Lemieux, R. U., Boullanger, P. H., Bundle, D. R., Baker, P. A., Nagpurkar, A., & Venot, A. (1978) *Nouv. J. Chim. 2*, 321-329.
Morell, A. G., & Scheinberg, I. H. (1972) *Biochem. Biophys. Res. Commun. 48*, 808-815.
Morell, A. G., Irvine, R. A., Sternlieb, I., & Scheinberg, I. H. (1968) *J. Biol. Chem. 243*, 155-159.
Neufeld, E. F., Sando, G. N., Garvin, A. J., & Rome, L. H. (1977) *J. Supramol. Struct. 6*, 95-101.
Ray, T. K. (1970) *Biochim. Biophys. Acta. 196*, 1-9.
Roseman, S. (1970) *Chem. Phys. Lipids 5*, 270-297.
Sarkar, M., Liao, J., Kabat, E. A., Tanabe, T., & Ashwell, G. (1979) *J. Biol. Chem. 254*, 3170-3174.
Schwartz, J., Sloan, J., & Lee, Y. C. (1970) *Arch. Biochem. Biophys. 137*, 122-127.
Stahl, P. D., Rodman, J. S., Miller, H. J., & Schlesinger, P. H. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 1399-1403.
Stockert, R. J., Morell, A. G., & Scheinberg, I. H. (1974) *Science 186*, 365-366.
Stowell, C. P., & Lee, Y. C. (1978) *J. Biol. Chem. 253*, 6107-6110.
Stowell, C. P., & Lee, Y. C. (1980) *Biochemistry* (preceding paper in this issue).
Theander, O. (1961) *Adv. Carbohydr. Chem. 17*, 223-299.
Van Lenten, L., & Ashwell, G. (1972) *J. Biol. Chem. 247*, 4633-4640.
Wallenfels, K., & Malhotra, O. P. (1961) *Adv. Carbohydr. Chem. 16*, 239-298.

Thermotropic Behavior of Phosphatidylethanolamine–Cholesterol and Phosphatidylethanolamine–Phosphatidylcholine–Cholesterol Mixtures†

Alfred Blume*

ABSTRACT: The thermotropic behavior of aqueous dispersions of phosphatidylethanolamine–cholesterol and phosphatidylethanolamine–phosphatidylcholine–cholesterol mixtures has been studied by high-sensitivity differential scanning calorimetry. The gel to liquid-crystalline phase transition of phosphatidylethanolamines is broadened and shifted to lower temperature when cholesterol is incorporated into the bilayer. When the cholesterol content is below 25 mol %, the calorimetric endotherms seem to consist of two components, a broad one at considerably lower temperature than the original transition and another component at only slightly lower temperature. This thermotropic behavior can be explained by the assumption of a homogeneous distribution of cholesterol in phosphatidylethanolamine bilayers. Scanning calorimetry of equimolar mixtures of phosphatidylethanolamines with phosphatidylcholines, which show either ideal or nonideal mixing properties, reveals that when cholesterol is added to these mixtures it shows no preferential affinity for either of the phospholipids.

The membranes of eukaryotic cells usually contain large amounts of sterols, cholesterol being the dominant component (Rouser et al., 1968). The physicochemical behavior of phospholipid–cholesterol mixtures has therefore been a subject of considerable research (Demel & de Kruijff, 1976). Since the first experimental findings of Ladbrooke et al. (1968) that the addition of increasing amounts of cholesterol to phosphatidylcholine bilayers gradually diminishes the gel to liquid-crystalline phase transition of this phospholipid, controversial results have been published regarding the exact percentage of cholesterol at which the transition is abolished and concerning the existence of possible phosphatidylcholine–cholesterol complexes and their stoichiometry (Darke et al., 1972; Shimshick & McConnell, 1973a; Engelman & Rothman, 1972; Hinz & Sturtevant, 1972; de Kruijff et al., 1972, 1973, 1974; Phillips & Finer, 1974; Tsong, 1975). Very recently reexaminations of the phosphatidylcholine–cholesterol system by high-sensitivity differential scanning calorimetry revealed that indeed an endothermic transition can be observed at a cholesterol content above 33 mol %, which, however, is very broad and shifted to higher temperature as compared to the original unperturbed transition (Mabrey et al., 1978; Estep et al., 1978). At a cholesterol content below 20–25 mol % the calorimetric scans could be decomposed into two different peaks. These findings were interpreted as evidence for the coexistence of a cholesterol-rich and a cholesterol-poor or a pure phospholipid phase, respectively. Similar results were obtained with sphingomyelin–cholesterol mixtures (Estep et al., 1979; Calhoun & Shipley, 1979). Other workers, however, have proposed a homogeneous phase for mixtures with less than 25 mol % cholesterol (Owicki & McConnell, 1979; Rubinstein et al., 1979). The same suggestions were made before by Verkleij et al. (1974) on the basis of freeze-fracturing experiments. Recent deuterium NMR experiments with mixtures of specifically deuterated phosphatidylcholines with cholesterol seem to confirm this view (Haberkorn et al., 1977; Jacobs & Oldfield, 1979).

