Secondary structure in very low density and intermediate density lipoproteins of human serum

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Abstract We have studied the secondary structures of the protein moieties of very low density lipoproteins (VLDL) and intermediate density lipoproteins (IDL) of human serum by circular dichroism (CD). Two potential complications in the application of this technique to lipoproteins have been evaluated. First, using chronographic potentiometry in CD measurements of VLDL fractions of different mean particle diameters, we have analyzed statistically the CD signals in order to define the limits imposed by light scattering with respect to both particle diameter and wavelength. We found that CD measurements can be made to as low as 210 nm on particles of 520 Å or smaller, and to 194 nm on particles of 450 Å and below. Second, we have evaluated the CD contribution of lipid chromophores. Despite the high ratio of lipid to protein, the relative CD effect of the lipids is smaller than for low density lipoproteins (LDL), due to the extremely small ellipticities of natural VLDL triglycerides. Thus, CD measurements can be obtained with confidence on the preponderant bulk of normal VLDL. For the first time we report the CD spectra of human VLDL and IDL. In contrast with human LDL and the lipoproteins of the hypercholesterolemic rabbit, the entire CD spectrum of human VLDL shows increased ellipticity with decreasing temperature, which is completely reversible. We have found that the protein moieties of human VLDL and IDL contain substantially more helix (approximately 50%) than does that of human LDL.—**Chen, G. C., and J. P. Kane.** Secondary structure in very low density and intermediate density lipoproteins of human serum. *J. Lipid Res.* 1979. **20:** 481–488.

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Studies employing optical rotatory dispersion and circular dichroism have shown that both the low density lipoproteins (LDL) (1–7) and high density lipoproteins (HDL) (8, 9) of human serum contain appreciable secondary structure, replacing earlier models in which the protein was assumed to be chiefly in the extended form at the lipid–water interface. Such studies have yielded contents of helical and beta forms of 24–33% and 23–44% (5, 7), respectively, for LDL, and 70% and 11% (9), respectively, for HDL. However, optical studies of conformation in the triglyceride-rich lipoproteins have been impeded by light scattering at-

tributable to the large diameters of some of these particles. Another problem in the application of these techniques is the fact that the contribution of lipid chromophores could be very important in triglyceride-rich lipoproteins in view of their high ratios of lipid to protein. Recently we have studied the effect of lipids on thermal changes in the optical activity of human LDL (6, 7) and have shown that a substantial part of these changes may be attributable to lipids, especially in the visible and far ultraviolet regions. Human LDL has a high content of cholesteryl esters and phospholipids which exhibit temperature-dependent CD below 220 nm in organic solvents (7).

In the experiments described below we have addressed both of these problems. We have evaluated the effects of light scattering on the CD measurements as a function of particle diameter and have defined the range of diameters within which this technique is applicable. Further, we have studied the CD contributions of lipid chromophores from triglyceride-rich lipoproteins and have found them to be relatively smaller than those of LDL, largely due to the extremely small CD of native triglycerides.

For the first time, we report conformational data on the bulk of very low density lipoproteins (VLDL) and the cholesteryl ester-rich intermediate density lipoproteins (IDL) of human serum and have compared them with the lipoproteins of the cholesterol-fed rabbit (10, 11). We have found that the protein moieties in both human VLDL and IDL contain appreciably more helix than does that of human LDL. In addition we have studied thermal effects on their CD and have described CD spectral features which appear to discriminate human VLDL from LDL.

Abbreviations: VLDL, very low density lipoproteins; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; CD, circular dichroism.

