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Induced Circular Dichroism of Incorporated Fluorescent Cholesteryl Esters and Polar Lipids as a Probe of Human Serum Low Density Lipoprotein Structure and Melting*

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Two fluorescent cholesteryl esters, cholesteryl *cis*-parinarate and cholestatrienyl oleate, have been incorporated into human serum low density lipoprotein (LDL) by two alternative procedures. The core location of the incorporated cholesterol esters and the surface location of *cis*-parinaric acid and cholestatrienol are verified by fluorescence energy transfer. In these LDL preparations, the incorporated cholesteryl esters exhibit a temperature-dependent induced circular dichroic spectrum at the absorption wavelengths of the chromophore with a temperature range which is similar to the calorimetrically determined thermal transition of LDL. We also examined the induced circular dichroism which arises when cholestatrienyl oleate is dissolved in pure cholesteryl oleate and observed a temperature dependence of the sign and magnitude of the circular dichroic spectrum which correlated with the temperature range of the calorimetric phase transitions. On the basis of these studies, we conclude that below the calorimetric transition, the cholesteryl esters of low density lipoprotein have an organization which is intermediate between isotropic and cholesteric, while above the transition, the organization is more nearly isotropic. Residual environmental chirality experienced by cholesteryl ester acyl chains (cholesteryl parinarate) in the particle core and free sterol (cholestatrienol) in the particle surface could represent specific lipid-protein interactions. These results are discussed in terms of the organization of low density lipoprotein.

Thermal transitions in low density lipoprotein have received considerable attention in recent years (1–7). The thermal transitions appear to affect the organization of the cholesteryl esters in the core of LDL[1] and may have considerable physiological importance because animals fed high cholesterol or saturated fat atherogenic diets have cholesteryl ester-rich, LDL-like particles with melting temperatures exceeding the animal's body temperature (8, 9).

Because optical probe techniques, especially fluorescence, are typically several orders of magnitude more sensitive than other physical techniques that have been used to study molecular organization and mobility and because fluorescently labeled macromolecules may be localized by fluorescence microscopy, we have been investigating methods for introducing fluorescent probes including cholesteryl esters into LDL. In the course of applying spectroscopic methods to study molecular details of LDL organization, we sought to repeat with chromophoric cholesteryl esters the observation of Chen and Kane (1) that the circular dichroism of chromophores in the core of LDL was sensitive to the cholesteryl ester thermal transition.

There exists significant literature concerning the effect of placing chromophores in chiral media. The term "liquid crystal-induced circular dichroism" has been used to describe the phenomena which result when chromophores are dissolved in cholesteric liquid crystals (10, 11). The magnitude of the induced CD has been related in model systems to the helical pitch of the cholesteric matrix and the sign of the induced CD to the chirality (10, 11). In the present study, we extend these observations by comparing the induced circular dichroism of several polar and nonpolar lipid chromophores in LDL and we examine the induced circular dichroism of one of these chromophores in neat cholesteryl oleate, which has been spread in a thin layer between quartz plates. Furthermore, we compare the temperature dependence of these phenomena to calorimetric measurements on the same preparations. The results of these studies are discussed in terms of the structural models of LDL.

MATERIALS AND METHODS

Fluorescent Probes

cis-Parinaric cid (9,11,13,15-*cis*, *trans*, *trans*, *cis*-octadecatetraenoic acid) was prepared and stored according to Sklar *et al.* (12). 5,7,9-Cholestatrienol was prepared from 7-dehydrocholesterol (Steraloids, Inc., Wilton, CT) by reduction with mercuric acetate in refluxing ethanol essentially according to Windaus and Linsert (13). Precautions against oxidation of the polyenoic sterol included the use of BHT and reflux under argon. 5,7,9-Cholestatrienol was purified on a Whatman preparative high pressure liquid chromatograph eluted with 4:1 hexane/ethyl acetate. Cholesteryl esters were prepared by the catalytic procedure of Patel *et al.* (14). Cholesteryl *cis*-parinarate was prepared from Sigma grade cholesterol and *cis*-parinaric acid and purified by column chromatography under argon by elution from silica gel with graded mixtures of hexane and ethyl acetate. After removal of the eluent under a stream of nitrogen, the purified product was dissolved in dry benzene and stored at −70 °C. Cholestatrienyl

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[1] The abbreviations used are: LDL, human serum low density lipoprotein; BHT, butylated hydroxytoluene; CD, circular dichroism; C-3-OL, cholestatrienyl oleate; DSC, differential scanning calorimetry; HAF, hexadecanoylaminofluorescein; LCICD, liquid crystal-induced circular dichroism; *cis*-PnA, *cis*-parinaric acid.

oleate was synthesized from cholestatriene and oleic acid (Nuchek Prep) and purified as above but stored in the eluent containing a trace of BHT at −70 °C under argon.

