Raman Spectroscopy of the Thermal Properties of Reassembled High-Density Lipoprotein: Apolipoprotein A-I Complexes of Dimyristoylphosphatidylcholine†

Thomas Gilman, John W. Kauffman,* and Henry J. Pownall

ABSTRACT: Isolated complexes of apolipoprotein A-I (apoA-I), the major apoprotein of human plasma high-density lipoproteins, and dimyristoylphosphatidylcholine (DMPC) have been prepared and studied by differential scanning calorimetry (DSC) and Raman spectroscopy. DSC studies establish that complexes having lipid to protein ratios of 200, 100, and 50 to 1 each exhibit a broad reversible thermal transition at T_c = 27 °C. The enthalpy of lipid melting for each of the three complexes is about 3 kcal/mol of DMPC. Raman spectroscopy indicates that the physical state of lipid molecules in the complexes is different from that in DMPC multilamellar liposomes. Analysis of the C–H stretching region (2800–3000 cm^{-1}) of the complexes and of the pure components in water suggests that below 24 °C (T_c for DMPC) there is considerably less lateral order among lipid acyl chains in the complexes than in DMPC liposomes. Above 24 °C, these types of interactions appear to contribute equally or slightly less to the complex structure than in pure DMPC. The temperature dependence of peaks in the C–C stretching region (1000–1180 cm^{-1}) reveals a continuous increase in the number of lipid acyl chain C–C gauche isomers over a broad range with increasing temperature. Compared to liposomes, DMPC in the complexes has more acyl chain trans isomers at temperatures above 24 °C; at temperatures above ca. 30 °C, trans isomer content is about the same for complexes and liposomes. A large change was observed in a protein vibrational band at 1340 cm^{-1} for pure vs. complexed apoA-I, indicating that protein hydrocarbon side chains are immobilized by lipid binding. The Raman data indicate that the reduction in melting enthalpy for complexed DMPC (~3 kcal/mol) compared to that for free DMPC (~6 kcal/mol) is due to reduced van der Waals interactions in the low-temperature lipid phase.

The human plasma lipoproteins have multiple metabolic roles, including lipid transport, activation of lipid-metabolizing enzymes, and control of lipid synthesis (Smith et al., 1978; Jackson et al., 1976; Morrisett et al., 1977). Within this context, considerable attention has been focused upon the correlation of physical structure and biological function of both real and model lipoproteins. The model lipid–protein system composed of apolipoprotein A-I (apoA-I) from human plasma high-density lipoprotein and dimyristoylphosphatidylcholine (DMPC)[1] has been investigated more thoroughly than any other lipid–apolipoprotein system. DMPC is a saturated lecithin having thermal transitions at 13.5 and 23.9 °C (Mabrey & Sturtevant, 1976). It has now been shown in many laboratories that apoA-I spontaneously associates with DMPC single or multibilayer vesicles, giving small disklike structures (Middelhoff et al., 1976; Pownall et al., 1978; Jonas et al., 1977; Jonas & Krajnovich, 1977; Tall et al., 1977). These investigations have produced a better understanding of the mechanism and dynamics of lipid–apolipoprotein association. The apoA-I–DMPC complexes have a variable stoichiometry (Tall et al., 1977) and can be isolated by gel filtration chromatography (Jonas et al., 1977; Pownall et al., 1978), or by centrifugation in a salt gradient (Tall & Lange, 1978). The properties of the isolated complexes have been investigated. Jonas et al. (1977) reported the isolation of a 235 000-dalton complex containing about 90 lipid molecules for each molecule of apoA-I. Although a bilayer arrangement of the lipids in this complex was excluded on the basis of its small size, the temperature dependence of the depolarization of a fluorescent probe, 1,6-diphenyl-1,3,5-hexatriene, revealed the persistence of a broad thermal transition at a temperature that was slightly higher than that of pure DMPC. On the basis of this finding, they concluded that the isolated complex retained some of the characteristics of the DMPC bilayer from which it was derived. Tall et al. (1977) measured the thermal transition of DMPC–apoA-I complexes directly by differential scanning calorimetry (DSC). They found that relative to pure DMPC the main transition of the complex was elevated and its corresponding enthalpy was greatly reduced. The pretransition of DMPC was not observed in the complexes.

We have been interested in the structure of DMPC–ApoA-I complexes and have recently used Raman spectroscopy to obtain a better description of the structure of lipid and protein molecules in lipoproteins. As pointed out by Pink et al. (1980), Raman spectroscopy has some special advantages for the study of lipid–protein interactions. It does not require the use of probe molecules, and it provides information on lipid chain conformation that is a short-time average (~10^{-13} s), thus avoiding the ambiguities of magnetic resonance techniques. It also provides unique information about lipid packing (Gaber & Peticolas, 1977) and about protein backbone and side-chain structure (Spiro & Gaber, 1977).