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MATERIAL AND METHODS

Human VLDL (d < 1.006 g/ml) was prepared from blood serum of normal men and of patients with endogenous lipemia by repetitive ultracentrifugation (12). All subjects had fasted at least 14 hr. The VLDL had pre-beta mobility upon electrophoresis in agarose gel by the method of Noble (13) and contained no detectable albumin upon analysis by immunodiffusion (14). Species of VLDL were separated by gel permeation chromatography on 2% agarose (15). Particle sizes of the lipoproteins were measured by electron microscopy using negative staining with 2% potassium phosphotungstate (16). A portion of each VLDL preparation was extracted with 25 volumes of 95% ethanol–diethyl ether 3:1 (v/v) to obtain the lipid moieties for optical studies. The organic phase was evaporated under N_2 and the residue was extracted with hexane, followed by trifluoroethanol to permit measurement in the far ultraviolet region (7). Triglycerides for optical studies were separated from the VLDL lipid extracts by chromatography on silicic acid columns (17). The organic solvents, all of spectral quality, were from Matheson Coleman and Bell, except diethyl ether which was from the Eastman Kodak Company. Human IDL (1.019 > d > 1.006 g/ml) was prepared by ultracentrifugation once at a density of 1.006 g/ml and twice at 1.019 g/ml from serum of normal subjects and from patients with primary dysbetalipoproteinemia (type III hyperlipidemia) or familial hypercholesterolemia, all after an overnight fast. These lipoproteins had beta mobility upon electrophoresis in agarose gel. Both human VLDL and IDL were dialyzed against 10 mM sodium phosphate containing 0.01% EDTA (pH 7.5) for 24 hr at 4°C. VLDL (d < 1.006 g/ml), IDL (1.019 > d > 1.006 g/ml), and LDL (1.050 > d > 1.019 g/ml) were prepared similarly from serum of New Zealand white rabbits fed a diet containing 2% cholesterol (w/w) for one month. Serum cholesterol levels in these animals varied from 900 to 1500 mg/dl. The rabbit VLDL was separated by gel permeation chromatography on 2% agarose (15). The resulting fractions were dialyzed against 50 mM KF containing 0.1% EDTA and 1 mM K_2HPO_4 (pH 7.5) for 24 hr at 4°C. The protein concentration was determined (18) using hydrated bovine serum albumin as a working standard because of its ready solubility in water. The apparent protein contents of rabbit LDL were multiplied by 0.91 to correct for hydration of the albumin standard and for the relative chromogenicities of the two proteins. Light scattering by lipids was eliminated by extracting the reaction mixture with chloroform before colorimetry. The contents of apolipoprotein B of all these lipoproteins were de-

termined using tetramethylurea (19). Contents of free and esterified cholesterol (20), phospholipids (21), and triglycerides (22) were determined in extracts of these lipoproteins (chloroform–methanol 2:1, v/v). CD was measured with a Jasco SS-10 spectropolarimeter using a thermostatted sample chamber between 3° and 37°C, under constant nitrogen flush. Fused cylindrical silica cells (Pyrocell S-18-260) with path lengths of 0.5–2 mm were used. Protein concentrations of the lipoprotein used were 0.05–0.2 mg/ml in order to keep photomultiplier voltage below 1.0.

To provide a large number of repetitive observations of ellipticity for statistical analysis, a chronographic technique was employed. A calibrated DC potentiometric chronograph (Nationwide Electronic Systems, Inc.) delivered potentiometric data at 5-sec intervals to a Teletype recorder over a period of 30–60 min. Means, standard deviations, and signal to noise ratios were calculated for these data. Because in repetitive measurements at a constant wavelength noise is expressed as a root mean square function, the ratio of mean signal to its standard deviation expresses the signal to noise ratio.[2]

CD values were expressed in terms of mean residue ellipticity, $[\theta]$, in (deg cm^2)/dmol (protein residue) using an assumed mean residue weight of 115. The contributions to CD of the individual lipid constituents were calculated from the ellipticity of corresponding amounts of pure lipids in organic solvents (7). The fractions of helix and of beta-form in the protein moiety of the lipoproteins were determined by the method of Chen, Yang, and Chau (23) at 1-nm intervals over the range of 205–240 nm.