Incorporation of Cholesteryl Esters into Lipoproteins

Method 1—This method takes advantage of lipid exchange factors in human plasma (15). A lipid dispersion of composition similar to LDL (1.2 mg of 1-stearoyl, 2-oleyl phosphatidylcholine, 0.6 mg of triolein, 0.5 mg of cholesterol, 1.1. mg of cholesteryl linoleate, 1.0 mg of probe cholesteryl ester, and 0.01 mg of BHT) is prepared by dissolving the lyophilized lipids in 300 μl of dry isopropanol under argon at ~60 °C; a volume of 100 ul of the isopropanol solution at 55 °C is injected from a jacketed 100-μl syringe into 1.0 ml of rapidly stirred Tris-buffered saline (0.15 M NaCl, 20 mM Tris-HCl, and 0.3 mM EDTA, pH 7.4) at 10 °C. The resulting dispersion is opalescent and consists of a fairly homogeneous population of particles with a diameter of about 300 Å. A total volume of 3.3 ml of this dispersion is added per 10.5 ml of human plasma, sealed in a Teflon stoppered tube under argon, and incubated for 36 h in a 37 °C shaking water bath. In order to obtain an amount of LDL sufficient for calorimetric studies, 3 volumes of plasma and lipid dispersion are used. LDL is obtained by sequential ultracentrifugation with KBr over the density range 1.019–1.063, refloated in a 7-ml centrifuge tube, and sliced in a final volume of 1 ml or less so that the LDL concentration is suitable, after dialysis, for analysis by differential scanning calorimetry. On the basis of criteria including light-scattering and gel filtration elution profiles, chemical composition, and *in vivo* clearance rates, the resulting LDL appear to be normal; this procedure and characterization will be described in detail elsewhere.[2]

Method 2—One to two mg of LDL (protein analysis according to Lowry *et al.* (16) containing 1% SDS) is lyophilized on starch as described by Gustafson (17) and Krieger *et al.* (18). A 100-μl heptane solution containing an amount of cholesteryl ester probe equivalent to 5–10% of the total of normal LDL cholesteryl ester is incubated with the LDL at 4 °C for 24 h and evaporated under a gentle stream of nitrogen. The LDL particles are rehydrated in 10 mM Tricine, pH 7.4, as described by Krieger *et al.* (18).

Incorporation of Polar Fluorescent Probes into LDL

cis-Parinaric acid is incorporated into LDL as previously described (19). Cholestatrienol was incorporated by applying a filter paper method used for cholesterol essentially as described by Nilsson and Zilversmit (20). Approximately 1 mg of cholestatriene was evaporated from solvent onto a small piece of Whatman 4 filter paper under argon and incubated with 2 mg of LDL (protein) for 2 h at 37 °C under argon. Recovery of LDL was greater than 90%. Controls showed that cholestatriene was not significantly dispersed in buffer by this procedure and that cholestatrienyl oleate could not be effectively incorporated into LDL by this method. An attempt was made to incorporate cholestatrienol from filter paper into LDL in plasma where it could be enzymatically converted to sterol ester. The isolated LDL showed a complex CD spectrum with both positive and negative components presumably representing both surface cholestatriene and core cholestatriene ester (see Figs. 4 and 6).

Spectroscopy

Absorption spectra were recorded on a Cary 15 scanning spectrophotometer. Circular dichroic spectra were recorded on a JASCO spectropolarimeter interfaced to a data processing computer in the laboratory of Professor Graham Palmer, Department of Biochemistry, Rice University. Temperature-dependent CD data were collected by recording an entire spectrum at a particular temperature or by continuously recording the ellipticity at a peak wavelength (normalized to the spectral value) while the temperature was controlled by a Haake circulating water bath, varied at a rate never exceeding 1 °C/min, and monitored by a Bailey BAT-8 digital thermometer with the thermocouple placed inside the cuvette. At each wavelength, λ, the molar ellipticity $[\phi]_\lambda$ was calculated according to the equation

$$[\phi]_\lambda = \phi_\lambda \times 100/cl \quad (1)$$

where ϕ is the ellipticity, c is the molar concentration, and l is the optical path length in cm. Fluorescence energy transfer measurements were performed with an SLM 8000 photon-counting spectrofluorometer as previously described (19) where details concerning the evaluation of energy transfer, calculations, and interpretation are provided.

Calorimetry

Differential scanning calorimetry was performed on LDL samples prepared by ultracentrifugal isolation using a Perkin-Elmer DSC-2 double pan calorimeter (see Fig. 3 for experimental conditions).

Measurements on Pure Cholesteryl Esters

Cholesteryl oleate was obtained from Sigma and used without further purification. The primary criterion of its purity was its calorimetric behavior exhibiting transitions between smectic, cholesteric, and isotropic phases at the appropriate temperatures (Fig. 7). Cholesteryl oleate containing 2% 5,7,9-cholestatrienyl oleate was prepared by dissolving the two components in benzene and removing the organic solvent by lyophilization. For CD and absorption measurements (Fig. 8), the melted sample was spread in a thin layer on one plate of a 0.01-cm Suprasil quartz cuvette (Hellma cells) which was maintained by a thermostatted plate at 60 °C. The cuvette was sealed with the opposite window and then clamped with the accessory cell holder. The sample was examined for uniformity by recording the absorption spectrum of both "top" and "bottom" regions of the cuvette which had absorbances within about 10% of one another. The absorbance was constant with time, indicating that the sample did not flow in the cuvette when it was heated to the isotropic phase.

RESULTS

The incorporation of the chromophoric cholesteryl esters into LDL may be evaluated from the absorbance of the incorporated chromophores (Fig. 1). Complementary experiments with lipid dispersions containing radiolabeled lipids have permitted us to follow and optimize the protein-mediated transfer of lipids (Method 1), including the cholesteryl esters, from lipid dispersion to LDL. The incorporation of cholesteryl esters into LDL from lipid dispersions is negligible in the absence of the plasma proteins. Variables included the time of incubation, the ratio of plasma to the lipid dispersion, and the composition of the lipid dispersion. Preliminary results suggest that the incorporation proceeds more rapidly when the phospholipid used in the dispersion is fluid at the incubation temperature. We, therefore, used synthetic phospholip-