Raman spectroscopy has been previously applied to model lipid–polypeptide systems (Lis et al., 1976a,b; Verma & Wallach, 1976a; Chapman et al., 1977; Susi et al., 1979) and to lipid–protein systems (Verma & Wallach, 1976b; Goheen et al., 1977; Mikkelsen et al., 1978; Rothschild et al., 1976;

† From the Biomedical Engineering Center, Northwestern University, The Technological Institute, Evanston, Illinois 60201 (T.G. and J.W.K.), and the Department of Medicine, Baylor College of Medicine and The Methodist Hospital, Houston, Texas 77030 (H.J.P). *Received July 12, 1979.* Supported by the Atherosclerosis, Lipids and Lipoproteins Section of the National Heart and Blood Vessel Research and Demonstration Center, Baylor College of Medicine, a grant-supported research project of the National Heart, Lung and Blood Institute, National Institutes of Health (Grant 17269), by the American Heart Association, and by a grant from the National Institutes of Health (HL 19459). H.J.P. is an Established Investigator of the American Heart Association.

[1] Abbreviations used: T_c, gel–liquid-crystalline transition temperature; DMPC, dimyristoylphosphatidylcholine.

0006-2960/81/0420-0656$01.00/0

Milanovich et al., 1976; Curatolo et al., 1978). This report describes the first Raman scattering study of an apolipoprotein–lipid complex.

Materials and Methods

Materials. Human plasma high-density lipoproteins (HDL) from normal subjects were isolated by the density flotation method of Scanu (1966). After removal of the KBr by dialysis, the HDL were lyophilized and delipidated at 4 °C by multiple extractions with diethyl ether/ethanol (3:1). After the mixture was dried, the apoHDL (~250 mg) was solubilized in 6 M guanidine hydrochloride and applied to a 2.5 × 190 cm column of Sephadex G-150. The column was eluted with a buffer of 3.0 M guanidine hydrochloride, 0.1 M Tris, pH 7.4, and 0.01% EDTA. The central portions of apoA-I fractions from two purifications were pooled, concentrated, and rechromatographed. The central portion of the apoA-I peak was combined; these fractions were devoid of other protein components as verified by amino acid analysis and the absence of reactivity to antibodies against human serum albumin, low-density lipoprotein, and apoA-I. Only one band appeared in sodium dodecyl sulfate–polyacrylamide gel electrophoresis even when the gels were overloaded.

DMPC was prepared by the method of Cubero Robles & van den Berg (1969) and purified by preparative high-pressure liquid chromatography on a Waters LC 500 liquid chromatography system (Patel & Sparrow, 1978). Thin-layer chromatography of the phospholipid on silica gel plates eluted with chloroform/methanol/water (65:25:4) gave only one spot as visualized by a phospholipid-specific spray (Dittmer & Lester, 1964) and by charring. Gas chromatographic analysis of the methyl esters obtained by transesterification of DMPC showed the ester composition to be >99% methyl myristate. All buffer salts were obtained from Fisher Scientific.

Sample Preparation. Liposomes were prepared at 30 °C by vortexing 200 mg of DMPC in 1 mL of buffer (150 mM NaCl and 0.010 M Tris, pH 7.4). Complexes of DMPC and apoA-I were formed by incubation of 100 mg of DMPC liposomes with the appropriate quantity of apoA-I in a final volume of 8 mL at 24 °C; accurate temperature control is essential (Pownall et al., 1978, 1979). After the sample obtained complete optical clarity (~1 h), it was concentrated to 1 mL and chromatographed on Sepharose CL 4B as previously described (Pownall et al., 1978). At average molar ratios of DMPC to apoA-I between 200 and 45 to 1, all lipid and protein coeluted as a single peak. The sample was concentrated to about 0.7 mL by placing it in a dialysis bag and covering it with dry Sephadex G-150. As the sample volume decreased, fresh Sephadex was placed around the bag, and the bag was knotted into a decreasingly smaller bag to prevent the sample from drying out. The composition and concentration of the complex were determined from analysis of the phosphorus content (Bartlett, 1959) and protein absorbance at 280 nm (ϵ = 32 500 M^{-1} cm^{-1}) of a diluted aliquot. A typical sample contained 120–150 mg of complex.

Differential Scanning Calorimetry. DSC was performed on a Perkin-Elmer DSC-2 calorimeter equipped with a subambient cooling accessory. A 65-μL sample of the complex was placed in a 75-μL capacity stainless-steel sample pan that was sealed and placed in the sample compartment of the calorimeter. A duplicate pan in which 65 μL of water was substituted for the sample was placed in the reference compartment. The DSC traces were measured at a scan rate of 5 °C/min and a sensitivity of 1 mcal/s. Duplicate samples from at least three preparations of each complex were scanned from 0 to 90 °C. The calorimeter was calibrated with an indium standard, and the enthalpy of melting of the complex was obtained by comparison of the areas under the endotherms of the sample with those of indium.

