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Induced Circular Dichroism of β-Carotene Dissolved in Mixtures of Cholesteryl Ester and Cholesterol or Triolein

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Mixtures of cholesteryl ester and cholesterol or triolein were tested by means of the differential scanning calorimetry (DSC) and the circular dichroism (CD). Cholesterol modified the monotropic nature of pure cholesteryl esters especially in cholesteryl linoleate and cholesteryl linolenate. β-Carotene dissolved in mixtures containing either of these cholesteryl esters exhibited an induced optical activity only when the mixtures were in the cholesteric mesophase. The helical sense of the cholesteric structure was always left-handed and the sign of the induced CD (ICD) was mostly negative. Triolein interrupted formation of any ordered structure only with a small amount. As for cholesteryl oleate neither of cholesterol and triolein was not enough to modify its monotropic nature. The behavior of the ICD of β-carotene in the mixtures was very different from that reported to be shown in reconstituted low-density lipoprotein (r-LDL). This suggests that the structure of the core cholesteryl ester of r-LDL is strongly modified by a coexisting component of r-LDL other than cholesterol, e.g., apoLDL.

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INTRODUCTION

Chen and Kane[1] have shown that the optical activity of carotenoids is the basis of nonlinearity of data for human plasma low-density lipoprotein (LDL) plotted according to the Drude equation and have suggested that the optical activity of β-carotene, which itself lacks optical asymmetry, is induced by environmental constraint in the lipoprotein complex. In order to study the effect of lipid composition (and structure) on the ICD of β-carotene Chen and her coworkers[2] have prepared r-LDLs which contain this carotenoid and have shown that the β-carotene exhibits a temperature-dependent CD which appears to be a consequence of cholesteryl ester phase transition in the lipoprotein core. This finding has been confirmed by Sklar and his coworkers[3] who measured the ICD of achiral chromophoric substances such as fluorescent cholesteryl esters and polar lipids as probes of human serum LDL structure. However, they have proposed that the cholesteryl ester in the core of LDL appears not to present a true cholesteric mesophase based on the experimental evidence that the magnitude of the ICD is far smaller in LDL than in pure cholesteryl ester. This suggests that the structure of the core cholesteryl esters is modified by components which coexist with them. According to Hatch and Lees[4] human serum LDL consists of 36% cholesteryl esters in which cholesteryl linoleate has a majority, 10% cholesterol, 20% phospholipids, 12% triglycerides and 22% proteins (apoLDL). It is thus necessary to check each coexistent component with respect to the ICD of achiral chromophore (and the structure of the core LDL). We deal in this paper first with cholesterol and triolein.

EXPERIMENTAL

Three kinds of cholesteryl esters of C_{18} aliphatic acids, e.g., cholesteryl oleate (18 : 1), cholesteryl linoleate (18 : 2) and cholesteryl linolenate (18 : 3) were purchased from Sedary Research Laboratories. Cholesterol and triolein were obtained from Applied Science Laboratories, Inc., and β-carotene from Sigma Chemical Co. These reagents were used without further purification. Each cholesteryl ester was mixed with cholesterol, as a carbon tetrachloride solution, at a predetermined ratio in a quartz cell of 1-mm pathlength. Triolein was also used instead of cholesterol. The solution was stirred with the top of a needle, under nitrogen gas, to attain uniform distribution of the constituents and vacuum dried. It was then kept at 5 °C at least two

weeks prior to initiating measurements in order to insure full growth of ordered structures. β-Carotene was added as a carbon tetrachloride solution to some of the aged preparations and vacuum dried; they appeared to resume their ordered structure rather quickly (1 night). A quartz spacer was inserted into each optical cell to shorten the pathlength to about 30 μm or less in advance. The preparations were marked as, for example, 1C-30′, 2T-03 and 3C-00, of which the first two refer to mixtures of cholesteryl oleate and 30% cholesterol (weight percentage against total neutral lipids) and of cholesteryl linoleate and 3% triolein plus β-carotene and the last to cholesteryl linolenate plus β-carotene.

The CD was measured with a Jasco J-40A spectropolarimeter and the absorptivity with a Jasco ORD/UV-5 spectropolarimeter at temperatures between 2°C and 55°C in a water-circulating jacket. Prior to initiating measurements the preparations containing β-carotene were inspected by the shape of its typical absorption spectrum centered around 470 nm since this carotenoid was rather easy to become denatured when once introduced in the optical cell. Measurements of the DSC were carried out with a Rigaku Denki Thermoflex. The transition temperature was obtained, according to Davis and Porter,[5] as the midpoints of the transition range which was about 1°C. All the scans were at the rate of 5°C/min.