Another important aspect of phospholipid–cholesterol interactions is the differential affinity of cholesterol to certain phospholipids in a mixture. In PE–PC[1] mixtures, for instance, which show monotectic behavior, cholesterol shows a preferential association with PC, irrespective of whether PC is the higher or the lower melting component (van Dijck et al., 1976a). It could be shown that cholesterol has a decreased

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[1] Abbreviations used: DSC, differential scanning calorimetry; PC, phosphatidylcholine; PE, phosphatidylethanolamine; DMPE, dimyristoylphosphatidylethanolamine; DPPE, dipalmitoylphosphatidylethanolamine; DLPE, dilauroylphosphatidylethanolamine; DPPC, dipalmitoylphosphatidylcholine; DSPC, distearoylphosphatidylcholine.

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affinity for phospholipids in the following order: sphingomyelin > phosphatidylcholine > phosphatidylethanolamine (Demel et al., 1977; van Dijck, 1979). However, Calhoun & Shipley (1979) showed that in an ideally mixed sphingomyelin–phosphatidylcholine system no preferential affinity of cholesterol for either of the phospholipids was observed. Because the phospholipids of biological membranes are a complex mixture of different phospholipid classes, which normally shows no monotectic behavior, the question of whether the differential affinity of cholesterol observed in monotectic systems has any relevance to biological membranes remains open.

Despite the widespread occurrence of phosphatidylethanolamines in eukaryotic membranes (Rouser et al., 1968), there is only one calorimetric study of the mixing behavior of cholesterol with pure phosphatidylethanolamines (van Dijck et al., 1976a). These workers showed by DSC that the addition of cholesterol to DMPE bilayers reduces the transition enthalpy till at a molar ratio of 0.5 (33 mol % cholesterol) a transition is no longer observable. In view of the recent reexaminations of the phosphatidylcholine–cholesterol system, it seemed worthwhile to reinvestigate in detail the behavior of phosphatidylethanolamine–cholesterol mixtures and of ideal and nonideal phosphatidylethanolamine–phosphatidylcholine–cholesterol mixtures by using high-sensitivity differential scanning calorimetry.

Experimental Procedures

Materials. DPPC, DSPC, DLPE, DMPE, DPPE, and cholesterol (all purissimum grade) were purchased from Fluka, Neu-Ulm, and used without further purification. The purity of the lipids was checked by thin-layer chromatography on microplates coated with "Silica Gel 60 HR" (Merck AG, Darmstadt). The buffer for the aqueous dispersions of the lipids was prepared by using water which was twice distilled from a quartz apparatus. The buffer contained 0.05 M Tris adjusted to pH 7.5 with hydrochloric acid.

Preparation of the Phospholipid Suspensions. Dispersions of phospholipids with and without cholesterol were prepared by mixing appropriate aliquots of chloroform stock solutions of the phospholipids. Chloroform was removed by evaporation in a stream of nitrogen at ca. 50 °C. This high temperature was essential to minimize effects due to the different solubilities of the lipids in chloroform. After 2 h under vacuum a defined volume of buffer was added to the sample. It was then heated to a temperature above the respective gel to liquid-crystalline phase transition, vortexed for ca. 2 min, and then kept at that temperature for an additional 30 min. The sample was cooled to ca. 5 °C before it was loaded into the calorimeter cell. Samples with high cholesterol content were usually sonicated in a Branson bath sonicator at room temperature for 1 min to completely remove the lipid film from the glass wall. Suspensions containing high amounts of cholesterol show a tendency toward settling after standing. This, however, had no effect on the calorimetric scans. Care was taken that these samples were homogeneous before they were loaded into the calorimeter cell.