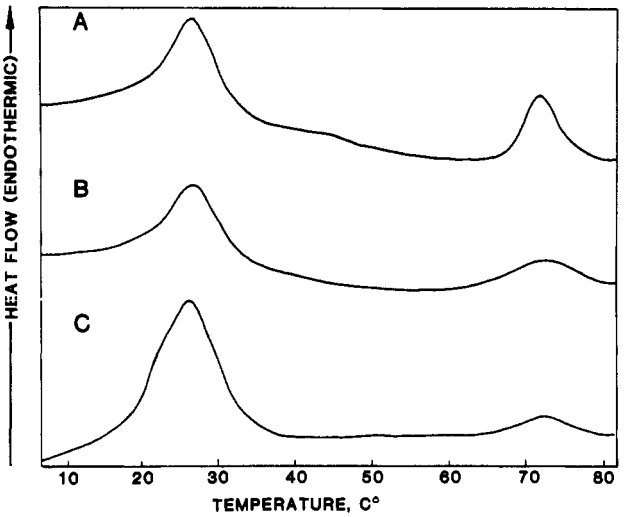

FIGURE 1: DSC traces of isolated complexes of DMPC and apoA-I having various lipid/protein ratios. (A) 50:1, 155 mg/mL DMPC; (B) 100:1, 108 mg/mL DMPC; (C) 200:1, 158 mg/mL DMPC. All samples were 0.065 mL and were run against an equal volume of water at a scan rate of 5 °C/min and a sensitivity of 1.0 mcal/s.

Table I: Calorimetric Parameters for Complexes of DMPC with ApoA-I[a]

complex stoichiometry	T_c^1 (°C)	ΔH_1 (kcal/mol of DMPC)	T_c^2 (°C)	ΔH_2 (kcal/mol of apoA-I)
50:1	27	3.3	74	94
100:1	27	3.4	75	110
200:1	27	3.7	74	95

[a] Error in temperatures is ±0.25 °C; estimated error in enthalpy measurements is ±15%. Temperatures were extrapolated to zero scan speed, a correction of less than 1 °C.

Raman Spectroscopy. The Raman spectrometer detection system consisted of a Spex 1401 double monochromator, an RCA C31034 Ga–As photomultiplier, and modified Spex counting electronics interfaced to a Nova 2 computer. Excitation was achieved with an Ar ion laser (Spectra Physics 164) tuned to either 514.5 or 488.0 nm. Laser power at the sample did not exceed 200 mW. Samples were enclosed in thin-walled quartz capillaries of 2-mm diameter and examined in 180° backscattering geometry with a line-focused beam. Temperature was controlled in a brass-lined chamber equilibrated with a thermostated fluid flowing through internal coils. Temperatures were corrected for laser heating by adding 1.5 °C, the difference between the melting temperature of DMPC by Raman intensity ratios and that measured by differential scanning calorimetry. In some cases, a buffer spectrum was subtracted from sample spectra. Scale factors were computed by using the region 2800–3200 cm^{-1}. The spectral resolution of the spectrometer was 6 cm^{-1}.

Results

Calorimetry. The calorimetric behavior of isolated complexes of DMPC and apoA-I having molar ratios of 50, 100, and 200 to 1 is shown in Figure 1. All three complexes exhibit a broad reversible endotherm which is maximal at T_c^1 ~27 °C. The enthalpies (ΔH_1) of these transitions are nearly the same in all three complexes (Table I). In the 50:1 complex (Figure 1A), another smaller transition at 45 °C is also observed; this transition is absent in the 100:1 and 200:1 com-