RESULTS AND DISCUSSION

Structure of the Preparations

Figure 1 shows some of the typical DSC curves of the preparations. On heating from −40°C (due to the system of the apparatus used the DSC running started at about −20°C.) all the cholesteryl linoleate and cholesteryl linolenate preparations exhibit only the crystal–isotropic liquid transition (upper panel); however, on cooling after premelting at 55°C these preparations exhibit the isotropic liquid–cholesteric and cholesteric–smectic transitions (middle panel). Such a monotropic nature of the neutral cholesteryl esters has already been observed by Davis and Porter[5] with pure cholesteryl esters under a similar experimental condition. Pure cholesteryl linolenate is also monotropic; however, its mixture with 30% cholesterol is liquid crystalline all the way until it melts when it is heated. On heating from −10°C (the DSC running from about 5°C) after 5-min preheating at 55°C and cooling the samples to this temperature in about 15 min

where they were held for 10 min, all the preparations except the cholesteryl oleate preparations which recrystallize rather rapidly below the cholesteric–smectic transition temperature exhibit the smectic–cholesteric and cholesteric–isotropic liquid transitions (lower panel). The cholesteryl oleate preparations, however, surrender their monotropic nature when heated from 15 °C instead of −10 °C. These experimental evidence suggest that the influence of cholesterol on the structure of cholesteryl ester becomes stronger with the increase of the number of unsaturated bonds in the aliphatic chain of the cholesteryl ester.

Table I summarizes temperatures and heats of transition for the preparations. As to pure cholesteryl esters the DSC results obtained on heating from −40 °C are in good agreement with those obtained by Davis and Porter[5] on a similar experimental condition. This

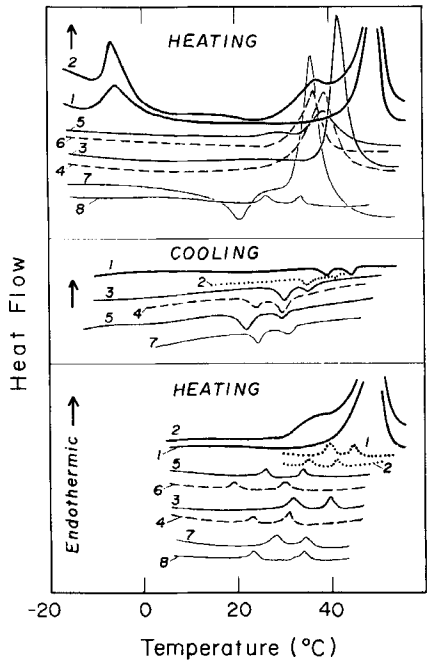

FIGURE 1 Differential scanning calorimetry of mixtures of cholesteryl ester and cholesterol. Prior to initiating measurements specimens were cooled to −40 °C (upper panel) or to −10 °C (lower panel); dotted lines in the lower panel cooled to 15 °C. Preparations: 1, 1C-00′; 2, 1C-30′; 3, 2C-00′; 4, 2C-00 (with 3% β-carotene); 5, 2C-30′; 6, 2C-30 (with 3% β-carotene); 7, 3C-00′; 8, 3C-30′. All scans were at the rate of 5 °C/min. For detail see the text.

TABLE I

Thermal characteristics of the mixtures of cholesteryl ester and cholesterol

Prepn	C–I		S–Ch		Ch–I		I–Ch		Ch–S	
	Temp. (°C)	ΔH (mCal/mg)	Temp. (°C)	ΔH (mCal/mg)	Temp. (°C)	ΔH (mCal/mg)	Temp. (°C)	ΔH (mCal/mg)	Temp. (°C)	ΔH (mCal/mg)
1C-00′	49.2	10.7	39.7	1.2	44.3	0.75	44.1	0.64	39.1	0.71
1C-00	46.1	11.0					41.2	0.69	36.1	1.0
1C-30′	45.8	8.0	35.0	0.5	41.2	0.4	41.0	0.28	34.6	0.42
1T-10′	45.0	9.7					27.2	0.13	24.1	0.33
1T-30′	40.6	5.2					—		—	
1T-50′	38.9	3.6					—		—	
2C-00′	41.4	8.2	32.7	1.0	39.2	1.0	35.6	0.65	31.2	0.97
2C-00	35.8	9.0	21.7	0.61	30.5	1.2	29.0	0.92	24.0	0.40
2C-10′	38.5	3.2	27.0	0.35	33.6	0.20	32.5	0.46	28.6	0.50
2C-20′	36.7	3.0	27.1	0.85	31.7	0.28	29.3	0.22	26.5	0.68
2C-30′	36.3	5.5	25.9	0.68	33.5	0.64	27.3	0.26	21.7	0.92
2C-50′	36.4	2.4					27.9	0.65	21.5	3.7 ?
3C-00′	35.5	6.9	28.4	0.66	34.0	0.49	30.3	0.53	25.1	0.73
3C-10′	—		25.7	0.35	34.3	0.14	—		—	
3C-30′	—		23.0	0.14	34.0	0.52	—		—	
3C-50′	—		—		31.5	1.0	—		—	