Calorimetry. All calorimetric scans were performed with a Privalov calorimeter (Privalov et al., 1975) with a scan rate of 2 K min^{-1}. For samples without cholesterol this high scan rate slightly broadens the transitions due to the instrumental dead time but it has no effect on the transition enthalpies. Control runs with a slower scan speed gave identical results. The lipid concentrations used were in the range from 0.2 to 2 mg of phospholipid per mL of buffer. The exact phospholipid concentration of the suspension was determined after the calorimetric run by a slightly modified procedure for phosphate analysis after the method of Bartlett (1959). At least three scans were taken from each sample to test the reproducibility and reversibility of the system. In the case of samples with high cholesterol content, the first scan sometimes gave slightly different curves with a higher heat capacity maximum than the second and consecutive scans. These usually showed good reproducibility and were therefore used for the evaluation of the transition enthalpies. For the extraordinary broad transitions the following procedure had to be applied for the determination of the transition enthalpies. The calorimetric scans were digitized with a Hewlett-Packard plotter (Model 9872A) connected to a Hewlett-Packard 9845A computer. The curves were replotted after computer subtraction of the instrumental base line, which had been determined before by scanning buffer vs. buffer. After that the base lines before and after the transition peak were usually completely horizontal and a linear interpolation in the temperature range of the transition could be applied. The area of the calorimetric peak was then determined with the help of a computer program using a numeric integration method. Molar transition enthalpies were calculated from the phospholipid content determined by phosphorus analysis and the sensitivity of the instrument known from an electrical calibration experiment.

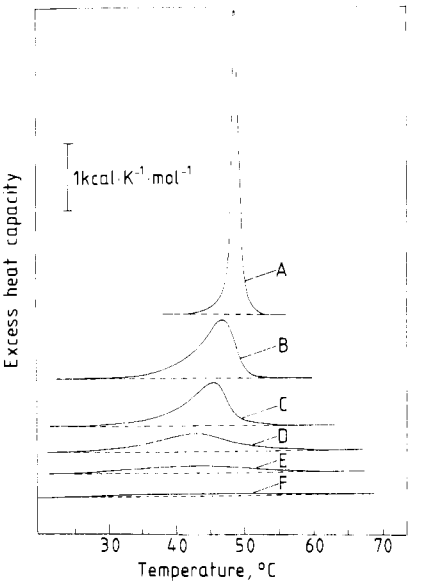

FIGURE 1: Excess heat capacity curves for DMPE–cholesterol mixtures with (A) 0, (B) 5, (C) 10, (D) 20, (E) 30, and (F) 40 mol % cholesterol.

Results

DMPE–Cholesterol. We investigated DMPE–cholesterol mixtures in the range from 5 to 50 mol % cholesterol. The calorimetric scans of these mixtures are shown in Figure 1. Pure DMPE has a considerably higher transition temperature (49 °C) than the corresponding PC with the same chain length of the fatty acid chains. The transition enthalpy, however, is very similar (6.4 kcal/mol). Addition of cholesterol to DMPE leads to a continuous decrease of the temperature of the maximum of the heat capacity curve with a concomitant broadening of the transition. Mixtures containing 50 mol % cholesterol show no detectable transition. The transition enthalpy decreases almost linearly with increasing cholesterol content (see Figure 2). Compared to the behavior of the related DMPC–cholesterol system (Mabrey et al., 1978), DMPE–cholesterol mixtures show quite different calorimetric scans. The peak width at half-maximum of the excess heat capacity curve is drastically increased already at a content of 5 mol % cholesterol from 1.2 to 6 °C, whereas it is almost

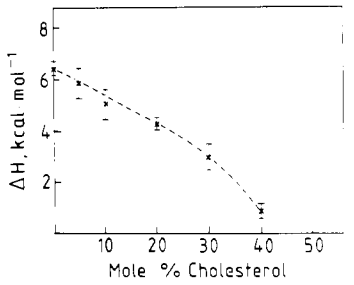

FIGURE 2: Plot of total transition enthalpy (kilocalories per mole of phospholipid) of DMPE–cholesterol mixtures vs. mole percent cholesterol.

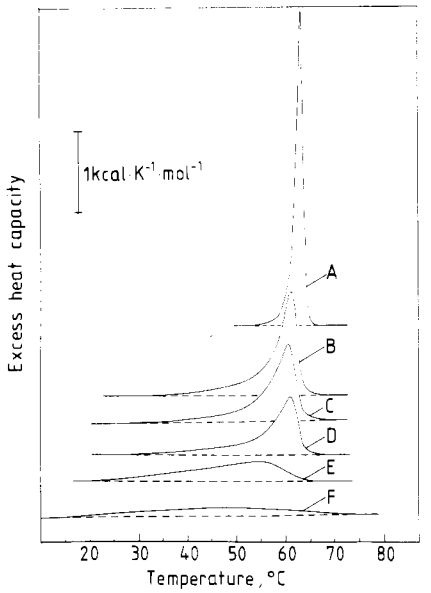

FIGURE 3: Excess heat capacity curves for DPPE–cholesterol mixtures with (A) 0, (B) 5, (C) 10, (D) 20, (E) 30, and (F) 40 mol % cholesterol.