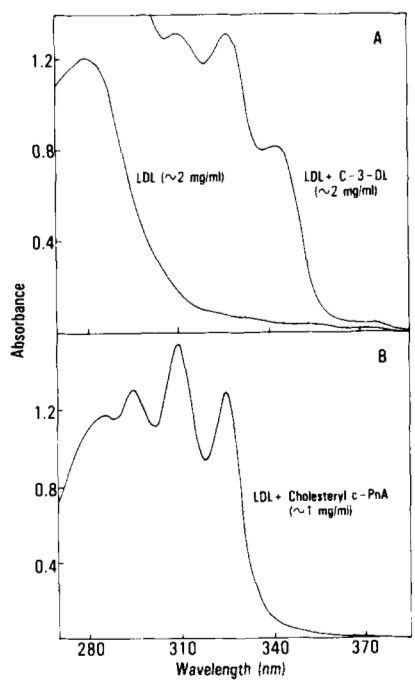

FIG. 1. **Absorbance of LDL containing chromophoric cholesteryl esters prepared by method 1.** *A*, LDL and C-3-OL LDL (~2 mg/ml); *B*, cholesteryl *cis*-parinarate LDL (~1 mg/ml).

[2] Craig, I. F., Via, D. P., Sherril, B. C., Sklar, L. A., Mantulin, W. M., Gotto, A. M., Smith, L. C., submitted for publication.

ids of the type 1-saturated, 2-unsaturated phosphatidylcholine that are representative of physiological phosphatidylcholines. We used cholesteryl linolenate (melting range ~35–40 °C) instead of cholesteryl oleate (melting range ~40–50 °C) because we felt that the physical state of the esters in the dispersion could in principle affect the rate of incorporation into LDL. Under optimal conditions, the incorporated chromophoric ester represented ~5% of the total LDL cholesteryl ester.

The location of the incorporated fluorescent lipid probes was verified by fluorescence energy transfer measurements as illustrated in Fig. 2. Results are shown for LDL into which cholesteryl esters have been incorporated by Method 1 (the exchange procedure) and polar lipids (*cis*-parinaric acid and cholestatrienol) have been incorporated by the partition procedures described above. Similar results are obtained for LDL labeled by Method 2 (solvent incorporation).

The strategy for such measurements is described in detail elsewhere (19). Briefly, HAF, a chromophore which binds to the surface of LDL, is a "fluorescent energy acceptor" for "donor chromophores" such as parinaric acid, cholestatrienol, and their sterol esters. This means that in the presence of "acceptor" the "donor" fluorescence is "quenched" or transferred from donor to acceptor. The distance at which energy transfer is 50% (R_0) can be calculated from spectroscopic variables and is 27 Å for transfer from the parinaric acid chromophore to HAF and 26 Å for transfer from the cholestatrienol chromophore to HAF. With R_0 values of this magnitude, it is possible to discriminate donor chromophores which are on the LDL surface along with the acceptor from donors which are in the core (19).

The *dashed line* in Fig. 2*A* is a calculation of the energy transfer as a function of acceptor concentration ("surface density") when both donor and acceptor chromophore are located near the surface of a spherical particle 220 Å in diameter and R_0 is 27 Å. The polar probes lie near the surface of the LDL particle, while the nonpolar cholesteryl esters which are quenched considerably less efficiently must, in part, be at distances exceeding R_0 from the particle surface. The temperature dependence (not shown) of neither energy transfer (a measure of the particle organization) nor fluorescence polarization or quantum yield is responsive to the calorimetric thermal transitions detected in these particles (Fig. 3).

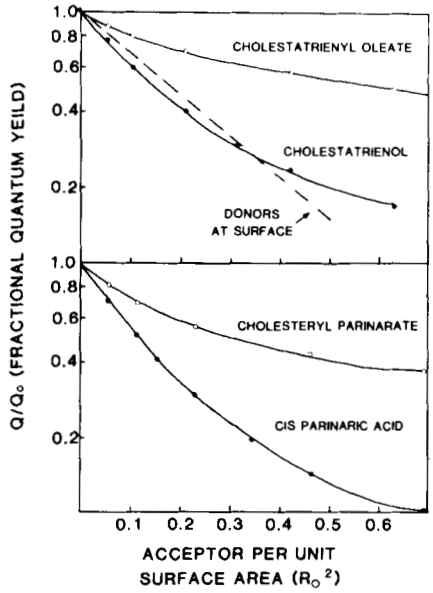

FIG. 2. **Fluorescence energy transfer localization of fluorescence lipids in LDL at 37 °C.** Upper panel, cholestatrienol and cholestatrienyl oleate. Lower panel, *cis*-Parinaric acid and cholesteryl *cis*-parinarate. Data are plotted in terms of reduction of the quantum yield of the fluorescent lipid donor (on a logarithmic scale) *versus* the surface density of fluorescent acceptor molecules expressed in terms of the number of acceptors per unit of surface area (R_0^2). R_0 is the distance at which energy transfer is calculated to be 50% and is 27 Å for the parinaric acid probes and 26 Å for the cholestatrienol probes when hexadecanoylaminofluorescein is the resonance energy transfer acceptor.

The thermal behavior of LDL prior to lipid incorporation is shown in Fig. 3*A*. The behavior of particles which have been treated by lipid dispersions to effect probe incorporation is shown in Fig. 3*B*. Normal human LDL typically exhibits thermal transition of core cholesteryl esters over the range of 20–35 °C (average midpoint Fig. 4*A* equals ~27 °C); the particles prepared by method 1 (exchange) have a thermotropic transition which has been depressed by about 10–15 °C (average midpoint equals ~14 °C).[3] Differences in heating and cooling curves result from the delayed instrumental response that occurs at the high scan rates required for measuring small amounts of sample (see legend to Fig. 3). There was insufficient LDL prepared by method 2 for calorimetry.