Measured at the scanning rate of 5 °C/min, and the heating started from −10 °C except for 1C-00′ and 1C-30′ (15 °C). Each value is the average of two or three runs. —transition not observed. 1C-00 contained 3% β-carotene and 2C-00, 4% β-carotene.

indicates, in a sense, that the cholesteryl esters used in this study were highly pure as in their case. When cholesterol is added all the transition temperatures of the cholesteryl linoleate and cholesteryl linolenate preparations go down both on heating and cooling processes. The shift of the transition temperatures is most striking at the first 10% addition of cholesterol (about 3°C). When the cholesterol content became 60% or more the heat transition was scarcely observed, suggesting that liquid crystal structures could not be assumed any more.

Effects of triolein, which is an isotropic liquid at 0°C, were more striking: The addition of 10% triolein was almost enough to make these cholesteryl esters isotropic liquids even at 0°C. β-Carotene also lowers the transition temperatures: The addition of 3% β-carotene corresponds to 5–10°C lowering of the smectic–cholesteric transition temperature (see Figure 1).

The cholesteryl linoleate and cholesteryl linolenate preparations, which were never cooled below 2 °C prior to initiating the CD measurement, exhibit the CD in a certain temperature range that depends on the preparation as may be seen in Figure 2, in which each panel represents a different typical shape of the CD curve and its change with temperature. This CD curve covers a wavelength range (600–300 nm) where no intrinsic absorption band of the lipids is situated. These preparations showed iridescent color due to the selective reflection of circularly polarized light of one sense only when they exhibited the CD. These two pieces of the experimental evidence suggest that the preparations are in the cholesteric mesophase. The CD (pitch-band CD) is always positive, suggesting according to Tachibana and Oda[6] that the helical sense of the cholesteric texture is left-handed.

The reflective wavelength of the cholesteric pitch band depends on the angle that the incident beam makes with respect to the nematic-like layers of the cholesteric texture. The wavelength at which the pitch-band CD shows the maximum intensity is the longest when this angle is 90° and is given by the equation,[7] $\lambda_m = nP$, where n is the average refractive index of the cholesteric medium and P is the cholesteric pitch. Probably due to many residual cholesteric regions, in the transmission volume, whose way of distribution differs from specimen to specimen the pattern of the pitch-band CD curve was not unique to the preparation.

Table II summarizes the cholesteric temperature ranges and intensities of the pitch-band CD for the cholesteryl linoleate and cholesteryl linolenate preparations. The temperature ranges naturally correspond to those obtained by the DSC technique (see Table I); however, the

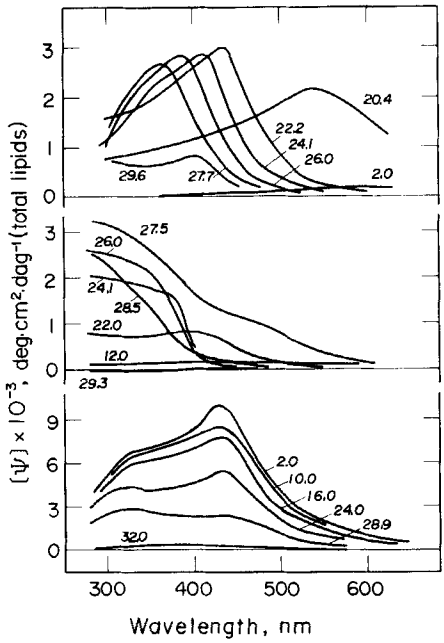

FIGURE 2 Dependence of the pitch-band CD on temperature for mixtures of cholesteryl ester and cholesterol. The numbers near the lines refer to the temperature. Preparations: Upper panel, 3C-00′B; middle panel, 2C-20′B; lower panel, 2C-40 (with 1.2% β-carotene, measured after the carotenoid had been denatured).

correspondence is only approximate. As an example the preparation 3C–10′A shows the pitch-band CD between 21°C and 28°C, whereas its cholesteric range as estimated from the DSC curve is between 25.7°C and 34.5°C. A difference seen between these two ranges may be inevitable because of the nature of the preparation and also of the difference in the experimental condition. The change of the cholesteric range is striking at the first 10% addition of cholesterol in accordance with the results obtained by the DSC measurement. The intensity of the pitch-band CD depends on the content of cholesterol in a complicated way and it is unable to describe the way of the dependence accurately. At any rate cholesterol acts as an obstacle on the native, monotropic nature of the cholesteryl esters and arouse their liquid crystalline nature; however, too much cholesterol is bad as too little cholesterol since cholesterol itself cannot assume liquid crystals. There seems to be a suitable amount of cholesterol and it is about 30% for cholesteryl linoleate and about 10% for cholesteryl linolenate. The