unchanged in DMPC–cholesterol mixtures up to 15 mol % cholesterol (Mabrey et al., 1978; Calhoun & Shipley, 1979). The endothermic peaks of DMPE–cholesterol mixtures with a cholesterol content in the range from 5 to 20 mol % are strongly asymmetric with the peak maximum at the high temperature side. It is possible to decompose these curves into two different transitions, a broad one with a peak maximum at lower temperature ranging from ca. 20 to 60 °C and another one with a maximum at higher temperature, which, however, is broadened and shifted to lower temperature with increasing cholesterol content. This decomposition, however, is not unambiguous, so that the resulting values for the transition enthalpies of the component transitions have a large error. This evaluation would lead to similar results as those obtained by Estep et al. (1978) for the resolution of the total transition enthalpy of DPPC–cholesterol mixtures.

DPPE–Cholesterol. Representative scans of DPPE–cholesterol mixtures are shown in Figure 3. Increasing cholesterol content again broadens the transition and decreases the transition enthalpy almost linearly, so that at 50 mol % cholesterol no transition is detectable (see Figure 4). Similar to the results obtained for DMPE, only 5 mol % cholesterol leads to a considerable increase in the width of the transition. The decrease of the temperature of the peak maximum with increasing cholesterol content is not as pronounced as that with DMPE. Below 30 mol % cholesterol the calorimetric curves are again strongly asymmetric with the peak maximum at the high temperature side. A decomposition into two different transitions seems to be possible but certainly is very difficult due to the great width of both transitions.

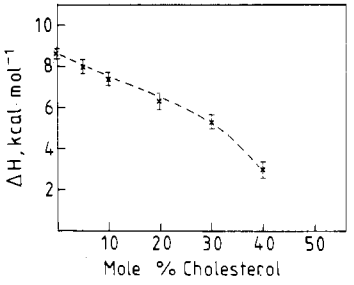

FIGURE 4: Plot of total transition enthalpy (kilocalories per mole of phospholipid) of DPPE–cholesterol mixtures vs. mole percent cholesterol.

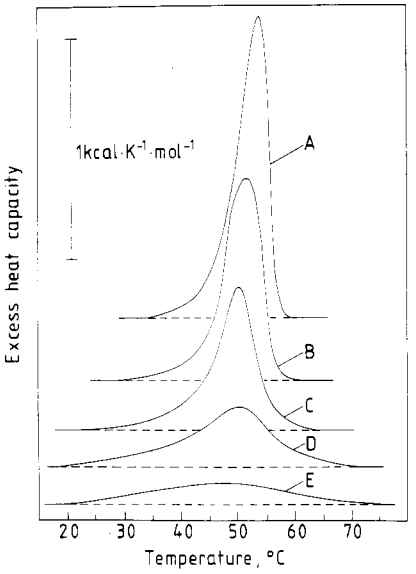

FIGURE 5: Excess heat capacity curves for DPPC–DPPE–cholesterol mixtures with (A) 0, (B) 10, (C) 20, (D) 30, and (E) 40 mol % cholesterol.

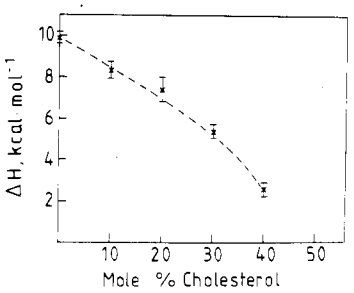

FIGURE 6: Plot of total transition enthalpy (kilocalories per mole of phospholipid) of DPPC–DPPE–cholesterol mixtures vs. mole percent cholesterol.

DPPC–DPPE–Cholesterol. The mixing behavior of DPPC with DPPE has been investigated by different methods, and it was found that mixtures of these two phospholipids exhibit almost ideal miscibility over the whole composition range (Shimshick & McConnell, 1973b; Blume & Ackermann, 1974; Lee, 1975). Addition of cholesterol to an equimolar mixture of DPPC and DPPE up to 20 mol % results in an increase in width and a slight decrease in the temperature of the heat capacity maximum. Further addition of cholesterol then decreases the transition enthalpy, but the width and the temperature of the peak maximum remain unchanged (see Figures 5 and 6). A decomposition of the calorimetric scans into two different transitions may be possible.