These calorimetric temperature ranges are comparable to the temperature range of the induced CD of cholesteryl ester probes in LDL as shown in Fig. 4. In Fig. 4*A*, CD spectra of cholestatrienyl oleate in LDL at temperatures above and below the calorimetric transition are shown. The temperature dependence of the induced CD of LDL particles which have been treated with cholestatrienyl oleate by method 2 (Fig. 4*B* midpoint ~20 °C) is similar to that of the change in heat capacity detected by calorimetry of the native LDL particles (3*A*). The molar ellipticity ranges from a value of $+12 \times 10^3$ at temperatures above the calorimetric transition (a value characteristic of cholestatrienyl oleate or cholestatrienol dissolved in organic solvent) and decreases steeply over the temperature range associated with the calorimetric transition. The temperature ranges of the change in the induced CD of cholestatrienyl oleate (Fig. 4*C*, midpoint ~14 °C) or cholesteryl *cis*-parinarate (Fig. 5) in LDL prepared by method 1 are similar to the observed calorimetric range (Fig. 3*B*). The molar ellipticities of cholestatrienyl oleate above and below the transition are similar in LDL prepared by either method. All of these thermal CD changes are reversible. Under similar conditions, we have not observed a temperature-dependent CD in the lipid dispersion used for introducing probes into LDL.

The temperature dependence of the circular dichroism of cholesteryl *cis*-parinarate is shown in Fig. 5. Whereas *cis*-parinaric acid or its ester has no intrinsic CD spectrum, the cholesteryl ester exhibits a positive CD in LDL even at a temperature (31 °C) above the calorimetric transition of LDL. At 8 °C, a negative CD spectrum for cholesteryl *cis*-parinarate is observed with peak values which are slightly shifted. At an intermediate temperature (20 °C), the spectrum exhibits both positive and negative peaks.

The CD behavior of the nonpolar sterol ester probes differs from that of polar lipid probes that are located in the surface of LDL. No induced CD was detected for *cis*-parinaric acid bound to LDL (not shown). Cholestatrienol appears to be insensitive to the organization of the core components and

[3] The decrease of the transition temperature of LDL prepared by Method I (exchange) apparently arises from an increase of the percentage of triglyceride in LDL which occurs when LDL is isolated from plasma which has been incubated for prolonged periods with our lipid dispersions. It seems unlikely that the introduction of the probe esters *per se* is responsible for the temperature change because cholesteryl parinarate has a higher melting range and cholestatrienyloleate has a lower melting range than the native LDL cholesteryl esters.

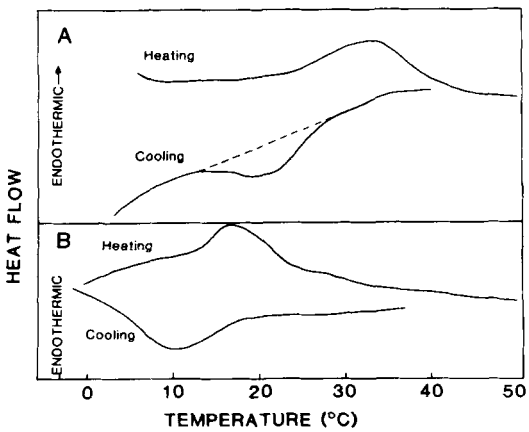

FIG. 3. **Differential scanning calorimetry of LDL.** *A*, native LDL: temperature was varied at the rate of 10 °C/min and the sensitivity was 0.5 mcal/s full scale. *B*, C-3-OL LDL, method 1: temperature was varied at the rate of 5 °C/min (sensitivity 0.5 mcal/s). These settings were required by the relatively low LDL concentration (20–40 mg/ml, LDL protein). The apparent difference in heating and cooling, particularly in *A*, is a reflection of the rapid rate of temperature scan.

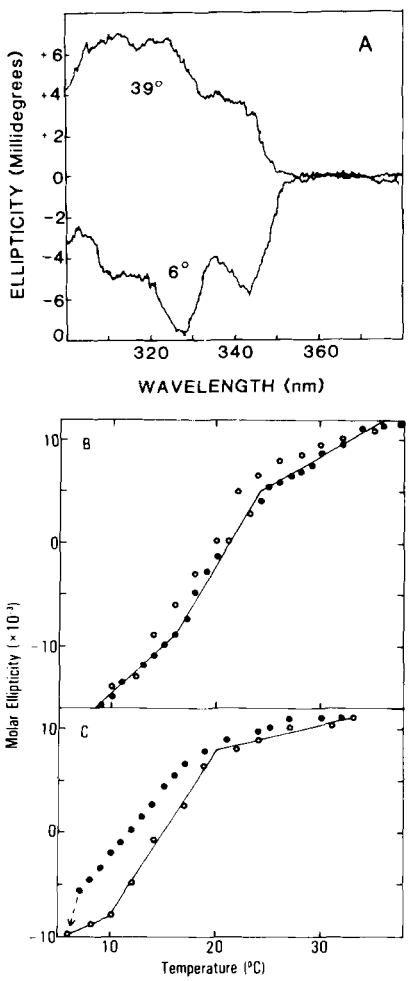

FIG. 4. **Temperature dependence of the CD of cholestatrienyl oleate in LDL.** Temperature dependence of the CD spectra of LDL (method 2) at 6 °C and 39 °C (*A*), and the molar ellipticity of LDL prepared by method 2 (*B*) and LDL prepared by method 1 (*C*). The circular dichroism was monitored continuously at a single wavelength (320 nm, *B*; 325 nm, *C*) as the temperature was varied. The data are replotted in the figure. Heating (○) and cooling (●). The maximum extinction coefficient of cholestatriene is ~12,000 at 325 nm.

