

ON THE STRUCTURE OF HUMAN SERUM HIGH-DENSITY
LIPOPROTEIN: STUDIES BY THE TECHNIQUE
OF CIRCULAR DICHROISM*

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Previous studies from this laboratory employing the technique of optical rotatory dispersion (ORD) have shown that human serum high-density lipoprotein (HDL) of d 1.063–1.21 gm/ml has a high content in α -helix which is to a large extent retained in its lipid-free form, apo HDL.¹

On the basis of the ORD parameters, an unordered structure was assigned to the nonhelical portion of the apoprotein, and this conclusion received corroboration from infrared spectroscopy analysis.² The present paper describes the optical properties of HDL and its products as studied by the technique of circular dichroism (CD). This method has the advantage over ORD of more clearly resolving bands relative to the optically active chromophores in the ultraviolet and far-ultraviolet regions. In agreement with the previous findings, both HDL and apo HDL exhibited a high α -helical content. The most significant observation was that the α -helical spectra were sufficiently different to permit a clear distinction between lipid-free and lipid-bound products. Moreover, they were found to differ in their optical behavior after either chemical modification by succinic anhydride or changes in the solvent medium.

The studies were carried out on the two HDL products, HDL₂ (d 1.063–1.125) and HDL₃ (d 1.125–1.21). Since the results were similar, this report will be limited to HDL₃.

Materials and Methods.—Methods for the isolation and purification of human serum HDL₃ have been reported previously.³ Apo HDL₃ was obtained from HDL₃ by extraction with 3:2 ethanol-ether mixture at -10° .⁴ Either HDL₃ or apo HDL₃ was succinylated by the reaction with succinic anhydride.⁵ Apo HDL₃ was reduced and alkylated as previously described.⁵ Relipidation of either apo HDL₃ or succinylated apo HDL₃ (S-apo HDL₃) was carried out with aqueous dispersions of HDL₃ phospholipids and the resulting apo HDL₃-phospholipid complex isolated by ultracentrifugation.⁵ The products studied had a protein:lipid ratio of 2:1 by weight. All the materials were extensively dialyzed against the desired buffers before analysis. On occasion, the preparations had to be clarified by centrifugation ($10,000 \times g$, 10 min).

Measurements of circular dichroism in the spectral region between 300 and 185 $m\mu$ were carried out at 27° in a Cary spectropolarimeter using quartz cells of 0.1-mm path length (Pyrocell, N.J.) with protein concentrations ranging between 1 and 2 mg/ml. All CD spectra were run at least twice. Baseline runs were made in the same cell, usually immediately after the sample run. The values of molar ellipticity $[\theta]$ in units of deg cm^2 /decimole were obtained from the relation: $[\theta] = \frac{\theta}{10} \cdot \frac{MRW}{lc}$, where θ is observed ellipticity; MRW (mean residue weight) is 112; l = cell path length in cm; and c is concentration of solute in gm/ml. In the spectral region studied, the absorption of HDL₃ lipids in trifluoroethanol was found to be negligible and was not included in the computations. This was also the case for the dispersions of HDL₃ phospholipids in aqueous solutions.

In the studies where urea, guanidine hydrochloride (G-HCl), and sodium dodecylsulfate (SDS) were used, the reagents, recrystallized from ethanol, were added as a solid to the protein solutions. Protein concentrations were determined by the Lowry method.⁶

Results.—Studies with HDL₃: The CD spectrum was characterized by two negative Cotton effects with maxima at 222 and 208 m μ , respectively, a crossover at 202 m μ , and a positive Cotton at 194 m μ . The same spectrum was obtained with HDL₃ preparations that had been (1) extracted with ethyl ether, (2) reacted with succinic anhydride, (3) dialyzed against pH 6, 8.6, and 10 buffers, (4) kept at 4°C for 1–2 weeks. Figure 1 represents the spectra of HDL₃ before

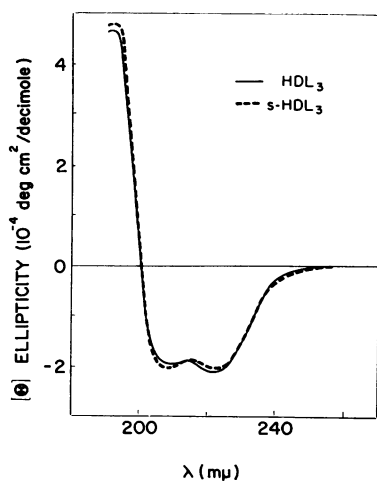


FIG. 1.—Ultraviolet circular dichroic spectra of HDL₃ and S-HDL₃.