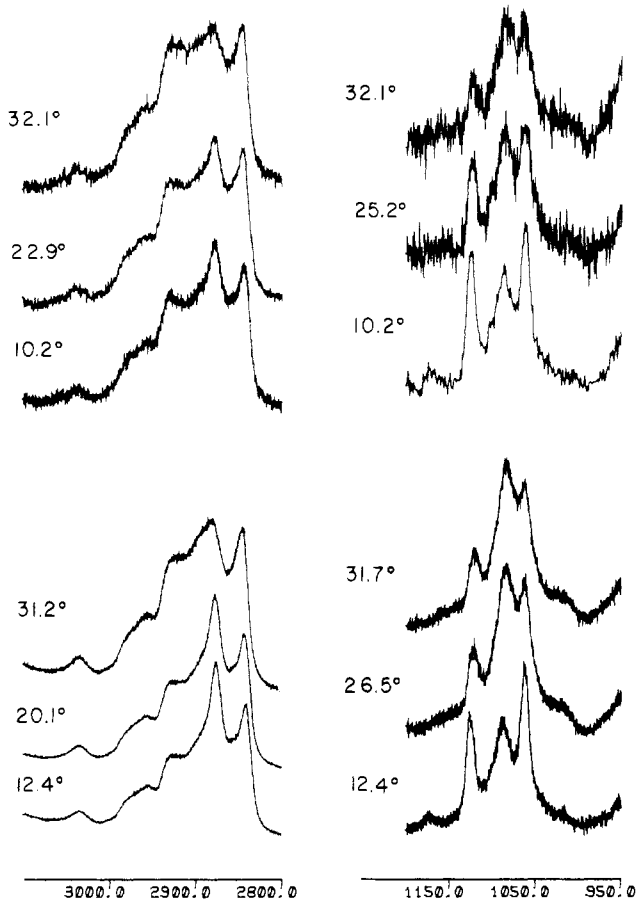

FIGURE 2: Typical Raman spectra: (upper left) 200:1 DMPC–apoA-I complex in the region 2800–3150 cm^{-1}; (upper right) 200:1 DMPC–apo-AI complex in the region 950–1200 cm^{-1}; (lower left) DMPC in the region 2800–3150 cm^{-1}; (lower right) DMPC in the region 950–1200 cm^{-1}. The temperatures at which the spectra were recorded are given to the left of each curve.

plexes. All three complexes exhibit an additional transition (T_c^2 ~74 °C). The temperatures and enthalpies of this transition are within experimental error of each other in all three complexes.

Raman Results Relevant to Lipid Structure. Results in this section are confined to those obtained on the sample with a high lipid/protein ratio (200:1). In this complex, underlying protein signal is minimal, and signal to noise ratio for the lipid peaks of interest to us is maximal. Two regions of the Raman spectrum of DMPC and of DMPC–ApoA-I complexes are sensitive to the structural changes accompanying the lipid phase transition. Figure 2 shows typical spectra of these regions, 950–1200 and 2800–3150 cm^{-1}.

In the region 2800–3150 cm^{-1}, differences between lipid and lipid complexed with protein are most apparent in the spectra below 24 °C. The parameter most often used to quantify changes in this spectral region, i.e., the ratio of peak intensity at 2880 cm^{-1} to that at 2850 cm^{-1}, is used in this analysis. From the plot in Figure 3A, we see that (a) I_{2880}/I_{2850} is significantly lower for the complex than for DMPC liposomes at temperatures below 24 °C, (b) the net change in the ratio from 20 to 30 °C is much smaller in the complex than in liposomes, and (c) the ratios for apoA-I–DMPC complexes and DMPC liposomes are nearly equal above 24 °C.

In the 950–1200 cm^{-1} region, differences between the spectra of pure lipid and those of complexed lipid are greatest at temperatures above 24 °C. We compare these spectra by using a ratio of the peak intensity at about 1130 cm^{-1} to that at about 1090 cm^{-1}. This ratio is plotted vs. temperature in

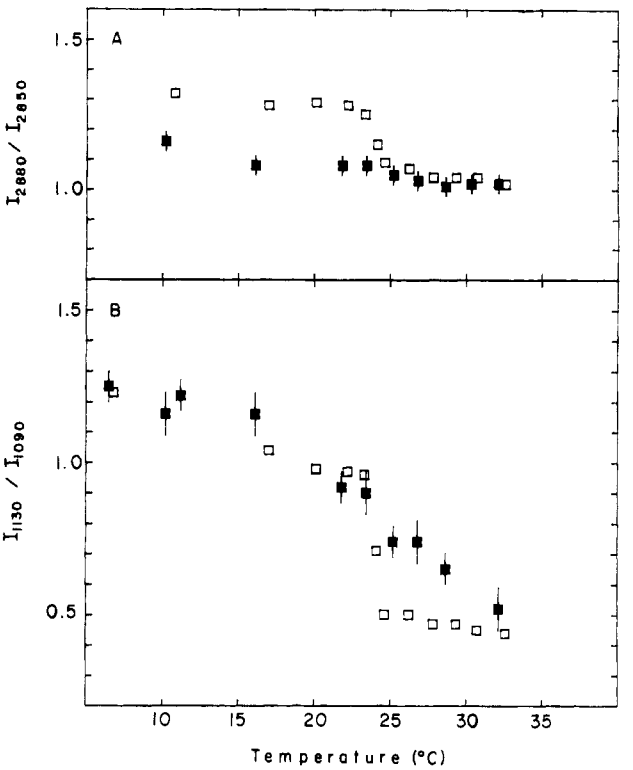

FIGURE 3: Raman intensity ratios for 200:1 DMPC–apoA-I complexes and for pure DMPC. Intensities were measured as peak height above background at the peak maximum (thus, the frequency values identifying peaks are approximate values). (A) I_{2880}/I_{2850} vs. temperature; (B) I_{1130}/I_{1090} vs. temperature.

Table II: Intensity Ratios of Lipid–Protein Complexes

sample	temp (°C)	I_{1340}/I_{1650}[a]
apoA-I in buffer	room temp	0.7
50:1 complex	28.2	1.5
50:1 complex	16.2	1.7
100:1 complex	30.6	1.5
100:1 complex	14.7	1.7

[a] This ratio was computed for spectra in which the buffer Raman spectrum had been subtracted out. The scaling factor for subtraction was determined by using the strong water background in the region 2800–3200 cm^{-1}.