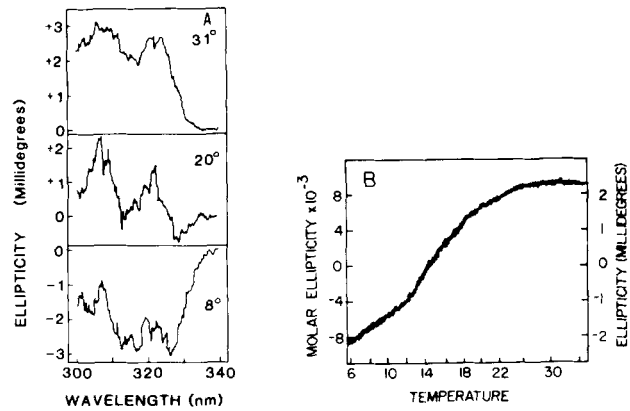

FIG. 5. **Temperature dependence of the circular dichroism of cholesteryl *cis*-parinarate in LDL prepared by enzymatic lipid exchange.** *A*, circular dichroic spectra at several temperatures as the sample was cooled. *B*, the raw data showing temperature dependence of the ellipticity at 325 nm while the sample was heated at the rate of ~2 °C/min. Note that the temperature intervals are 5 °C above 25 °C and 2 °C below 22 °C. The cholesteryl *cis*-parinarate absorption peaks were at 324, 309, and 293 nm with a maximum absorbance of 1.5 (Fig. 1B). Assuming an extinction coefficient of 60,000 at 310 nm, the concentration of cholesteryl *cis*-parinarate was 2.5×10^{-5} M; the concentration of LDL was 1.2 mg/ml.

exhibits a negative CD spectrum over the entire temperature range of 5–40 °C (Fig. 6).

We compared thermal CD and DSC measurements of cholesteryl oleate containing the chromophoric cholesteryl esters. The melting properties of cholesteryl oleate containing 2% cholestatrienyl oleate (Fig. 7*B*) are essentially identical with those of pure cholesteryl oleate (Fig. 7*A*). When either sample is cooled from the isotropic phase (from temperatures above 50 °C), two characteristic thermal transitions, corresponding to the isotropic to cholesteric transition and the cholesteric to smectic transition, are observed (upper curves). If either sample is held at a temperature below the lower transition for more than 5–10 min, the subsequent heating curve shows, in addition to the sharp transitions above, a broad transition (45–50 °C) associated with the melting of a fraction of the cholesteryl oleate from the C_I crystalline phase to the isotropic phase (middle curves) (21). If, however, the samples are cooled from the isotropic phase and immediately heated from a temperature just below the smectic phase, then only the smectic to cholesteric and the cholesteric to isotropic phase transitions are observed (lower curves). In each of these scans, the mixture containing probe chromophore exhibits the transitions at no more than 1–2 °C lower than the pure ester.

The temperature dependence of the induced circular dichroism of 2% cholestatrienyl oleate in cholesteryl oleate is shown in Fig. 8. The thermal data of Fig. 8*B* represent the conversion of crystalline cholesteryl oleate to the isotropic phase (see figure legend). The molar ellipticity at the peak wavelength varies as the sample is heated from about −140,000 at 36 °C to about −100,000 at 46 °C, but between 46 and 50 °C, the molar ellipticity abruptly changes from −100,000 to +12,000, going through 0 at 49 °C. The magnitude of the ellipticity is not affected by raising the temperature above 50 °C. The replacement of the negative CD spectrum by a much smaller positive spectrum occurs over the temperature range associated with the crystalline to isotropic phase transition of cholesteryl oleate.

As the sample is cooled from 60 °C (Fig. 8*C*), the circular dichroism remains positive with a molar ellipticity of about 12,000 that persists down to at least 46 °C. When the temperature is lowered to 44 °C, a negative spectrum appears superimposed on a wavelength-dependent base-line (apparently

resulting from the chiral optical quality of the thin layer phase of cholesteryloleate indicated by the *dashed lines* in the figure). The molar ellipticity is on the order of −200,000. The negative peaks appear at the same wavelengths as the positive

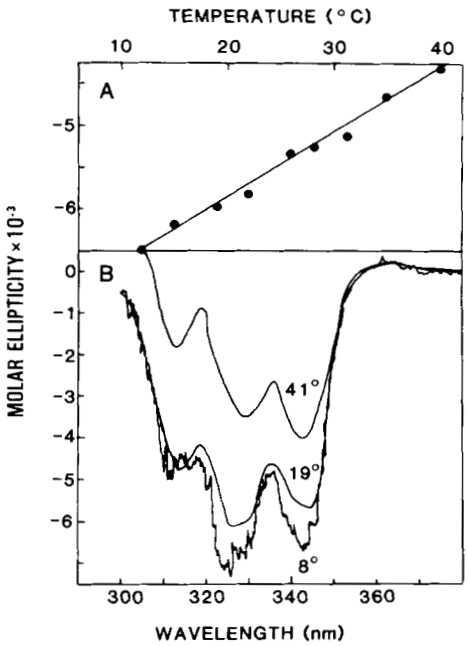

FIG. 6. **Temperature dependence of the circular dichroism of cholestatrienol in LDL.** *A*, temperature dependence of the molar ellipticity of cholestatrienol at 325 nm. *B*, spectra at several temperatures. The 8 °C spectra is an average of four scans. The spectra at 19 °C and 41 °C are smoothed so that all of the spectra can be distinguished from one another.