DMPE–DSPC–Cholesterol. DMPE–DSPC mixtures show a mixing behavior which is definitely not ideal and arises from the differences in head group structure and the different chain

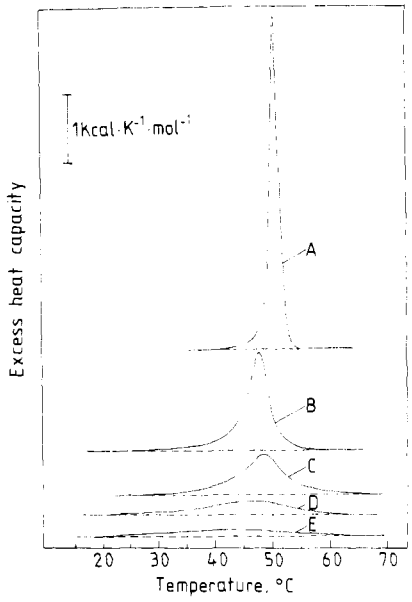

FIGURE 7: Excess heat capacity curves for DMPE–DSPC–cholesterol mixtures with (A) 0, (B) 10, (C) 20, (D) 30, and (E) 40 mol % cholesterol.

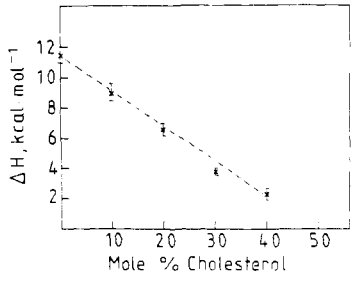

FIGURE 8: Plot of total transition enthalpy (kilocalories per mole of phospholipid) of DMPE–DSPC–cholesterol mixtures vs. mole percent cholesterol.

lengths of the two lipids (Blume & Ackermann, 1974; Lee, 1978). The nonideality is probably greater for the gel phase than for the liquid-crystalline phase (Lee, 1978). This was also observed for phosphatidylcholines with unequal chain lengths, and it was assumed that repulsive interactions between unlike molecules might be the reason for this behavior (von Dreele, 1978). With a decrease of the repulsive interactions in the liquid-crystalline phase the number of like–like lipid pairs will decrease also, increasing the degree of mixing. The high transition enthalpy of 11.5 kcal/mol for an equimolar DMPE–DSPC mixture (the mean value would be 8.6 kcal/mol) might be attributed to this process accompanying the phase transition. Similar high transition enthalpies were observed for other nonideal mixtures, for instance for DMPC–DSPC (Mabrey & Sturtevant, 1976). The addition of cholesterol to DMPE–DSPC mixtures results in a considerable broadening of the transition with a concomitant decrease of the temperature of the peak maximum. The transition enthalpy decreases almost linearly. Mixtures with 50 mol % cholesterol show no transition (see Figures 7 and 8).

DLPE–DSPC–Cholesterol. The DLPE–DSPC mixing behavior is certainly nonideal due to the great difference in fatty acid chain length. However, it is not monotectic; i.e., in an equimolar mixture separate peaks for the transitions of the two lipids are not observed (see Figure 9). The transition is highly asymmetric with the peak maximum at the high temperature side. The transition enthalpy for an equimolar mixture amounts to 7.8 kcal/mol. This is not much larger than

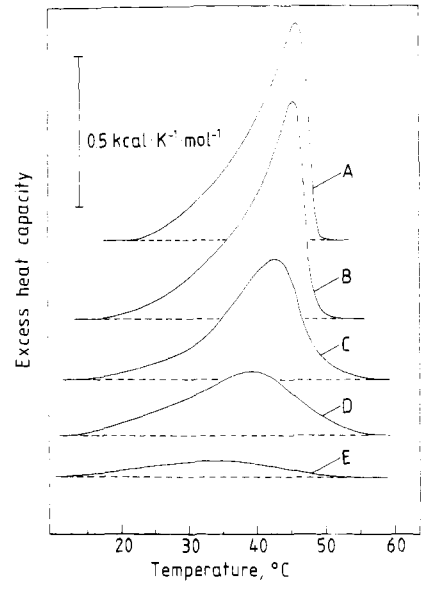

FIGURE 9: Excess heat capacity curves for DLPE–DSPC–cholesterol mixtures with (A) 0, (B) 10, (C) 20, (D) 30, and (E) 40 mol % cholesterol.