Figure 3B. From this figure, we see that (a) the ratios for pure lipid and for complexes are similar below 24 °C, and (b) the ratio is significantly higher in the lipid–protein complex at temperatures just above 24 °C, but (c) the net change in this ratio over the temperature range 20–35 °C is about the same for complexed and free lipid.

The measured intensity ratios, of course, represent averages over all lipid present. In most cases, we were not able to detect, within our experimental error, any changes in Figure 3 as the lipid–protein stoichiometry was changed. An exception was changes in 3A at the highest protein content, which were obviously due to the presence of underlying protein signal.

Raman Results Relevant to Protein Structure. The Raman spectrum of apoA-I in buffer is shown in Figures 4 and 5. Intensity at 935 cm^{-1} and at ca. 1300 cm^{-1} has been assigned to an α-helical backbone conformation (Lippert et al., 1976; Spiro & Gaber, 1977). Intensities at lower frequencies within the amide III region (less than 1300 cm^{-1}) indicate the presence of other conformations as well. A promiment feature in the spectrum is the broad band at 1340 cm^{-1}. This peak is very different in the Raman spectrum of lipid–protein complexes. In terms of peak intensities, it increases by more

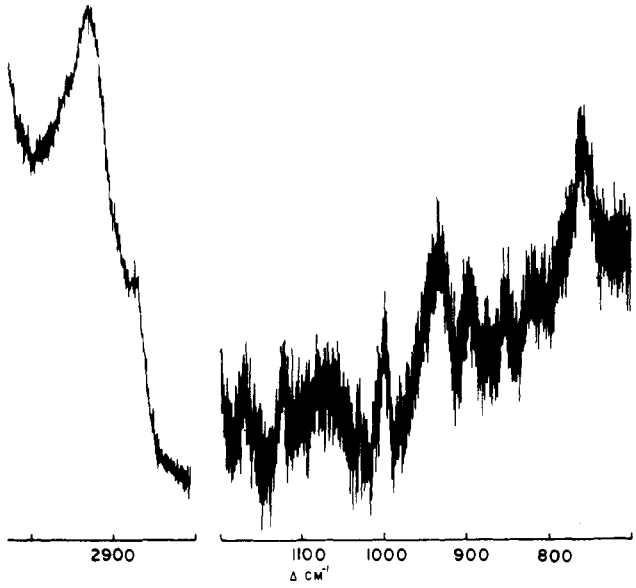

FIGURE 4: Raman spectrum of apoA-I (50 mg/mL) in standard buffer for the regions 700–1200 and 2800–3150 cm^{-1}. These are both from the same spectrum with the ordinate expanded in 700–1200 cm^{-1} to show the details of the weak scattering in this region. The steeply rising base line for 2800–3150 cm^{-1} is due to water scattering.

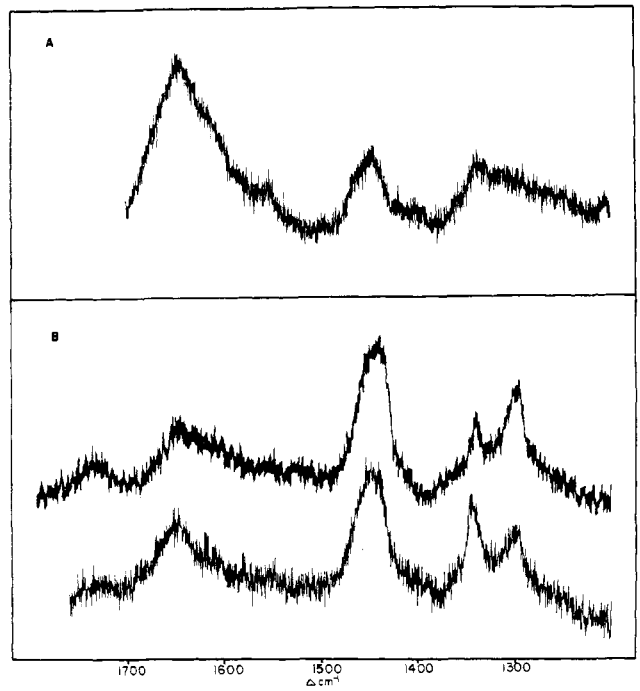

FIGURE 5: Raman spectra of pure apoA-I (A) and of apoA-I–DMPC complexes (B) in the region 1200–1700 cm^{-1}. In (B), the top spectrum is the 100:1 lipid–protein complex at 30.6 °C; the bottom spectrum is the 50:1 lipid–protein complex at 28.2 °C. The (A) trace was recorded at room temperature and is from the same spectrum as that in Figure 4.

than a factor of two relative to amide I, as detailed in Table II. This increase in peak intensity is probably due in part, possibly due entirely, to a narrowing of the peak. In the lipid–protein complexes, this peak has a width of less than 20 cm^{-1}. Though it is difficult to quantify the peak width in the pure apoA-I spectrum, the spectrum in Figure 5A is consistent with a peak of 40-cm^{-1} width at half-height at 1340 cm^{-1}.