RESULTS

Fig. 1 shows the gel chromatographic elution pattern of one sample of VLDL prepared from the serum

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[2] Signal to noise ratio:

$$f(t) = s(t) + n(t)$$

where $f(t)$ is a measure of function over interval t, s is desired signal, and n is noise

$$n(t) = f(t) - s(t)$$

$$N^2 = \frac{1}{(t_1 - t_0)} \int_{t_0}^{t_1} n^2(t)dt$$

$$N^2 = \frac{1}{(t_1 - t_0)} \int_{t_0}^{t_1} [f(t) - s(t)]^2 dt$$

$$N^2 = \frac{1}{(t_1 - t_0)} \int_{t_0}^{t_1} [X - \bar{X}]^2 dt$$

where $\bar{X}$ is the mean signal; thus $N^2 =$ the variance, and $N =$ standard deviation.

of a patient with hyperprebetalipoproteinemia, illustrating the effect of particle size on light scattering. **Table 1** lists the particle sizes and detailed chemical compositions of the four fractions marked by arrows in Fig. 1. Clearly, when the VLDL particles are larger than 500 Å in diameter, the apparent absorbance at 280 nm is mainly due to light scattering. As the diameters of the VLDL particles decrease, their triglyceride content decreases and the percentages of the contents of total protein and of apolipoprotein B increase. CD measurements were made on these four fractions to as low a wavelength as possible, keeping the absorbance of the solution below 2.0. As shown in **Tables 1** and **2**, signal to noise ratios of about 5 were observed at wavelengths as low as 210 nm with particle diameters of VLDL less than 520 Å, whereas with particle diameters below 450 Å signal to noise ratios were about 8 at 210 nm and 3 at 194 nm. In the fraction of largest mean particle diameter, $[\theta]_{210}$ was immeasurable and the signal to noise ratio at 222 nm was 4. Thus the estimate of mean residue ellipticity at 222 nm for this fraction should be considered approximate and its true value may not be different from that observed in the fractions of smaller mean particle diameters. Because of the limitations imposed by light scattering, studies of the effect of temperature on the CD of VLDL were made on particles circa 420 Å in diameter.

Table 3 shows the lipid and protein compositions of the preparations of human VLDL, human IDL, and of VLDL, IDL, and LDL from hypercholesterolemic rabbits corresponding to the CD spectra below. Mean diameters of VLDL particles were less than 450 Å in each sample. CD measurements could be obtained on these preparations in 10 mM phosphate
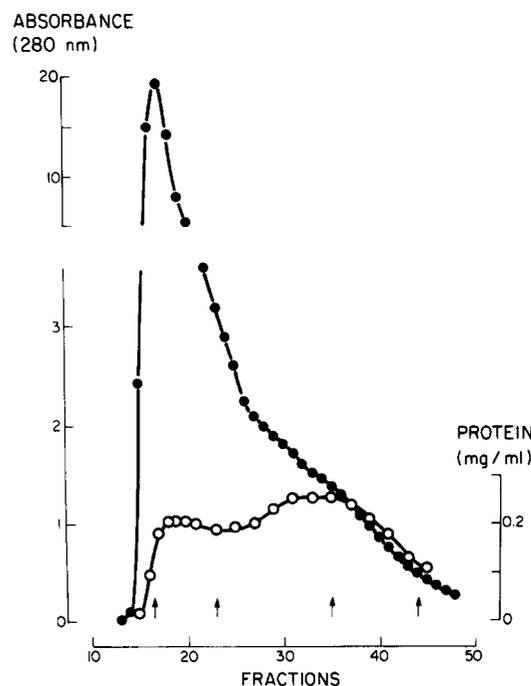
Fig. 1. Chromatographic elution pattern of human VLDL on 2% agarose gel. The column, 70 × 2.5 cm, was equilibrated with 0.1 M NaCl, 0.04% EDTA (pH 8.6), and 0.02% NaN₃. Individual points represent fractions of 9.2 ml each. Arrows denote fractions of mean particle diameters 710, 520, 430, and 410 Å, respectively (left to right). Open circles represent protein determination, closed circles, O.D.$_{280}^{1cm}$.

buffer or in 50 mM KF to wavelengths as low as 192 nm. The magnitude of ellipticity of human VLDL from different donors was found to vary within a relatively limited range $[-[\theta]_{222}:13.0-17.0 \times 10^3,$ $-[\theta]_{208}:13.1-16.7 \times 10^3$ (deg cm²)/dmol (protein residue)]. **Fig. 2** shows the CD spectra of a representative preparation of normal human VLDL between 190