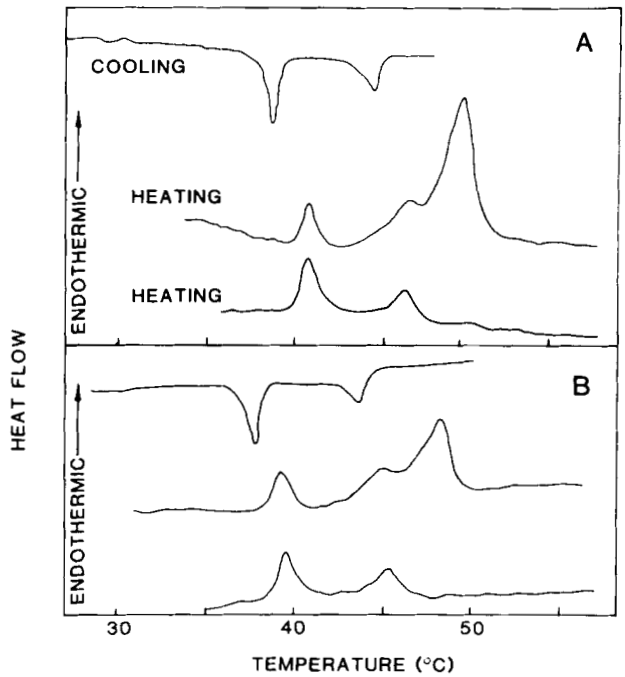

FIG. 7. **Differential scanning calorimetry of cholesteryl oleate (*A*) and 2% 5,7,9-cholestatrienyl oleate in cholesteryl oleate (*B*).** The sample was prepared as described under "Materials and Methods." Upper curves in each panel: sample cooled from 55–25 °C. Middle curves: the heating curve which followed cooling the sample to 25 °C where it was held at 25 °C for 15 min prior to heating. Lower curves: the heating curve which followed cooling the sample to 30 °C prior to reheating immediately to 60 °C. All scans were at the rate of 2.5 °C/min.

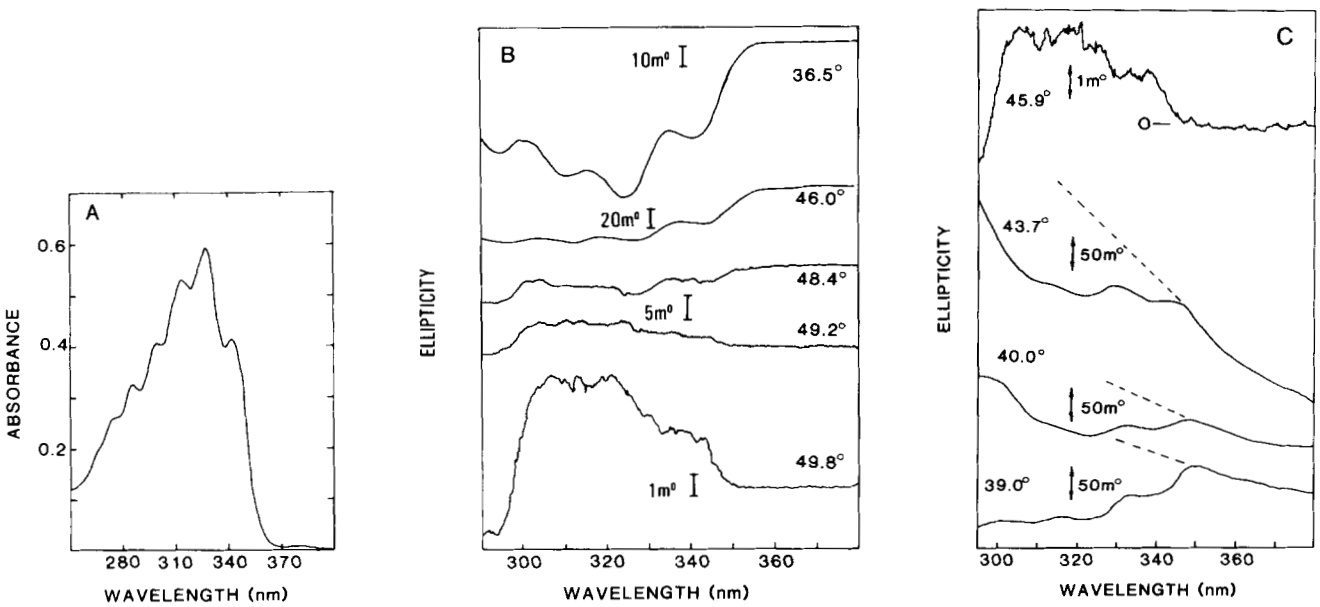

FIG. 8. **Absorption and circular dichroism spectroscopy of 2% 5,7,9-cholestatrienyl oleate in cholesteryl oleate.** Sample prepared as under "Materials and Methods." *A*, the absorption spectrum of the sample. The major absorption bands of cholestatrienyl oleate are at 342, 325, and 310 nm. The small peaks at shorter wavelength probably represent an impurity of about 10% of the probe ester, likely to be a conjugated diene sterol. The absorption bands at shorter wavelength do not interfere with the spectroscopic measurements at longer wavelength. *B*, the melting of crystalline cholesteryl oleate to the isotropic phase (46–50 °C). Circular dichroism spectra are shown for the sample which had been previously heated to 55 °C and then cooled at the rate of ~10 °C/h and held at 35 °C for 1 h prior to initiating measurements. The spectra at 36.5 and 46 °C represent a single scan, while the other spectra represent a computer average of two scans. *C*, the cooling of cholesteryl oleate from isotropic to cholesteric and smectic phases. The sample in *B* was cooled from 55 °C and spectra were recorded at ~1 °C intervals. The spectrum was essentially unchanged between 54 and 45.9 °C (average of two spectra). At 45 °C, the wavelength-dependent base-line was so steep that it was not possible to obtain a spectrum. Spectra obtained below 44 °C show negative peak values of several hundred millidegrees. Note the scale factors for each spectrum.