TABLE II

Characteristics of the pitch-band CD of the mixtures of cholesteryl ester and cholesterol

Prepn	Cholesteric range (°C)	Specific ellipticity at 24°C		Maximum ellipticity and Temperature		
		λ_m (nm)	$[\Psi]_{470\,nm} \times 10^{-3}$	λ_m(nm)	$[\Psi]_{470\,nm} \times 10^{-3}$	Temp. (°C)
2C-00′	33 ~ 40		0		not determined	
2C-10′	24 ~ 31		0	370	0.93	26.0
2C-20′A	22 ~ 28	370	1.62	350	2.14	26.0
2C-20′B	22 ~ 29		not determined		not determined	
2C-20	below 2 ~ 28	435	12.4	430	18.0[a]	below 2.0
2C-30′	20 ~ 29	350	0.35	360	0.50	27.0
2C-30	19 ~ 27	370	8.50	370	10.3	22.0
2C-40′	19 ~ 32	350	1.58	410	1.98	21.0
2C-40	below 2 ~ 29	430	5.44	430	9.38	20.0
2C-50′	19 ~ 30	365	1.07	400	1.73	19.3
2C-60′	24 ~ 30		0	370	1.44	26.7
2C-70′	24 ~ 30		0	370	0.2	26
3C-00′A	26 ~ 32	450	2.3	450	3.3	26.8
3C-00′B	20 ~ 30	410	2.85	435	3.0	22.2
3C-10′A	21 ~ 28	430	0.88	450	2.1	23.6
3C-10′B	18 ~ 30	430	9.6	420	9.8	25.9
3C-20′	15 ~ 23		0	380	5.5	17.0
3C-30′	20 ~ 31	400	1.44	370	3.98	26.0
3C-50′	below 2 ~ 32	300	0.4	370	1.94	28.3

2C-20, 2C-30 and 2C-40 contained β-carotene and were measured after β-carotene had been denatured.
[a] Ellipticity at 2°C. The unit for $[\Psi]_{470\,nm}$, deg · cm^2 · dag^{-1} (total lipids).

latter is more sensitive to the cholesterol, which again agrees with the DSC results. As to the reflective wavelength it is about 360 nm for cholesteryl linoleate and 430 nm for cholesteryl linolenate and appears not to depend on the cholesterol content.

The addition of β-carotene makes the cholesteric temperature range shifted to the low-temperature side as expected from the experimental evidence obtained by the DSC measurements; however, the shift of the lower limit of this temperature range is often far larger than expected when the measurements are made after the β-carotene was denatured to avoid overlapping of its ICD with the pitch-band CD in order to better locate the latter. This is probably due to possible denaturation of the neutral lipids during the lapse of time (2 weeks or more).

As for the cholesteryl oleate preparations they show no liquid crystals when the measurements is initiated from 2°C since they are monotropic. On cooling quickly after premelting the specimen at 55°C to 37.5°C, however, the preparation containing 30% cholesterol

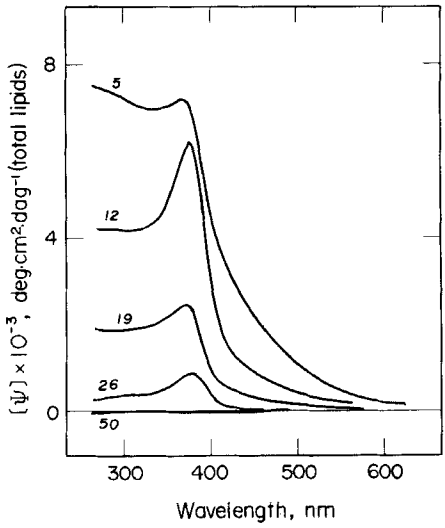

FIGURE 3 Circular dichroism of cholesteryl oleate with 30% cholesterol on cooling from the isotropic state. The numbers near the lines refer to the time in min after premelting and cooling the specimen to 37.5 °C.

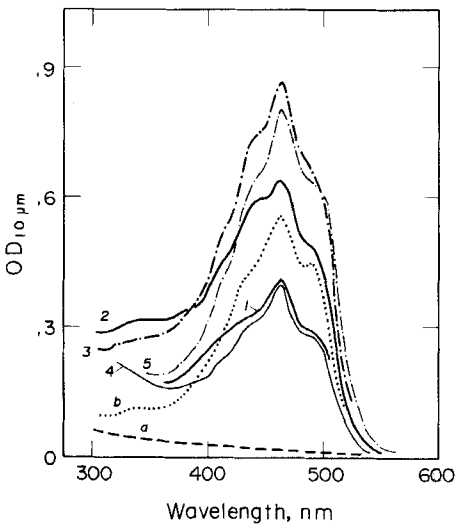

FIGURE 4 Intrinsic absorption spectra of β-carotene dissolved in mixtures of cholesteryl ester and cholesterol. Preparations: 1, 1C-00; 2, 2C-00; 3, 2C-20A; 4, 3C-00A; 5, 3C-10A; a, 1C-00′ (containing no β-carotene); b, 1% CCl_4 solution of β-carotene (1-mm pathlength).