TABLE 1. The size, chemical composition^a and CD data^b of four subfractions of human VLDL separated by gel permeation chromatography

Fraction	17	23	35	44
Mean particle diameter ± SE (Å)	710 ± 35	521 ± 20	436 ± 12	421 ± 17
Triglyceride	62	55	47	39
Phospholipid	10	15	17	17
Cholesterol	5	6	7	7
Cholesteryl ester	20	16	18	23
Total protein	3	8	11	14
Apolipoprotein B^c	20	29	37	40
$[\theta]_{222\ nm}$ ± SD	(−18,320 ± 4,220)	−14,060 ± 1,190	−13,650 ± 750	−12,940 ± 600
Signal/noise^d	4	12	18	21
$[\theta]_{210\ nm}$ ± SD		−16,670 ± 3,315	−14,300 ± 1,800	−13,060 ± 1,700
Signal/noise^d		5	8	8

^{a} In weight percent.
^{b} $[\theta]$ is in (deg cm²)/dmol (protein residue) at 25°C, uncorrected for contribution of lipid chromophores.
^{c} Percent of total protein.
^{d} The standard deviations (SD) for buffer baseline at 222 nm are ~470 "apparent" (deg cm²)/dmol (protein residue) for fractions 44, 35, and 23 and ~1,000 in fraction 17 because of increased amplification of the signal. The SD at 210 nm is ~520 for fractions 35 and 44, and ~1040 for fraction 23.

TABLE 2. The ellipticity[a] at 194 nm[b] of normal human VLDL, IDL, and LDL at three temperatures

Lipo-protein	4°C	25°C	38°C	P[c]
VLDL	23,090 ± 6,470	21,110 ± 6,440	20,663 ± 6,420	<0.001
IDL	19,620 ± 6,180	19,150 ± 6,960	18,450 ± 6,270	$0.01 < P < 0.025$
LDL	19,750 ± 4,520	22,093 ± 4,490	24,783 ± 4,750	<0.001

[a] In (deg cm²)/dmol (protein residue).
[b] The standard deviation for buffer baseline at 194 nm is ~3,500 "apparent" (deg cm²)/dmol (protein residue).
[c] Probability, Student's t test (unpaired), between 4° and 38°C.

and 245 nm at three temperatures. The contour of the CD spectrum of human VLDL resembles that of most globular proteins containing a moderate amount of helix, with minima at 210 and 222 nm and a peak at 194 nm. Thermal changes in the CD of human VLDL are completely reversible between 4° and 39°C. There is no detectable change in CD between 230 and 245 nm from 4° to 39°C. Below that wavelength the magnitudes of the ellipticity at both of the minima increase with decreasing temperature and, unlike LDL, the ellipticity of VLDL increases at 194 nm as temperature decreases. Statistical examination of thermal dependency in normal VLDL is presented in Table 2. The difference in ellipticity between 4° and 38°C was significant at $P < 0.001$ by Student's unpaired t test. Both the hexane-soluble and trifluoroethanol-soluble lipid fractions from this VLDL preparation show a small negative CD below 230 nm and 210 nm, respectively. However, there is no detectable CD between 190 and 240 nm for the triglyceride separated from the VLDL lipid extracts. After subtraction of the ellipticity corresponding to amounts of pure lipids in organic solvents, equivalent to those found in the VLDL, the minimum at 208–210 nm is decreased. For example, the ellipticities at 208 nm are decreased by 1.13, 1.33, and 1.75 × 10³ (deg cm²)/dmol (protein residue) at 39°, 28°, and 4°C, respectively, or about 10% at all three temperatures. Furthermore, the positive ellipticity increases in the range of 190–200 nm after correction. For example, the ellipticity

at 194 nm is increased by about 15% at all three temperatures. This is accompanied by a small blue-shift of the extrema. CD spectra of six VLDL specimens from patients with endogenous hyperlipemia were similar to those of two normal subjects.