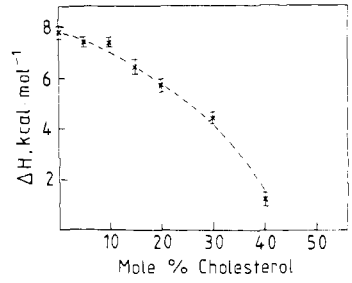

FIGURE 10: Plot of total transition enthalpy (kilocalories per mole of phospholipid) of DLPE–DSPC–cholesterol mixtures vs. mole percent cholesterol.

the calculated mean value of 7.4 kcal/mol. The difference in the nonideality between the gel and the liquid-crystalline phase seems for this system to be smaller than that for the DMPE–DSPC mixture. Addition of cholesterol up to 10 mol % leads only to a slight change in the form of the calorimetric curve. The transition enthalpy remains almost unaffected. The addition of 20 mol % cholesterol or more then finally leads to the usually observed broadening of the transition, the lowering of the temperature of the peak maximum, and the decrease in the transition enthalpy (see Figures 9 and 10).

Discussion

Phosphatidylethanolamine–Cholesterol Mixtures. Phosphatidylethanolamines have higher transition temperatures than the corresponding phosphatidylcholines, but the transition enthalpies are nearly the same (Blume & Ackermann, 1974; Mabrey & Sturtevant, 1976). The higher transition temperatures are thought to be due to the formation of intermolecular hydrogen bonds between the NH_3^+ protons of the PE head group and the neighboring phosphate oxygens as proven for crystalline DLPE (Hitchcock et al., 1974). These interactions are also responsible for the different thermal behavior of PE–cholesterol mixtures as compared to the PC–cholesterol system. We could confirm the general observation by van Dijck et al. (1976a) that cholesterol decreases the transition enthalpy of DMPE; however, even beyond a cholesterol content of 33 mol % a transition could be detected with our calorimeter. So in this respect the behavior of PE–cholesterol mixtures is similar to those of PC– and sphingomyelin–cholesterol

mixtures. Similarly, the scans of mixtures with less than 25 mol % cholesterol seem to consist of two different transitions, a narrower one close to the transition temperature of pure PE, which is slightly shifted to lower temperature with increasing cholesterol content, and a broad one, which in contrast to PC–cholesterol mixtures, however, is at lower temperature. According to Estep et al. (1978) the broad peak can be due either to a transition of cholesterol-rich domains or to a transition of the boundary regions surrounding these domains. Therefore, these authors proposed that phase separation occurs in PC–cholesterol mixtures. However, the calorimetric data can also be explained by assuming a homogeneous distribution of cholesterol in the lipid matrix when those lipid acyl chains in contact with only one cholesterol molecule still can undergo a transition with low cooperativity and reduced transition enthalpy (Cornell et al., 1979). Measurements of the diffusion coefficient of a fluorescent probe (Rubinstein et al., 1979) and the freeze-fracture studies by Verkleij et al. (1974) support this latter explanation. Also recent deuterium NMR measurements of PC–cholesterol mixtures in the liquid-crystalline phase (Haberkorn et al., 1977; Jacobs & Oldfield, 1979) and in the gel phase (R. G. Griffin, personal communication) reveal only one signal for the specifically deuterated PC. The existence of two phases, however, should lead to two different signals, at least in the gel state, where the diffusion is slow. We think that also in PE–cholesterol mixtures there is no phase separation below the transition temperature. That the broad transition in these mixtures is at lower temperature can be explained by taking into account the intermolecular hydrogen bonds responsible for the higher transition temperature of the pure PE. The formation of these hydrogen bonds is certainly perturbed in the vicinity of the cholesterol molecules so that the transition arising from those lipids in contact with cholesterol is shifted to lower temperature. Even those molecules not in contact with cholesterol seem to be slightly influenced because their transition is also broadened and shifted to lower temperature (see Figures 1 and 3).

Phosphatidylcholine–Phosphatidylethanolamine–Cholesterol Mixtures. van Dijck et al. (1976a) found a preferential affinity of cholesterol for PC in PC–PE mixtures which show monotectic behavior. If this were also true for the nearly ideally mixed DPPC–DPPE system, we would expect a progressive increase of the temperature of the heat capacity maximum, because the remaining lipid population would be enriched with the higher melting DPPE. However, we observed that the peak maximum is shifted to lower temperature with increasing cholesterol content. Apparently cholesterol shows no preferential affinity for DPPC in this system. A random arrangement seems to be more likely. The asymmetry of the calorimetric curves at low cholesterol content may be an indication that again two transitions are superimposed.

We also studied the influence of cholesterol on DMPE–DSPC and DLPE–DSPC mixtures which both show nonideal mixing behavior due to the differences in acyl chain lengths. A preferential association of cholesterol with PC should again lead to the appearance of a calorimetric peak belonging to the less perturbed PE. This especially should be clearly visible in the DLPE–DSPC system because of the large difference (23 °C) in transition temperature of the two lipids, whereas DMPE and DSPC melt almost at the same temperature. However, in both mixtures the calorigrams show no peak which can be attributed to less perturbed DMPE or DLPE, respectively.