(1C-30′) shows opalescent color for while and the pitch-band CD centered around 380 nm appears only during its presence (opalescent color) as shown in Figure 3. Sometime after the opalescent color vanishes a spherulitic (and crystalline) structure appears and grows very rapidly; the spherulite often becomes several millimeters in diameter. It thus is known that cholesteryl oleate recrystallizes at a temperature that situates in the cholesteric range of the cooling process (41.0–34.6 °C for 1C-30′, see Table I).

Induced Optical Activity of β-Carotene dissolved in the Preparations.

Figure 4 shows the absorption spectra of β-carotene dissolved in the preparations. β-Carotene was supposedly oriented in the liquid crystalline texture and exhibited only an apparent absorption. Therefore the absorptivity is expressed only in terms of the optical density reduced to 10-μm pathlength. The shape of the absorption curve is very similar to that shown by a carbon tetrachloride solution of this carotenoid.

When β-carotene is added most of the cholesteryl linoleate and cholesteryl linolenate preparations display a new CD overlapping the pitch-band CD in a certain temperature range that depends on the preparation as may be seen in Figure 5. The curve of this new CD, which can be separated from the original CD curve by subtracting an assumed pitch-band CD curve (dotted lines in the figure), is situated

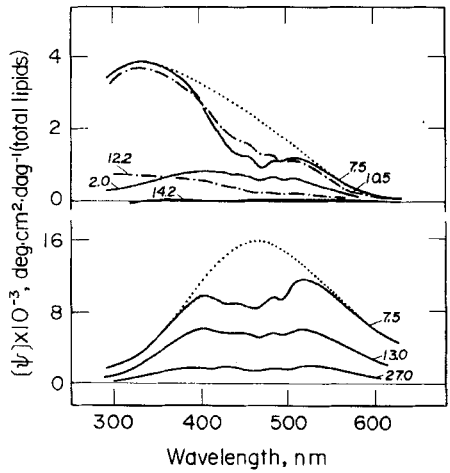

FIGURE 5 Circular dichroism of mixtures of cholesteryl ester and cholesterol containing β-carotene. The numbers near the lines refer to the temperature. Preparations: upper panel, 2C-20A; lower panel, 2C-30B.

exactly in the same wavelength range where the intrinsic absorption of β-carotene is observed. β-Carotene, which itself is achiral, does not exhibit such a CD when it is in an isotropic solution. Therefore it may be safe to conclude that this new CD is due to the optical activity of β-carotene induced in the achiral environment of the cholesteric mesophase as Chen and Kane[1] have suggested. The shape of the ICD curve and that of the intrinsic absorption curve of β-carotene appear to be very like, which suggests according to Sato and Hatano[8] that the β-carotene molecules are supposedly not bound to the host molecules (cholesteryl ester molecules).

Due to the linear dichroism and linear birefringence in the transmission volume the CD curve exhibited by the specimen was only apparent. Schneider and Maestre[9] have been able to demonstrate that the average of two curves taken at rotation angles, the angle around the axis parallel to the incident beam and perpendicular to the surface of a film specimen, 90° apart gives the inherent CD curve of the specimen provided that the specimen is thin (0.01–0.1 mm) and only partly oriented (less than 5% orientation). This procedure was just applicable to our preparations; in some cases three curves, each being the average of two original curves measured at different set of rotation angles 90° apart, were averaged as shown in Figure 6.

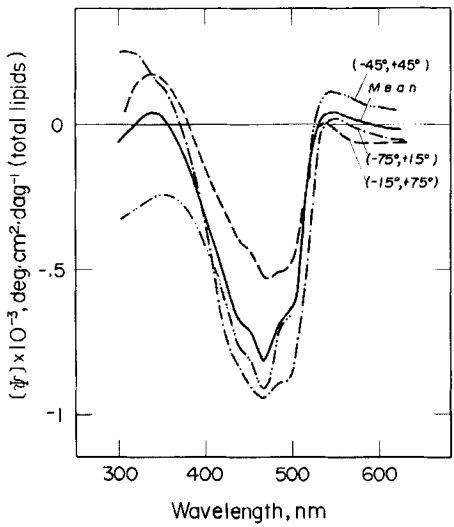

FIGURE 6 Method to determine the inherent CD curve of an oriented specimen. The numbers in the parentheses refer to the rotation angles. See the text. Preparation, 3C-10A. Measured at 2°C.