Fig. 3 shows the CD spectra of the IDL from a normal subject. As with human VLDL, there is no detectable change in CD between 230 and 245 nm from 5° to 35°C. Between 200 and 240 nm the spectrum becomes more negative with decreasing temperature, and the minimum around 210 nm becomes more distinct. Ellipticity of IDL at 194 nm changes very little with temperature (Table 2). A slight blue-shift of the crossover point is noted with decreasing temperature, and, like VLDL, after subtraction of the CD attributable to lipids, the minimum at 208–210 nm is decreased. For example, the ellipticities at 208 nm are decreased by 0.74, 0.98, and 1.44 × 10³ (deg cm²)/dmol (protein residue) at 35°, 27°, and 5°C, respectively. These thermal effects are completely reversible. Similar results were obtained with IDL from subjects with dysbetalipoproteinemia and with familial hypercholesterolemia.

Fig. 4 shows the CD spectra at 3°, 25°, and 37°C of a subfraction of VLDL from a hypercholesterolemic rabbit, from the same region of the gel eluate as the human VLDL described above. The CD spectrum of the rabbit VLDL shows double minima between 205 and 230 nm and a peak at 196 nm. The minimum around 208 nm is strikingly more distinct than that

TABLE 3. Lipid and protein compositions of human and rabbit serum lipoproteins

	Normolipidemic VLDL (Human)	Normolipidemic IDL (Human)	Normolipidemic LDL[a] (Human)	Hypercholesterolemic Rabbit		
				VLDL	IDL	LDL
			weight %			
Triglyceride	57	24	3	6	8	0.5
Phospholipid	17	12	21	18	14	15
Cholesterol	6	13	10	14	13	14
Cholesteryl ester	13	33	44	53	53	56
Total protein	7	18	22	9	12	15
Apolipoprotein B[b]	39	74	92	46	76	90

[a] Data taken from Chen and Kane (7).
[b] Percent of total protein.
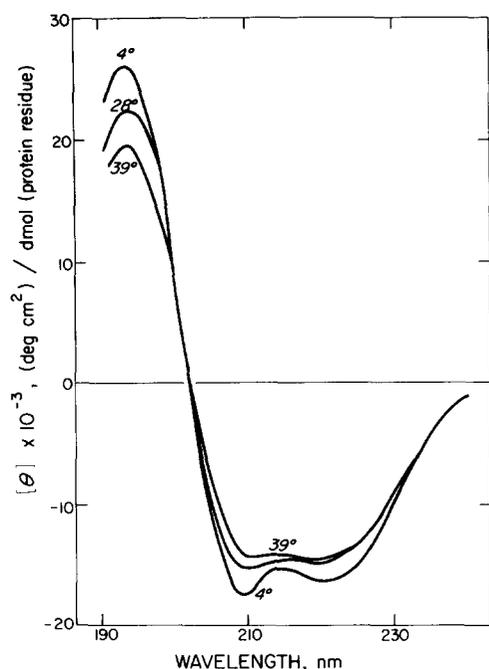
Fig. 2. Effect of temperature on the CD spectrum of normal human VLDL in 10 mM sodium phosphate buffer containing 0.01% EDTA, pH 7.5.
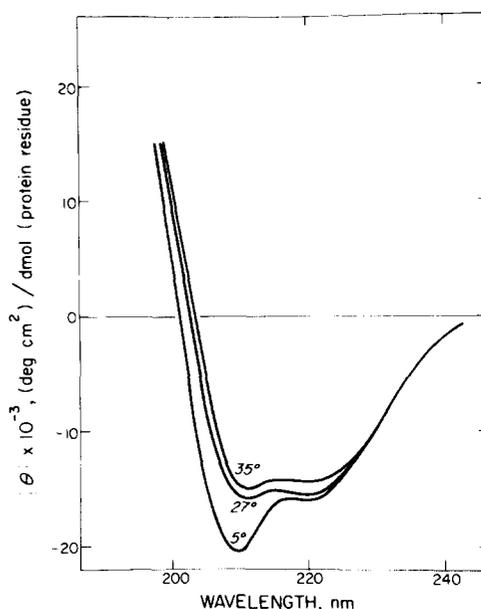