Discussion

Calorimetric Studies of ApoA-I–DMPC Complexes. The thermal properties of the complexes formed by DMPC and apoA-I or apoHDL have been previously reported (Tall & Lange, 1978; Tall et al., 1977). The major features of our data are in agreement with Tall's studies. These are (a) melting of the DMPC acyl chains in isolated complexes at 27 °C; (b) a lower enthalpy of melting of DMPC in complexes than in multibilayer liposomes; and (c) unfolding of apoA-I between 40 and 60 °C. Only in the 50:1 complex did we observe the endotherm at about 45–55 °C, which is characteristic of free apoA-I. Therefore, in this complex, a small fraction of the apoA-I is not associated with DMPC.

In the 200:1 complex, there is a very weak shoulder on the melting endotherm at 24 °C. This indicates the presence of free DMPC. Thus, the full range of possible stoichiometries has been covered, from lipid-rich complexes, where addition of more lipid causes free lipid to be present in equilibrium with the complexes, to protein-rich complexes, where free protein begins to be found (Tall et al., 1977). The amounts of free protein and free lipid present in the 50:1 and the 200:1 complexes, respectively, are quite small relative to the protein or lipid present in the complexes, and, thus, our conclusions about complexed protein and lipid from Raman studies of these stoichiometries are not affected.

Within experimental error, the temperatures and enthalpies of lipid melting (Table I) are not much different in the 50:1, 100:1, and 200:1 complexes. This finding suggests that the DMPC in all three complexes is in a similar physical state. Similarly, the small differences in the temperatures and enthalpies of complex denaturation suggest that the protein is in the same physical state in all three complexes.

Effect of ApoA-I on the Structure of DMPC. The Raman peak at ca. 1130 cm^{-1} has been assigned to carbon–carbon stretching of all trans segments of the acyl chains while the intensity at 1090 cm^{-1} is thought to be due mainly to C–C vibrations of gauche isomers. On this basis, it has been suggested that the ratio I_{1130}/I_{1090} represents the ratio of trans to gauche conformers present in the lipid hydrocarbon chains (Yellin & Levin, 1977). There are several difficulties in applying this correlation in a quantitative sense [see, e.g., Pink et al. (1980)]. However, the qualitative conclusions made here should be valid since a monotonic decrease in the ratio with increasing gauche conformers is generally accepted. From Figure 5B, we conclude that (a) the trans content for lipid molecules in the complexes and in liposomes is about equal at temperatures below 24 °C, (b) free lipid undergoes an abrupt transition at 24 °C during which many new gauche rotamers are formed; for lipid in the complexes, there is a gradual increase in the number of gauche rotamers with temperatures above 24 °C, and (c) the net change in trans content over the temperature range 20–35 °C is, however, about the same for complexed and free lipid.

The peak at 1065 cm^{-1} is also usually assigned to trans hydrocarbon segments. The ratio I_{1065}/I_{1090} follows the same general trend as I_{1125}/I_{1090} but would indicate less trans content at temperatures below 24 °C (data not shown, but see Figure 2). The explanation for this may lie in the mixed character of the band at 1065 cm^{-1}. In the calculations performed by Snyder (1967), the potential energy distribution for this band had a significant amount of C–H wag content in addition to the C–C stretch coordinate. The 1125-cm^{-1} band was also mixed but contained only contributions from C–C bond motions. We propose, then, that the C–H wag contribution to the 1065-cm^{-1} band is sensitive to lateral interactions among the acyl chains rather than only to trans–gauche isomerization along a single chain. This is supported by a reexamination of the data for *n*-heptadecane (Gaber et al., 1978). This

hydrocarbon can exist in two phases in which the chains are packed in a different crystal structure (different lateral interactions for the chains) while they are in an essentially all-trans conformation. Their Figure 6 shows that the phase transformation leaves the 1130-cm^{-1} band unchanged in intensity but that the 1065-cm^{-1} band decreases in intensity by about 20%. Thus, it is clear that the 1065-cm^{-1} band is sensitive to lateral interactions.

It has been well established experimentally that the ratio I_{2880}/I_{2850} is sensitive to interchain interactions of the lipid acyl chains (Larsson & Rand, 1973; Gaber & Peticolas, 1977; Gaber et al., 1978). Progress has been made in obtaining a good theoretical understanding of this sensitivity (Snyder et al., 1978; Snyder & Scherer, 1979). The ratio I_{2880}/I_{2850} decreases with both increasing interchain disorder (decreased lateral interactions) and increasing gauche rotamer content (Gaber & Peticolas, 1977). Our interpretation of I_{2880}/I_{2850} is free of complications involving gauche rotamers; as Figure 3B indicates, these are about the same for the two samples below 24 °C. Therefore, our data in Figure 3A indicate that below 24 °C molecules of DMPC in the lipid–protein complexes are packed in a disordered array compared to the gel-state phase of pure lipid.