Figure 7 shows three typical cases representing the temperature dependence of the ICD curve of β-carotene. When the specimen is heated the sign of the ICD is always negative (Case 1, lower panel), always positive (Case 2 upper panel), or variable (Case 3, middle panel). In any case the ICD disappears at the temperature at which the cholesteric mesophase of the mother preparation melts. Cases 2 and 3 are rare cases: The former is observed with 2C-10, which, however, turns to exhibit the normal behavior (Case 1) when the measurement is repeated. The latter is observed with 1C-30 on the above-mentioned special condition that produces the cholesteric mesophase (initiating the measurement from 15 °C after premelting and cooling the specimen to this temperature for 10 min).

As has been mentioned already the cholesteryl oleate preparations were usually spherulitic (and crystalline). Because of the linear dichroism and linear birefringence due to the strong orientation of the lipids

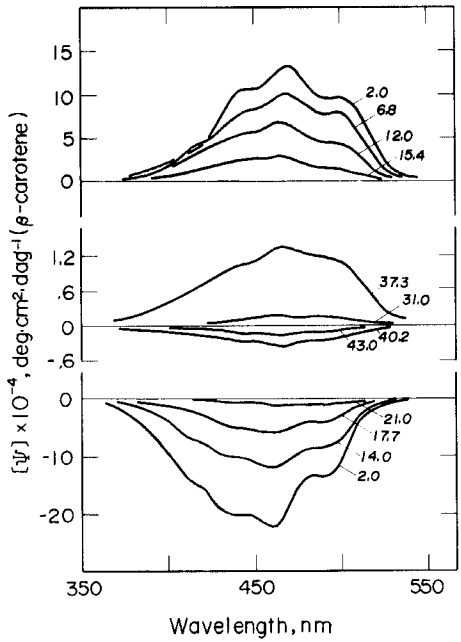

FIGURE 7 Temperature dependence of the induced circular dichroism of β-carotene dissolved in mixtures of cholesteryl ester and cholesterol. The numbers near the lines refer to the temperature. Lower panel, Case 1 (preparation, 2C-30A); middle panel, Case 3 (preparation, 1C-30); upper panel, Case 2 (preparation, 2C-10B).

the CD was so intensely dependent on the rotation angle that it was unable to detect a small inherent CD (ICD) if any.

The sign of the ICD depends on the helical sense of the cholesteric medium, the polarizing direction of the electric transition of dyes within the solute and the position of the reflective wavelength of the cholesteric mesophase (λ_0) relative to that of the intrinsic absorption band of dyes (λ_{ab}).[11,12] (λ_0 is the same as λ_m in this study.) In a left-handed cholesteric mesophase the ICD sign for a transition moment having a preferred orientation perpendicular to the long-axis of the mesophase molecule is negative if $\lambda_0 < \lambda_{ab}$. This is just the case for the ICD of the mixtures of cholesteryl ester and cholesterol in the normal case.

The helical sense of the cholesteric mesophase is always left-handed in our preparations as has already been mentioned. Therefore there is a good chance that the occurence of the abnormal cases (Cases 2 and 3) is due to the different position of λ_0 relative to that of λ_{ab} (as compared with what is in Case 1), or to the change of the relative position of λ_0 during temperature rising. Unfortunately the position of λ_0 could not be located because of too close approaching of the two CD curves, the ICD and pitch-band CD curves. Since the sign of the ICD is different depending on whether λ_0 is larger or smaller than λ_{ab} it may be natural to expect that the intensity of the ICD is very low if λ_0 is close to λ_{ab}. Actually Saeva and Wysocki[12] have pointed

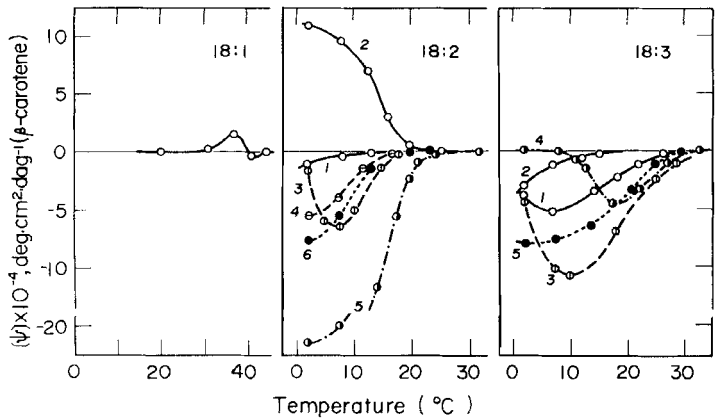

FIGURE 8 The effect of temperature on the specific ellipticity of β-carotene dissolved in mixtures of cholesteryl ester and cholesterol. Left panel: 1C-30. Middle panel: 1, 2C-10A; 2, 2C-10B; 3, 2C-20A; 4, 2C-20B; 5, 2C-30A; 6, 2C-40. Right panel: 1, 3C-00A; 2, 3C-00B; 3, 3C-10B; 4, 3C-30; 5, 3C-50.

out that the ICD intensity increases as the pitch of a cholesteric mesophase increases although such a relationship cannot continue indefinitely since the intensity must go to zero when the pitch increases to infinity to transform to the nematic phase as they have admitted. In opposition to this expectation the experimental result for 2C-30B indicates that the ICD intensity is very high despite of the apparent coincidence of these two wavelengths (see Figure 5, lower panel). This cannot be elucidated at the present moment. It seems that the ICD of β-carotene displays every possible singular behaviors when these two wavelengths are nearly or completely coincident.