Fig. 3. Effect of temperature on the CD spectrum of normal human IDL in 10 mM sodium phosphate buffer containing 0.01% EDTA, pH 7.5.

around 222 nm. When the temperature is lowered from 37° to 3°C, the entire CD spectrum between 190 and 220 nm shifts downward and the minimum around 208 nm is further accentuated. This change is completely reversible. A blue-shift of the crossover point is noted with decreasing temperature, and there is no detectable change in CD between 220 and 240 nm from 3° to 37°C. After subtraction of the CD attributable to lipids, the magnitudes of ellipticity at 208 nm are decreased by 2.17, 2.85, and 4.18 × 10³ (deg cm²)/dmol (protein residue) at 37°, 25°, and 3°C, respectively, which are 12% of the uncorrected magnitude at 37° and 25°C, and 16% at 3°C.

Fig. 5 shows the CD spectra at three temperatures of IDL of hypercholesterolemic rabbits. The CD spectrum also shows a double minimum between 205 and 230 nm and a peak at 196 nm. When the temperature is lowered from 37° to 2°C, the entire CD spectrum between 190 and 240 nm shifts downward and the minimum at 208 nm is markedly enhanced. The thermal effect on CD is much less apparent above 215 nm. A blue-shift of the crossover point is noted with decreasing temperature. These changes are completely reversible.

Fig. 6 shows the CD spectra of LDL of the hypercholesterolemic rabbits at three temperatures. At 23°C, the contour of the CD spectrum shows double minima around 208 and 222 nm, of equal magnitude, and a peak at 196 nm. Above 230 nm, there is no detectable

change in CD between 2° and 37°C. Below that wavelength, however, the entire CD spectrum becomes more negative when the temperature is lowered from 37° to 2°C and the minimum around 208 nm, observed at 23°C, becomes more distinct. This minimum is absent at 37°C. Again, these changes are completely reversible. A small blue-shift of the crossover point is also noted with decreasing temperature. After subtraction of the ellipticity attributable to lipids in the
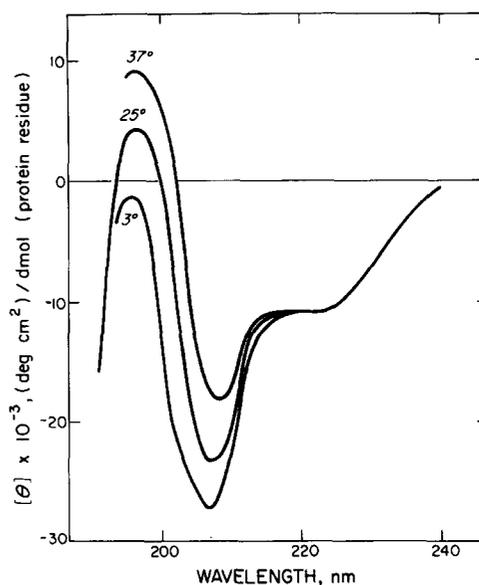
Fig. 4. Effect of temperature on the CD spectrum of hypercholesterolemic rabbit VLDL in 50 mM KF plus 1 mM K₂HPO₄ containing 0.01% EDTA, pH 7.5.
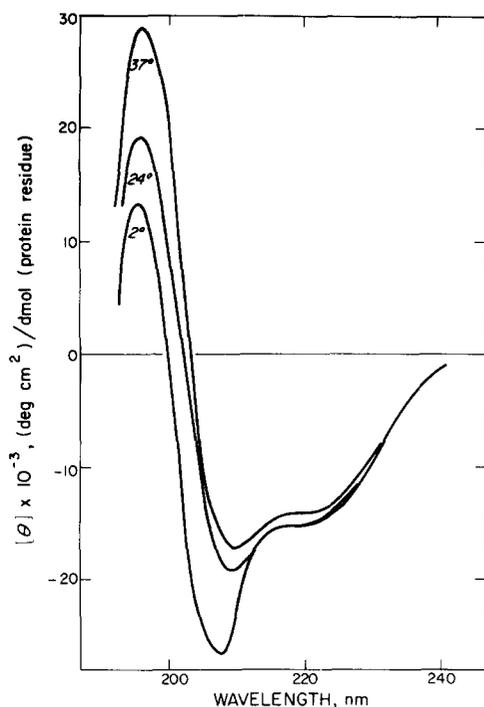
Fig. 5. Effect of temperature on the CD spectrum of hypercholesterolemic rabbit IDL in 50 mM KF plus 1 mM K_2HPO_4 containing 0.01% EDTA, pH 7.5.