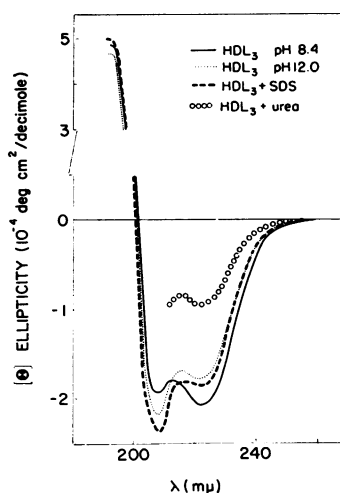


FIG. 2.—Effect of solvent medium on the circular dichroism spectrum of HDL₃.

and after succinylation. The α -helical content of HDL₃ estimated from the ellipticity value at 222 m μ was about 65 per cent.

HDL₃ dissolved in 8 M urea-phosphate buffer of pH 8.4 or 7 M G-HCl exhibited a marked decrease of the ellipticity value of the $n \rightarrow \pi^*$ transition. The spectrum below 210 m μ could not be followed because of solvent opacity (Fig. 2).

A significant change in spectrum was observed with HDL₃ preparations, in either buffers of pH between 11 and 12, or in neutral buffers made 0.1 to 0.2 M with SDS (Fig. 2 and Table 1). These changes were characterized by an increase of the negative ellipticity maxima at 208 m μ .

Studies with apo HDL₃: As compared with HDL₃, the apo HDL₃ spectrum (Fig. 3 and Table 1) was characterized by a 208-m μ band that was more intense than that at 222 m μ , and by a shift of the crossover point to 200 m μ . The same pattern was observed with apo HDL₃ after reduction and alkylation. Following succinylation (Fig. 3), the positions of all electronic transitions were shifted toward shorter wavelengths. The spectrum was characterized by a shallow band with a negative maximum around 220 m μ , a deep Cotton at 205 m μ , a

crossover point at 197 m μ , and a positive maximum at 190 m μ . A progressive blue shift was also noted in nonsuccinylated preparations as the pH of the solutions was increased from 10 to 12. At pH 12 the apo HDL₃ spectrum was not significantly modified in SDS solutions (0.1 to 0.2 M). In 8 M urea, on the

TABLE 1. Comparative parameters of ultraviolet circular dichroism between HDL₃ and apo HDL₃.

Materials	Position of the Cotton Effects				Molar ellipticity,	
	$n \rightarrow \pi^*$	$\pi \rightarrow \pi^*$		$n \rightarrow \pi^*/\pi$		
		parallel	perpendicular	parallel	π^*	
HDL ₃	222	208	194	1.10		
HDL ₃ -SDS	222	208	193	0.80		
apo HDL ₃	222	208	193	0.83		

contrary, there was no longer evidence for the α -helical parameters noted in apo HDL₃ dissolved in aqueous buffers (Fig. 3).

Studies on apo HDL₃-phospholipid complex (Fig. 4): After recombination with phospholipids, apo HDL₃ exhibited a CD spectrum very similar to that of HDL₃. Like HDL₃ it was more resistant than apo HDL₃ to spectral changes

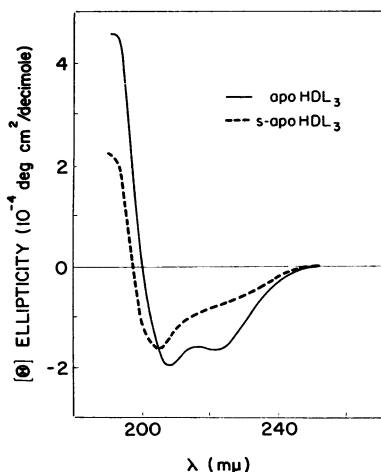


FIG. 3.—Ultraviolet circular dichroic spectra of apo HDL₃ before and after succinylation.

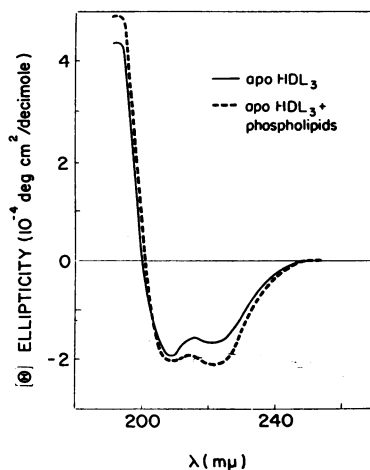


FIG. 4.—Ultraviolet circular dichroic spectra of apo HDL₃ before and after mixing with an aqueous dispersion of phospholipids.

induced by either pH variations, urea, or chemical modifications by succinic anhydride.