Summarizing our results, we propose that PE–cholesterol mixtures are homogeneous in the liquid-crystalline state as well as in the gel state, but they are certainly not ideal. The differences in the calorimetric scans of DMPE– and DPPE–cholesterol mixtures (see Figure 1 and 3) may be attributed to slight variations in the nonideality. In the ternary PE–PC–cholesterol systems the nonideality of the phospholipid mixture itself and the interaction of cholesterol with two different types of phospholipids have to be considered. Because these interactions obviously depend on the acyl chain length as well as on the nature of the polar group of the phospholipid, a complex mixing behavior is observed. The interpretation of the calorimetric scans of these mixtures is complicated by the fact that the transition temperature of PE alone is reduced drastically by the addition of cholesterol due to the perturbation of the intermolecular hydrogen bonds. We cannot, however, detect any preferential affinity of cholesterol for either PC or PE in the mixtures and propose that they are homogeneous but nonideal at all cholesterol concentrations investigated.

The phospholipids of eukaryotic membranes, for instance, erythrocyte membranes, are a complex mixture of different classes with a wide distribution in the fatty acid composition. The extracted phospholipids without cholesterol show continuous transitions and no monotectic behavior (van Dijck et al., 1976b). In view of our results and those of Calhoun & Shipley (1979) for sphingomyelin–PC–cholesterol mixtures, it is questionable whether the preferential association of cholesterol in monotectic mixtures has any biological relevance. The saturated synthetic phospholipids that we and Calhoun and Shipley used for our model systems are certainly different from those of biological membranes, which are mostly unsaturated. We would expect, though, that mixtures of biological phospholipids behave similarly, as long as they are homogeneous and not monotectic.

Acknowledgments

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References

Bartlett, G. R. (1959) *J. Biol. Chem. 234*, 466.
Blume, A., & Ackermann, Th. (1974) *FEBS Lett. 43*, 71.
Calhoun, W. I., & Shipley, G. G. (1979) *Biochemistry 18*, 1717.
Cornell, B. A., Chapman, D., & Peel, W. E. (1979) *Chem. Phys. Lipids 23*, 223.
Darke, A., Finer, E. G., Flook, A. G., & Phillips, M. C. (1972) *J. Mol. Biol. 63*, 265.
de Kruijff, B., Demel, R. A., & van Deenen, L. L. M. (1972) *Biochim. Biophys. Acta 255*, 331.
de Kruijff, B., Demel, R. A., Slotboom, A. J., van Deenen, L. L. M., & Rosenthal, A. F. (1973) *Biochim. Biophys. Acta 307*, 1.
de Kruijff, B., van Dijck, P. W. M., Demel, R. A., Schuijff, A., Brants, F., & van Deenen, L. L. M. (1974) *Biochim. Biophys. Acta 356*, 1.
Demel, R. A., & de Kruijff, B. (1976) *Biochim. Biophys. Acta 457*, 109.
Demel, R. A., Jansen, J. W. C. M., van Dijck, P. W. M., & van Deenen, L. L. M. (1977) *Biochim. Biophys. Acta 465*, 1.
Engelman, D. M., & Rothman, J. E. (1972) *J. Biol. Chem. 247*, 3694.
Estep, T. N., Mountcastle, D. B., Biltonen, R. L., & Thompson, T. E. (1978) *Biochemistry 17*, 1984.
Estep, T. N., Mountcastle, D. B., Barenholz, Y., Biltonen, R. L., & Thompson, T. E. (1979) *Biochemistry 18*, 2112.

