*, 2930–2935.
Rottem, S., & Markowitz, O. (1979b) *FEBS Lett. 107*, 379–382.
Ryu, E. K., & MacCoss, M. (1979) *J. Lipid Res. 20*, 561–563.
Selinger, Z., & Lapidot, Y. (1966) *J. Lipid Res. 7*, 174–175.
Slater, G., & Caillé, A. (1981) *Phys. Lett. A 86A*, 256–258.
Snyder, B., & Freire, E. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 4055–4059.
Spink, C. H., Müller, K., & Sturtevant, J. M. (1982) *Biochemistry 21*, 6598–6605.
Thompson, W. (1969) *Biochim. Biophys. Acta 187*, 150–153.
van Dijck, P. W. M., de Kruijff, B., van Deenen, L. L. M., de Gier, J., & Demel, R. A. (1976) *Biochim. Biophys. Acta 455*, 576–587.
van Hoeven, R. P., & Emmelot, P. (1972) *J. Membr. Biol. 9*, 105–126.
Wagner, H., Hörhammer, L., & Wolff, P. (1961) *Biochem. Z. 334*, 175–184.