peaks observed in the isotropic phase and correspond to peaks in the absorption spectrum of cholestatrienyl oleate (Fig. 8*A*). The first appearance of the strongly negative dichroism (~45 °C) and the sloping base-line correspond to the temperature range of the cholesteric phase. Between 44 °C and 40 °C, the spectrum is of constant magnitude while below 40 °C, the ellipticity increases and the base-line tends to become more regular (as in Fig. 8*B*). This temperature range is similar to that of the thermal conversion of the cholesteric phase to the smectic phase at 38–40 °C (Fig. 7). The differences in the CD spectra at temperatures below 45 °C are primarily qualitative, but in any of these low temperature mesophases, the CD is negative with a molar ellipticity of several hundred thousand, whereas in the isotropic phase they are positive and do not exceed ~12,000. These results are reproduced when the temperature is cycled.

DISCUSSION

Induced circular dichroism is a well known phenomenon which occurs when chromophores, either chiral or achiral, are present in chiral environments. Among the most common types of induced CD are those which arise when ligands are bound to chiral pockets in proteins (22, 23) and those which arise from dissolving chromophores in chiral solvents (24). Chen and Kane (1) showed that the ellipticity of β-carotene was a function of the temperature-sensitive chirality of LDL, presumably arising in the core of the particle. In this work, we have used lipid chromophores which are localized exclusively on the surface or in the core of LDL and examined the resulting induced CD. From these results, we are able to develop a qualitative analysis of the chiral features and the liquid crystalline nature of the LDL particle.

We compared the sign and magnitude of the induced circular dichroism of cholestatrienyl oleate incorporated into LDL and into pure cholesteryl oleate which we examined as a thin layer between quartz plates (9, 10). The circular dichroism ($[\phi]_{max} \simeq 12{,}000$) of cholestatrienyl oleate at high temperature in LDL, in isotropic phase cholesteryl oleate, or in organic solvents is very similar. As LDL undergoes its characteristic calorimetric transition, the circular dichroism decreases in magnitude and changes sign, but the molar ellipticity at low temperature in LDL is considerably smaller ($[\phi]_{max} > -12{,}000$) than that observed when the chromophore is in either cholesteric, smectic, or crystalline phases of cholesteryl oleate ($[\phi]_{max} \sim -100{,}000$ to $-200{,}000$). Furthermore, the induced CD of cholestatrienyl oleate in pure cholesteryl oleate changes sign from positive to negative at the temperature associated with the disappearance of the isotropic phase and from negative to positive as the isotropic phase is formed. These results strongly indicate that the change in sign of the induced CD in LDL arises from a structural transition in the cholesteryl ester containing core of the particle. The magnitude of the induced CD in LDL implies that the low temperature phase of LDL is not as ordered as the cholesteric phase of cholesteryl oleate.

We observed that cholesteryl *cis*-parinarate, which normally has no CD, exhibits an induced positive CD in "isotropic" LDL, and that a shifted spectrum with a negative CD appears as the particle undergoes its thermal transition. The positive CD of cholesteryl parinarate in "isotropic" LDL could, in principle, arise either from some residual lipid chirality in the LDL core which affects acyl chains but not the sterol rings of cholesteryl esters or from a specific lipid-protein interaction between sterol ester acyl chains and apo B. The temperature dependence again reflects the changing structure of the LDL core.

The CD of chromophores incorporated into the surface of the LDL particles behaves as if the chirality of the core components is not transmitted to the surface of LDL. *cis*-Parinaric acid, which exhibits an induced CD when bound to albumin (22, 25), but not to lipid vesicles, exhibits no detectable induced CD when bound to the surface of LDL. Remarkably, cholestatrienol, which exhibits an intrinsic positive CD in isotropic media, exhibits a negative CD in the surface of LDL. Although this ellipticity is temperature dependent, it is not sensitive to the LDL core reorganization. In principal, this induced CD could also result either from lipid-lipid interactions (*e.g.* sterol-sterol coupling which provides a chiral environment for the chromophore) or lipid-protein interactions (in which surface sterols interact with apo B sterol binding sites). It will be necessary to develop further models to elaborate the requirements of lipid and lipid-protein organization which give rise to induced CD.

These CD data do not readily lend themselves to a detailed structural interpretation. Since a complete theoretical description of induced CD is not available (see for example Refs. 26 and 27), we can only suggest that both cholesteryl ester acyl chains and steroid nuclei experience similar chiral environments in LDL below the phase transition. Whether this chirality arises from an interdigitation of chiral steroid nuclei and achiral acyl chains or the propagation of a helicoid and, thereby, achiral liquid crystalline phase throughout the core of LDL cannot yet be distinguished. Furthermore, a model in which the ester acyl chains interact either with sterols or proteins could account for the observed residual chirality of the "isotropic" (high temperature) phase of LDL. Above the phase transition, the environments of the sterol rings and the acyl chains of cholesteryl esters appear to be dissimilar.