Haberkorn, R. A., Griffin, R. G., Meadows, M. D., & Oldfield, E. (1977) *J. Am. Chem. Soc. 99*, 7353.
Hinz, H. J., & Sturtevant, J. M. (1972) *J. Biol. Chem. 247*, 3697.
Hitchcock, P. B., Mason, R., Thomas, K. M., & Shipley, G. G. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 3036.
Jacobs, R., & Oldfield, E. (1979) *Biochemistry 18*, 3280.
Ladbrooke, B. D., Williams, R. M., & Chapman, D. (1968) *Biochim. Biophys. Acta 150*, 333.
Lee, A. G. (1975) *Biochim. Biophys. Acta 413*, 11.
Lee, A. G. (1978) *Biochim. Biophys. Acta 507*, 433.
Mabrey, S., & Sturtevant, J. M. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 3862.
Mabrey, S., Mateo, P. L., & Sturtevant, J. M. (1978) *Biochemistry 17*, 2464.
Owicki, J. C., & McConnell, H. M. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 4750.
Phillips, M. C., & Finer, E. G. (1974) *Biochim. Biophys. Acta 356*, 199.
Privalov, P., Plotnikov, V. V., & Filimonov, V. V. (1975) *J. Chem. Thermodyn. 7*, 41.
Rouser, G., Nelson, G. J., Fleischer, S., & Simon, G. (1968) in *Biological Membranes* (Chapman, D., Ed.) pp 5–69, Academic Press, London.
Rubinstein, J. L. R., Smith, B. A., & McConnell, H. M. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 15.
Shimshick, E. J., & McConnell, H. M. (1973a) *Biochem. Biophys. Res. Commun. 53*, 446.
Shimshick, E. J., & McConnell, H. M. (1973b) *Biochemistry 12*, 2351.
Tsong, T. Y. (1975) *Biochemistry 14*, 5415.
van Dijck, P. W. M. (1979) *Biochim. Biophys. Acta 555*, 89.
van Dijck, P. W. M., de Kruijff, B., van Deenen, L. L. M., de Gier, J., & Demel, R. A. (1976a) *Biochim. Biophys. Acta 455*, 576.
van Dijck, P. W. M., van Zoelen, E. J. J., Seldenrijk, R., van Deenen, L. L. M., & de Gier, J. (1976b) *Chem. Phys. Lipids 17*, 336.
Verkleij, A. J., Ververgaert, P. H., de Kruijff, B., & van Deenen, L. L. M. (1974) *Biochim. Biophys. Acta 373*, 495.
von Dreele, P. H. (1978) *Biochemistry 17*, 3939.

Binding of Heavy Meromyosin and Subfragment-1 to Thin Filaments in Myofibrils and Single Muscle Fibers†

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ABSTRACT: The binding of fluorescently labeled heavy meromyosin (HMM) and heavy meromyosin subfragment-1 (S-1) to thin filaments of myofibrils and of rabbit psoas muscle fibers was measured under conditions of rigor and contraction. The fragments diffused rapidly into the myofibrillar space and bound specifically to the thin filaments. The fragments bound strongest and in a uniform fashion to myofibrils in which the competition from indigenous myosin was abolished by removing it with Hasselbach–Schneider solution. Under these conditions, the rigor K_a values for HMM and S-1 were 1.5×10^6 M^{-1} and 4.8×10^4 M^{-1}, respectively. The stoichiometry of binding was measured by independently estimating the concentration of actin sites. S-1 was found to be capable of saturating all available actin sites in a myofibril or a fiber, but HMM could only occupy 50% of the sites.

The study of the binding of myosin fragments to actin has attracted attention for two important reasons. First, association of the myosin–product complex with actin constitutes one of the intermediate steps in the actomyosin ATPase cycle. In addition, binding studied yield information about the interaction between the two heads of the myosin molecule and about the role such interactions (if any) play in the contractile process (Taylor, 1977; Tonomura & Inoue, 1977). To date, the results of these investigations have suggested the values for the rigor binding to actin of the single-headed fragment of myosin (S-1)[1] (Margossian & Lowey, 1975, 1976; Marston & Weber, 1975; Highsmith et al., 1976) and of the double-headed fragment HMM (Takeuchi & Tonomura, 1971; Eisenberg et al., 1972; Margossian & Lowey, 1973; Highsmith, 1978) but were unable to determine unambiguously whether the interactions between the heads occur (Highsmith, 1978; Greene & Eisenberg, 1980). All these studies have been carried out in solution, taking advantage of the fact that myosin fragments are soluble at low ionic strength. In muscle, however, actin filaments are arranged in a well-defined spatial configuration with fixed interfilament distances, where steric effects may be important in the binding of myosin heads. Further, in vitro experiments uniformly utilized purified actin preparations, and it is not at all clear what effect the presence of the regulatory proteins tropomyosin and troponin might have on the affinity of a cross bridge to actin. Tropomyosin makes actomyosin interactions cooperative (Bremel et al., 1972) presumably through propagated conformational change in the actin molecules (Weber & Murray, 1973), and it is conceivable

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[1] Abbreviations used: HMM, heavy meromyosin; S-1, heavy meromyosin subfragment-1; EGTA, 2,2′-ethylenedioxybis(ethyliminodiacetic acid); IAF, 5-iodoacetamidofluorescein; 1,5-IAEDANS, *N*-(iodoacetyl)-*N*′-(1-sulfo-5-naphthyl)ethylenediamine; $NaDodSO_4$, sodium dodecyl sulfate; H-S, Hasselbach–Schneider solution; P_i, inorganic phosphate.

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