Figure 8 shows how the specific ellipticity of β-carotene at the peak wavelength, $[\Psi]_{470\,nm}$, varies when the specimen is heated. The temperature range where the ICD of β-carotene is observed is shifted by several degrees to the low-temperature side in comparison with the temperature range for the cholesteric mesophase as obtained for the preparations which do not contain β-carotene. This is natural since the β-carotene lowers the transition temperatures as has already been mentioned. Further interpretation of this figure will be made later.

Table III lists the preparations containing β-carotene and their characteristics. Several distinct differences arise depending on whether β-carotene is dissolved in mixtures of cholesteryl ester and cholesterol (or triolein) or in r-LDL: (1) The optical activity of β-carotene is not usually induced in pure cholesteryl oleate, whereas it is most strongly induced in r-LDL which contains this ester. According to Chen and her coworkers[2] the specific ellipticity at the peak wavelength, $[\Psi]_{470\,nm}$, is $-47{,}900$ [deg · cm^2 · dag^{-1} (β-carotene)] and the ellipticity decreases when cholesterol is incorporated. Their preparations in question consist of cholesteryl oleate, apoLDL, cholesterol and β-carotene. It thus is known that apoLDL plays an important role in modifying the monotropic nature of cholesteryl ester to help it assume a chiral texture that induces the optical activity of β-carotene. Even the possibility that the optical activity is induced by apoLDL instead of cholesteryl ester cannot be ruled out.

Sklar and his coworkers[3] have shown that 2% 5,7,9-cholestatrienyl oleate in pure cholesteryl oleate, which itself is achiral, exhibits an ICD when the specimen had been previously heated to 55 °C and cooled at the rate of 10 °C/h and held at 35 °C for 1 h prior to initiating measurements. The molar ellipticity at the peak wavelength (325 nm) varies as the specimen is heated from $-140{,}000$ at 36 °C to $+12{,}000$ going through zero at 49 °C corresponding to the cholesteric–isotropic liquid transition. This positive ICD is not temperature dependent. The appearance of the ICD in pure cholesteryl oleate,

however, should seem to be due to the special pretreatment of the specimen and is only exceptional as in our 1C-30 preparation.

They have also observed the temperature-dependent negative ICD of the cholestatrienyl oleate in LDL between 5°C and 40°C. The molar ellipticity is considerably smaller ($[\Psi]_{max} > -12{,}000$) than that observed in pure cholesteryl oleate and a similar positive ICD is again observed at high temperature. They have proposed that the positive ICD in "isotropic" LDL could result either from lipid-lipid interactions or lipid-protein interactions.[3] Chen and her coworkers[2] have not observed such a sign change and this discrepancy cannot be explained at the present moment. The pitch-band CD of r-LDL has not been

TABLE III

Induced circular dichroism of β-carotene dissolved in the mixtures of cholesteryl ester and cholesterol or triolein

Prepn	Cholesteryl ester	Cholesterol (wt %)	Triolein (wt %)	β-Carotene (wt %)	Specific ellipticity ($[\Psi]_{470\ nm} \times 10^{-3}$) Value at 2°C	Max. value (and Temp., °C)
1C-00	18 : 1	0		0.7	0	0
1C-30		30		2.3	—	14.2 (37.3), −3.3 (40.2)
1C-50		50		2.5	0	0
1T-10			10	4.5	0	0
1T-30			30	1.3	0	0
2C-00	18 : 2	0		1.2		not determined
2C-10A		10		1.0	−10	—
2C-10B		10		3.0	+110	—
2C-20A		20		1.6	−15	−65 (7)
2C-20B		20		1.7	−54	—
2C-30A		30		0.7	−215	—
2C-30B		30		3.4	−190[a]	
2C-40		40		1.2	−74	−74 (ca. 2)
2C-50		50		1.0	0	0
2T-03			3	1.4	−4	—
2T-10			10	1.2	0	0
3C-00A	18 : 3	0		1.6	−38	−50 (7)
3C-00B		0		0.7	−28	−59 (10)
3C-10A		10		1.3	−59	−59 (ca. 2)
3C-10B		10		1.6	−68	−105 (10)
3C-20		20		1.2	−12	—
3C-30		30		0.6	0	−48 (17)
3C-50		50		2.0	−79	−79 (ca. 2)

1C preparations except 1C-30 were measured before the spherulitic structure appeared after they had been premelted and cooled; 1C-30 was measured on a special condition (see the text).