In the case of S-apo HDL₃, addition of phospholipids did not restore the HDL₃ spectrum completely. There was, however, a significant shift of the various electronic transitions toward longer wavelengths.

Discussion.—The present studies have shown that human serum HDL₃ and its delipidated product apo HDL₃ have circular dichroic bands in the position described for the α -helix⁷⁻¹⁰ and that they differ from each other in the relative

value of the maxima at 222 and 208 $m\mu$ (see Table 1). In descriptive terms, HDL₃ had a spectrum similar to that of poly-L-glutamic acid, whereas apo HDL₃ resembled that of poly- γ -methyl glutamate.⁷ Previous ORD studies had failed to identify two types of spectra in HDL₃ preparations before and after delipidation. This may be attributed to the greater resolving power of the CD technique, which facilitates detection of contributions of chromophores active in closely spaced regions of the spectrum.¹¹ The reasons for the two " α -helical" spectra are not apparent. One possibility is that there are no true conformational differences between the protein, with and without bound lipids, but that the observed spectral changes are due to changes in the environment around the active chromophores. This, however, appears improbable in view of the fact that a spectral shift HDL₃ \rightarrow apo HDL₃ was noted in HDL₃ preparations (containing lipids) treated with SDS or dissolved in high alkaline media. Such an observation plus the direct spectral analysis on phospholipids appear to rule out the possibility, postulated for other lipoprotein systems,¹² that the lipids contribute significantly in the over-all spectral properties of HDL₃. Thus, we favor the idea that upon delipidation HDL₃ protein exhibits spectral changes that may be a reflection of changes in length, tightness, or orientation of the various helical segments of the polypeptide chains.

Another important difference between HDL₃ and apo HDL₃ is in their sensitivity to chemical modification by succinic anhydride (Figs. 1 and 4). Whereas S-apo HDL₃ underwent a marked spectral shift of all its electronic transitions toward lower wavelengths, suggestive of a rather disordered structure, its parent S-HDL₃ was essentially unchanged in respect to HDL₃. Since under the conditions of succinylation employed, the number of sites reacting with succinic anhydride is the same in either lipidated or delipidated HDL₃,¹³ we may conclude that lipids protected the polypeptide chains from undergoing conformational transition. This conclusion is supported by the observed relative resistance of HDL₃ to spectral changes produced by variations in the nature of the solvent medium (pH, urea, and guanidine-HCl) and also by the acquisition of such a resistance in apo HDL₃ products relipidated by phospholipids.

Previous studies from this and other laboratories,^{3, 4, 14, 15} have shown that HDL₃ apoprotein has a multiunit structure. Experiments have also been presented to suggest that in HDL₃ the various subunits are held together by lipid bridges³⁻⁵ and that the bonding may be largely hydrophobic.⁵ If the above information is combined with the results of the current and previous ORD data,¹ one may postulate that in HDL₃ the helices relating to the various polypeptide chains are stabilized by the interaction of their hydrophobic interior with the nonpolar moiety of the phospholipids. Thus, one of the structural roles of phospholipids in HDL₃ may be that of stabilizing the secondary structure of the polypeptide chains, a structure that is in turn dependent upon the nature of each peptide backbone. This concept, which is also of relevance in biological problems such as that of lipoprotein biosynthesis, is currently undergoing experimental testing.

Summary.—(1) Human serum high-density lipoprotein of d 1.125–1.21 (HDL₃) and its delipidated product apo HDL₃ had circular dichroic spectra

indicative of a high α -helical content. The two products were distinguishable by the relative intensity of the bands at 222 and 208 $m\mu$ and the position of the crossover point.

(2) A spectral shift, apo HDL₃ \rightarrow HDL₃, was observed by mixing apo HDL₃ with aqueous dispersions of phospholipids.

(3) Both HDL₃ and apo HDL₃-phospholipid complex were more resistant than apo HDL₃ to spectral changes induced by variations in the nature of the solvent medium.

(4) The results were taken to indicate that lipids of HDL₃ serve a structural role by stabilizing the predominantly α -helical configuration of the various polypeptide chains, probably through hydrophobic interactions.

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Abbreviations used: HDL₃, lipoprotein of d 1.125–1.21 gm/ml; apo HDL₃, protein of HDL₃ obtained by extraction with organic solvents; S-HDL₃ and S-apo HDL₃, succinylated HDL₃ and apo HDL₃, respectively; SDS, sodiumdodecylsulfate; G-HCl, guanidine hydrochloride.

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