Our results are in contrast to the behavior of DMPC in complexes with myelin proteolipid apoprotein (Curatolo et al., 1978), in which a more sharply defined change was observed in the ratio I_{2880}/I_{2850} at T_c. Below T_c, the intensity ratio was unchanged with the addition of protein; above T_c, the ratio was higher in the protein–lipid recombinants than in DMPC liposomes. Our results also indicate that the glucagon–DMPC system is not a good model for the serum lipoproteins (Taraschi & Mendelsohn, 1979). The temperature dependence of the Raman spectra of glucagon–DMPC complexes is different from that of our apoA-I–DMPC complex; moreover, structural changes which accompany the melting of the glucagon–DMPC system arise because glucagon dissociates from DMPC at temperatures above T_c (Epand et al., 1977 a,b). This is not the case for apoA-I (Pownall et al., 1978, 1979).

We note that our results are consistent with, and can be taken as support for, earlier theoretical studies on the gel to liquid crystal phase transition for saturated phosphatidylcholines (Nagle, 1973, 1976). In these studies, it was concluded that rotational isomeric changes account for less than half the total enthalpy of melting. In our lipid–protein complexes, the enthalpy per mole of lipid is reduced to half the value for free lipid while the Raman results indicate the total change in isomerization is not reduced.

Effect of Lipid–Protein Interaction on the Structure of ApoA-I. The band at 1340 cm^{-1} in protein spectra has been assigned to C–H deformation modes of protein hydrocarbon side chains, or to tryptophan (Chen et al., 1974; Carew et al., 1975; Koenig, 1975; Frushour & Koenig, 1975; Yu & East, 1975; Lin & Koenig, 1976; Chen & Lord, 1976a,b). If the large increase in peak intensity of the 1340-cm^{-1} band is due primarily to a narrowing of the peak, as we suspect, then this indicates that protein apolar residues undergo a marked decrease in conformational and motional freedom upon association with lipid. This is consistent with the mode of apoprotein interaction proposed by Segrest et al. (1974), which is based on the potential of apolipoproteins to form an amphipathic helix. In this model, it is proposed that the apolar side chains of the protein interact with the lipid apolar regions while polar side chains (on the opposite side of the amphipathic helix) remain in contact with the aqueous phase. The picture of protein apolar side chains interacting with (and being immobilized by) lipid acyl chains is consistent with our other results. One would expect such an interaction to disrupt lipid packing and to inhibit gauche isomer formation.

A molecular description of the observed calorimetric behavior of lipid–protein complexes emerges from our Raman scattering data. ApoA-I disrupts the packing of the DMPC liquid crystalline low-temperature phase, leading to less perfect lateral interactions among chains. The enthalpy of the phase transition is reduced because less heat is required to overcome the reduced lateral chain interactions. The net change in gauche isomers through the transition is about the same for complexed and pure lipid, but protein inhibits the formation of gauche isomers so that it takes place over a wider range. These effects on lipid thermal behavior may be due to interactions between protein apolar residues and the lipid acyl chains, evidenced in reduced conformational and motional freedom for the side chains.

Acknowledgments

We thank Quein Pao for her expert technical assistance, Susan McNeely for her artistic contributions, and Sarah Myers, Sharon Bonnot, and Mary Pahl for preparing the manuscript.