The sterol esters used in the present study are fluorescent. Since the fluorescence emission of cholestatrienyl oleate in LDL is highly polarized, it is conceivable that fluorescence-detected circular dichroism (30) will make it possible to observe changes in the organization of LDL present in cells. Normal CD measurements could also provide further insight into particles undergoing lipolysis or exposed to cholesteryl ester or triglyceride exchange proteins. The ability to introduce chromophoric lipids into lipoproteins and to characterize their location and organization by both CD and fluorescent methods should be a great asset in deciphering molecular details of lipoprotein structure and metabolism and their interactions with cells. In summary, we have demonstrated that the induced CD of lipid chromophores is sensitive to some structural features in LDL. Above the calorimetric transition, the core of LDL has isotropic features, while below the transition, the core has chiral properties which do not represent a true cholesteric phase. Hamilton *et al.* (28, 29), examining the motional properties of the cholesteryl esters of LDL by nuclear magnetic resonance, concluded that the core of LDL was not a true cholesteric phase.

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Addendum—Since this manuscript was submitted, a paper by Chen and co-workers (31) showing that the induced CD of β-carotene was sensitive to the structure of the core of solvent-reconstituted LDL (18) has appeared.

REFERENCES

1. Chen, G. C., and Kane, J. P. (1974) *Biochemistry* **13,** 3330–3335
2. Deckelbaum, R. J., Shipley, G. G., Small, D. M., Lees, R. S., and George, P. K. (1975) *Science* **190,** 392–394
3. Deckelbaum, R. J., Shipley, G. G., and Small, D. M. (1977) *J.*

Biol. Chem. **252,** 744–754
4. Atkinson, D., Deckelbaum, R. J., Small, D. M., and Shipley, G. G. (1977) *Proc. Natl. Acad. Sci. U. S. A.* **74,** 1042–1046
5. Laggner, P., Degovics, G., Muller, K. W., Glatner, O., Kratky, O., Kostner, G., and Holasek, A. (1977) *Hoppe-Seyler's Z. Physiol. Chem.* **358,** 771–778
6. Luzzati, U., Tardieu, A., and Aggerbeck, L. P. (1979) *J. Mol. Biol* **134,** 435–473
7. Pownall, H. J., Shepherd, J., Mantulin, W. W., Sklar, L. A., and Gotto, A. M. (1980) *Atherosclerosis* **36,** 299–314
8. Tall, A. R., Small, D. M., Atkinson, D., and Rudel, L. L. (1978) *J. Clin. Invest.* **62,** 1354–1363
9. Kirchhausen, T., Untracht, S. H., Fless, G. M., and Scanu, A. M. (1979) *Atherosclerosis* **33,** 59–70
10. Saeva, F. D., Sharpe, P. Z., and Olin, G. R. (1973) *J. Am. Chem. Soc.* **95,** 7656–7659
11. Saeva, D. F., and Olin, G. R. (1973) *J. Am. Chem. Soc.* **95,** 7882–7884
12. Sklar, L. A., Hudson, B. S., and Simoni, R. D. (1977) *Biochemistry* **16,** 819–828
13. Windaus, A., and Linsert, O. (1928) *Justus Liebigs Ann. Chem.* **465,** 148
14. Patel, K. M., Sklar, L. A., Currie, R., Pownall, H. J., Morrisett, J. D., and Sparrow, J. T. (1979) *Lipid* **14,** 816–818
15. Pattnaik, N. M., Montes, A., Hughes, L. B., and Zilversmit, D. B. (1978) *Biochim. Biophys. Acta* **530,** 428–438
16. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275
17. Gustafson, A. (1965) *J. Lipid Res.* **6,** 512–517
18. Krieger, M., Brown, M. S., Faust, J. R., and Goldstein, J. L. (1978) *J. Biol. Chem.* **253,** 4093–4101
19. Sklar, L. A., Doody, M. C., Gotto, A. M., and Pownall, H. J. (1980) *Biochemistry* **19,** 1294–1301
20. Nilsson, A., and Zilversmit, D. B. (1972) *J. Lipid Res.* **13,** 32–38
21. Shipley, G. G. (1974) in *Fundamentals of Lipid Chemistry* (Burton, R. M., and Guerra, F. C., eds), pp. 585–613, BI-Science Publications, Webster Groves, Missouri
22. Sklar, L. A., Hudson, B. S., and Simoni, R. D. (1977) *Biochemistry* **16,** 5100–5108
23. Blout, E. R., and Stryer, L. (1959) *Proc. Natl. Acad. Sci. U. S. A.* **45,** 1591–1593
24. Hayward, L. D., and Totty, R. N. (1971) *Can. J. Chem.* **49,** 624–631
25. Berde, C. B., Hudson, B. S., Simoni, R. D., and Sklar, L. A. (1979) *J. Biol. Chem.* **254,** 391–400
26. Mason, S.-F. (1975) *Chem. Phys. Lett.* **32,** 201–203
27. Craig, D. P., Power, E. A., and Thirunamachandran, T. (1974) *Chem. Phys. Lett.* **27,** 149–153
28. Hamilton, J. A., Oppenheimer, N., and Cordes, E. H. (1977) *J. Biol. Chem.* **252,** 8071–8080
29. Hamilton, J. A., Cordes, E. H., and Glueck, C. J. (1979) *J. Biol. Chem.* **254,** 5435–5441
30. Tinocc, I., Ehrenberg, B., and Steinberg, I. Z. (1977) *J. Chem. Phys.* **66,** 916–920
31. Chen, G. C., Krieger, M., Kane, J. P., Wu, C. C., Brown, M. S., and Goldstein, J. L. (1980) *Biochemistry* **19,** 4706–4712