rabbit IDL and LDL, the magnitudes of ellipticity at 208 nm are decreased by almost the same amount; e.g., 1.49, 2.04, and 2.99 × 10³ (deg cm²)/dmol (protein residue) for IDL and 1.40, 1.87, and 2.67 × 10³ (deg cm²)/dmol (protein residue) for LDL at 2°, 25°, and 37°C, respectively. That is about 11–13% of the uncorrected values at all three temperatures.

Table 4 lists the contents of secondary structure corresponding to the spectra described above, calculated by the method of Chen et al. (23) at intervals of 1 nm over the range of 205–240 nm. At 25°C about half of the protein moiety of each is in the helical form and 15–25% is in the beta form. Between 3° and 37°C, the apparent change in helical contents of these lipoproteins is smaller than the precision of the measurement. Similarly, the content of the beta form in the human VLDL, IDL, and LDL did not change appreciably between 3° and 37°C; whereas for the three lipoproteins of hypercholesterolemic rabbits, especially the VLDL and IDL, the apparent content of beta form showed a marked thermal dependence. Since the CD of VLDL and IDL of hypercholesterolemic rabbits exhibited profound thermal effects around 205–210 nm, we calculated the secondary structure by the same method over the range of 215–240 nm. For all lipoproteins, the helical contents were nearly the same as those calculated over the range of 205–240 nm, but the content of beta form differed

greatly. For example, the content of beta form changed from 3 to 20%, 6 to 15%, and 13 to 14% at 3°, 25°, and 37°C, respectively, for VLDL of hypercholesterolemic rabbits. The changes for the human VLDL and IDL were much smaller. The apparent content of beta form varied with the range of wavelength used by the same method; thus the reported value should be considered somewhat uncertain. Furthermore, when the CD spectra were corrected for the contribution of lipids as described above, the change in the content of helix and beta form was again negligible when calculated by the method of Chen et al. (23).

DISCUSSION

The application of chronographic potentiometry to CD measurement of VLDL permits statistical analysis of signal such that reliable data can be obtained on all but the largest particles. Between particle diameters of 520 and 400 Å the spectra are quite similar despite progressive changes in chemical composition with decreasing mean particle diameter. From Fig. 1 it is apparent that preparations of VLDL from which the largest particles were removed would still contain the preponderance of the apolipoprotein mass, even in VLDL from subjects with endogenous lipemia. Moreover, in normal VLDL such preparations usually contain over 95% of the apolipoprotein mass.
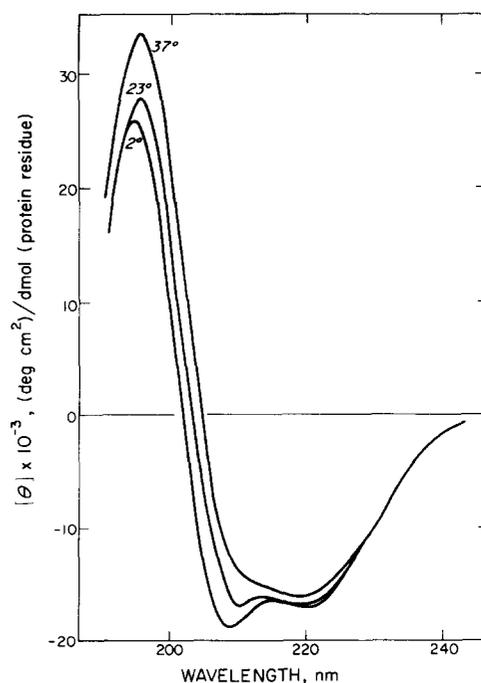
Fig. 6. Effect of temperature on the CD spectrum of hypercholesterolemic rabbit LDL in 50 mM KF plus 1 mM K_2HPO_4 containing 0.01% EDTA, pH 7.5.