References

Bartlett, G. R. (1959) *J. Biol. Chem. 234*, 466.
Carew, E. G., Asher, I. M., & Stanley, H. E. (1975) *Science (Washington, D.C.) 188*, 933.
Chapman, D., Cornell, B. A., Eliasz, A. W., & Perry, A. (1977) *J. Mol. Biol. 113*, 517.
Chen, M. C., & Lord, R. C. (1976a) *J. Am. Chem. Soc. 98*, 990.
Chen, M. C., & Lord, R. C. (1976b) *Biochemistry 15*, 1889.
Chen, M. C., Lord, R. C., & Mendelsohn, R. (1974) *J. Am. Chem. Soc. 96*, 3038.
Cubero Robles, E., & van den Berg, D. (1969) *Biochim. Biophys. Acta 187*, 520.
Curatolo, W., Verma, S. P., Sakura, J. D., Small, D. M., Shipley, G. G., & Wallach, D. F. H. (1978) *Biochemistry 17*, 1802.
Dittmer, J. C., & Lester, R. L. (1964) *J. Lipid Res. 5*, 126.
Epand, R. M., Jones, A. J. S., & Shreier, S. (1977a) *Biochim. Biophys. Acta 465*, 260.
Epand, R. M., Jones, A. J. S., & Sayer, B. (1977b) *Biochemistry 16*, 4360.
Frushour, B., & Koenig, J. L. (1975) *Biopolymers 14*, 379.
Gaber, B. P., & Peticolas, W. L. (1977) *Biochim. Biophys. Acta 465*, 260.
Gaber, B. P., Yaeger, P., & Peticolas, W. L. (1978) *Biophys. J. 21*, 161.
Goheen, S. C., Gilman, T. H., Kauffman, J. W., & Garvin, J. E. (1977) *Biochem. Biophys. Res. Commun. 79*, 805.
Jackson, R. L., Morrisett, J. D., & Gotto, A. M., Jr. (1976) *Physiol. Rev. 56*, 259.
Jonas, A., & Krajnovich, D. J. (1977) *J. Biol. Chem. 252*, 2194.
Jonas, A., Krajnovich, D. J., & Patterson, B. W. (1977) *J. Biol. Chem. 252*, 2200.
Koenig, J. L. (1975) *Biopolymers 14*, 379.
Larsson, K., & Rand, R. P. (1973) *Biochim. Biophys. Acta 326*, 245.
Lin, V. J. C., & Koenig, J. L. (1976) *Biopolymers 15*, 203.
Lippert, J. L., Tyminske, D., & Desmeules, P. J. (1976) *J. Am. Chem. Soc. 98*, 7075.
Lis, L. J., Kauffman, J. W., & Shriver, D. W. (1976a) *Biochim. Biophys. Acta 436*, 513.

Lis, L. J., Goheen, S. C., Kauffman, J. W., & Shriver, D. W. (1976b) *Biochim. Biophys. Acta 443*, 331.
Mabrey, S., & Sturtevant, J. M. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 3862.
Middelhoff, G., Rosseneu, M., Peeters, H., & Brown, W. V. (1976) *Biochim. Biophys. Acta 441*, 57.
Mikkelsen, R. B., Verma, S. P., & Wallach, D. F. H. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 5478–5482.
Milanovich, F. P., Yeh, Y., Baskin, R. J., & Harney, R. C. (1976) *Biochim. Biophys. Acta 419*, 243.
Morrisett, J. D., Jackson, R. L., & Gotto, A. M., Jr. (1977) *Biochim. Biophys. Acta 472*, 93.
Nagle, J. F. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70*, 3443.
Nagle, J. F. (1976) *J. Membr. Biol. 27*, 233.
Patel, K. M., & Sparrow, J. T. (1978) *J. Chromatogr. 150*, 542.
Pink, D. A., Green, T. J., & Chapman, D. (1980) *Biochemistry 19*, 349.
Pownall, H. J., Massey, J. B., Kusserow, S. K., & Gotto, A. M., Jr. (1978) *Biochemistry 17*, 1183.
Pownall, H. J., Massey, J. B., Kusserow, S. K., & Gotto, A. M., Jr. (1979) *Biochemistry 18*, 574.
Rothschild, K. J., Andrew, K. R., De Grip, W. J., & Stanley, H. E. (1976) *Science (Washington, D.C.) 191*, 1176.
Scanu, A. M. (1966) *J. Lipid Res. 7*, 285.
Segrest, J. P., Jackson, R. L., Morrisett, J. D., & Gotto, A. M., Jr. (1974) *FEBS Lett. 38*, 247.
Smith, L. C., Pownall, H. J., & Gotto, A. M., Jr. (1978) *Annu. Rev. Biochem. 47*, 751.
Snyder, R. G. (1967) *J. Chem. Phys. 47(4)*, 1316.
Snyder, R. G., & Scherer, J. R. (1979) *J. Chem. Phys. 71*, 3229.
Snyder, R. G., Hsu, S. L., & Krimm, S. (1978) *Spectrochim. Acta, Part A 34A*, 395.
Spiro, T. G., & Gaber, B. P. (1977) *Annu. Rev. Biochem. 46*, 553.
Susi, H., Sampugna, J., Hampson, J. W., & Ard, J. S. (1979) *Biochemistry 18*, 297.
Tall, A. R., & Lange, T. (1978) *Biochem. Biophys. Res. Commun. 80*, 206–211.
Tall, A. R., Small, D. M., & Shipley, G. G. (1977) *J. Biol. Chem. 252*, 4701–4711.
Taraschi, T., & Mendelsohn, R. (1979) *J. Am. Chem. Soc. 101*, 1050.
Verma, S. P., & Wallach, D. F. H. (1976a) *Proc. Natl. Acad. Sci. U.S.A. 73*, 3558.
Verma, S. P., & Wallach, D. F. H. (1976b) *Biochim. Biophys. Acta 436*, 307.
Yellin, N., & Levin, I. W. (1977) *Biochemistry 16*, 642.
Yu, T., & East, E. J. (1975) *J. Biol. Chem. 256*, 2196.