[a]Value at 7.5°C. The unit for $[\Psi]_{470\ nm}$, deg · cm^2 · dag^{-1} (β-carotene).

discovered by them,[2] it may be situated beyond the wavelength range they have covered and supposedly below 300 nm.

β-Carotene exhibits the ICD most strongly in cholesteryl linoleate preparations. The specific ellipticity at the peak wavelength (470 nm, same as in r-LDL) is much larger in our preparations than in the corresponding r-LDLs: The largest ellipticities are $-215{,}000$ and $-26{,}200$, respectively. Similarly the largest ellipticity in the cholesteryl linolenate preparations is $-105{,}000$, whereas it is $-15{,}000$ in the corresponding r-LDLs. The finding that the ICD of chromophore is far weaker in r-LDL corresponds to the observation by Sklar and his coworkers and leads to the idea that the structure of the core of r-LDL (and of LDL, Chen and her coworkers[2] have demonstrated that the characteristics of r-LDL and those of LDL are quite alike.) differs from that of the mixture of cholesteryl ester and cholesterol. Furthermore it is suspected that the core LDL structure is not a true cholesteric mesophase and this suspicion corresponds to those by Hamilton and his coworkers[13] who examined the motional properties of the cholesteryl esters of LDL using the NMR technique and by Sklar and his coworkers.[3] However, it should be noticed that the ICD intensity depends on the position of the reflective wavelength relative to that of the intrinsic absorption band of the chromophoric compounds.

The magnitude of the specific ellipticity of β-carotene becomes the largest when a proper amount of cholesterol is contained in preparation; the proper amount to be added to preparation is some 30% for cholesteryl linoleate and about 10% for cholesteryl linolenate. This condition naturally agreees with the best condition for the formation of the cholesteric mesophase of the preparation. In contrast with this result the magnitude of the specific ellipticity of β-carotene dissolved in r-LDL decreases constantly with increasing content of the cholesterol; according to Chen and her coworkers[2] the ellipticity varies from $-26{,}200$ to $-15{,}900$ with the addition of 0.16 mg cholesterol to 1 mg protein in their r-[cholesteryl linoleate + β-carotene] LDL which contains 1 mg protein and 1.4 mg ester.

Some of the preparations display the maximum magnitude of the ICD at a certain temperature between 2°C and 30°C (see Figure 8). This temperature is dependent on the preparation and naturally coincides with the temperature at which the cholesteric mesophase of the preparation is best assumed. The ICD intensity in r-LDL, however, decreases constantly with temperature (measurements started at 2°C.) and vanishes to zero at a temperature corresponding to the liquid crystalline-isotropic liquid transition.[2] This suggests that the lower limit of the chiral mesophase extend far below 2°C.

Triolein which is an isotropic liquid at 0°C interrupts formation of any ordered structure of cholesteryl linoleate and cholesteryl linolenate; an amount of 10% triolein was almost enough to make these cholesteryl esters isotropic liquids at room temperature. Chen and her coworkers[2] have also studied the effect of triolein on the ICD of β-carotene incorporated into their r-[cholesteryl linoleate + β-carotene] LDLs. They have been able to demonstrate that only a trace of triolein reduces the magnitude of the specific ellipticity as much as by 30%, and that when triolein amounts for 14% of the neutral lipids of the r-LDL the ICD disappears. It thus is known that the effect of triolein is also very striking on the structure of the core LDL. Cholesteryl oleate, which is most hard to be modified its monotropic nature among the three kinds of cholesteryl esters tested, strongly resisted triolein. The spherulitic (and crystalline) structure was produced even with the addition of 50% triolein.

CONCLUSION

It has been demonstrated that cholesterol modifies the monotropic nature of cholesteryl linoleate and cholesteryl linolenate to help them assume stable liquid crystals, and that the basis of the ICD of β-carotene is the cholesteric structure of these neutral lipids. The proper amount of the cholesterol to be added in relation to the formation of the cholesteric mesophase is some 30% for cholesteryl linoleate and about 10% for cholesteryl linolenate. The addition of cholesterol alone is not enough to modify the monotropic nature of cholesteryl oleate. Triolein interrupts formation of any ordered structure in cholesteryl linoleate and cholesteryl linolenate only with a small amount, say 10%, but not in cholesteryl oleate. The sense of the cholesteric structure assumed is left-handed, and the ICD of β-carotene incorporated into such a medium is essentially negative. The characteristics of the ICD of β-carotene is very different depending on whether β-carotene is dissolved in mixture of cholesteryl ester and cholesterol or in r-LDL. This suggests that the chiral structure of cholesteryl esters that induce the optical activity of β-carotene in the core of LDL is strongly affected by apoLDL.

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