TABLE 4. The fractions of helix and beta form of human and rabbit serum lipoproteins at three temperatures

	3°C		25°C		37°C	
	f_H	f_β	f_H	f_β	f_H	f_β
Normolipidemic VLDL (human)	0.55	0.15	0.50	0.16	0.48	0.14
Normolipidemic IDL (human)	0.58	0.17	0.51	0.22	0.48	0.19
Normolipidemic LDL (human)[a]	0.33	0.23	0.31	0.26	0.29	0.26
Hypercholesterolemic VLDL (rabbit)	0.50	0.03	0.47	0.06	0.43	0.13
Hypercholesterolemic IDL (rabbit)	0.60	0.04	0.53	0.18	0.49	0.21
Hypercholesterolemic LDL (rabbit)	0.57	0.19	0.53	0.26	0.48	0.33

[a] Data taken from Chen and Kane (7).

The contours of the CD spectra of human VLDL and IDL resemble that of human LDL; however, the magnitudes of the ellipticities of VLDL and IDL at both the minima and the peaks are much larger than those of LDL (Figs. 2 and 3), indicating a much higher content of helix. A discriminating characteristic of VLDL in these studies is increased ellipticity of the positive band at 194 nm with decreasing temperature (Table 2).

The CD spectra of VLDL and IDL of hypercholesterolemic rabbits showed a markedly more intense minimum at 208 nm than at 222 nm, whereas the minima of LDL were of nearly equal magnitude (Figs. 4, 5, and 6). The entire CD spectrum of the three rabbit lipoproteins showed increasingly negative ellipticity with decreasing temperature throughout the region of 190–240 nm similar to that of human LDL but contrasting with that of human VLDL. This similarity of human LDL and the cholesteryl ester-rich lipoproteins of the hypercholesterolemic rabbit may reflect either lipid–lipid interaction involving cholesteryl esters or the interaction of apoprotein with lipid. Cholesterol and cholesteryl esters, which comprise more than half of the mass of these lipoproteins, also show an increasingly negative CD band in that region with decreasing temperature (7).

Another interesting observation in these studies is the well-defined minimum at 208–210 nm observed at low temperature in the CD spectra of human IDL, and of VLDL and IDL of the cholesterol-fed rabbit. Though lipid chromophores contribute to the CD in that region, we found that the corrections for these chromophores, based on lipids in organic solvents, are of smaller relative magnitude than those for human LDL despite the smaller protein content of the former, because the CD contribution of naturally occurring triglycerides was found to be negligible. Correction for the effect of these lipid chromophores only slightly diminishes the thermal effect in the region of 208 nm, indicating that some additional factor is responsible. It is possible that this phenomenon may represent induced CD resulting from lipid–lipid

interactions involving cholesteryl esters. Also the high content of the arginine-rich apolipoprotein, observed in those lipoproteins showing the greatest thermal effect, i.e., human IDL and VLDL and IDL from the hypercholesterolemic rabbit, may be important in this respect because this protein has been shown to be highly helical in aqueous dispersion (10, 11).

The CD spectrum below 250 nm can be used to estimate the secondary structure of proteins (23). This method of analysis is best for proteins containing a moderate amount of helix. Estimates of content of beta form are still somewhat uncertain. In order to yield reliable estimates, the CD of the amide bonds must dominate the spectrum below 240 nm and overshadow the contributions of the nonpeptide chromophores. Despite the fact that the lipoproteins studied contain only 10–20% (by weight) of protein (Table 3), they all showed very strong CD spectra with minima at 208 and 222 nm characteristic of helix (Figs. 2–6). Helical content based on the method of Chen et al. (23) was found to be 48% for human VLDL and IDL and 43–49% for the cholesteryl ester-rich VLDL, IDL, and LDL of hypercholesterolemic rabbits at 37°C. Thus all of these lipoproteins contain appreciably more helix than does human LDL.∎

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