OBSERVATIONS ON THE CONFORMATION OF HUMAN BETA LIPOPROTEIN: EVIDENCE FOR THE OCCURRENCE OF BETA STRUCTURE

BY A. M. GOTTO, R. I. LEVY, AND D. S. FREDRICKSON

LABORATORY OF MOLECULAR DISEASES, NATIONAL HEART INSTITUTE,
BETHESDA, MARYLAND

A knowledge of the structure of the protein moiety of beta lipoprotein is of obvious interest in view of its possible relationships to the manner in which lipids are transported by this macromolecule. Investigation of the lipid-free protein has recently become feasible with the solubilization of an apoprotein derivative by succinylation.[1-5] As described herein, the preparation of a nonsuccinylated beta apoprotein is now possible. The preparation of this apoprotein, which retains aqueous solubility at a concentration of sodium decyl sulfate of only 0.1–0.2 mM, led to a reinvestigation of the conformation of native beta lipoprotein in order to determine what changes were caused by delipidation. Our results, described in this communication, indicate a significant quantity of β-structure (pleated sheet) in β-lipoprotein and β-apoprotein. Possible reasons for different conclusions concerning configuration by other workers[6,7] are discussed.

Methods.—Optical rotatory dispersion and circular dichroism: Optical rotatory dispersion (ORD) and circular dichroism (CD) were measured in 0.05 M potassium phosphate buffer (with 0.01% EDTA), pH 7.9, with a Cary 60 spectropolarimeter at 24°. Protein concentrations of β-lipoprotein and β-apoprotein varied from 0.05 to 1.0 mg/ml, and quartz cells (0.5–5.0 mm of light path) were used. Values were corrected for the refractive index of the solvent and were used to calculate the reduced mean molar ellipticity $[\theta']$ in deg cm^2 decimole^{-1} and reduced mean residue rotation $[m']$. All measurements were made in triplicate. Reproducibility of measurements was within 3% for CD to 210 mμ (5% for 200–210 mμ) and for ORD to 220 mμ.

For purposes of comparison, solutions containing the random, β-, and helical-structures of poly-L-lysine (Pilot Chemical Co., mol wt 110,000) were prepared by the methods of Sarkar and Doty.[10] Although the CD spectra of these solutions were very similar to those described by others,[10,11] the values of $[\theta']$ for this preparation were only 55–60% of those reported for preparations of lower molecular weight.

Infrared (IR) spectroscopy: Measurements were made in calcium fluoride cells (0.1 mm in path length) with a Beckman IR 7 infrared spectrometer calibrated against water. Actual frequencies were 3 cm^{-1} less than the readings given by the instrument. Unless otherwise indicated, uncorrected values are presented here. The solvent system was 0.05 M potassium phosphate buffer (with 0.01% EDTA) in 99.8% D$_2$O at pH 7.9, and protein concentrations varied from 4 to 20 mg/ml. The β-form of poly-L-lysine was prepared for 10 min at 50° by heating a solution adjusted to pH 11.4 with NaOD for 10 min at 50° as previously described,[10] except that the protein concentration was increased tenfold to 10 mg/ml. The precipitate that formed when the pH was raised was removed by centrifugation. Turbidity and aggregation occurred when the supernatant solution was heated at 50°, but this did not interfere with the measurement of its infrared spectrum.

Preparation of β-lipoprotein and β-apoprotein: Human serum β-lipoprotein was prepared by ultracentrifugation between densities 1.019 and 1.063 or by precipitation with heparin and manganese followed by ultracentrifugation as previously described.[3] All preparations used were immunochemically pure. The techniques for succinylation, de-

lipidation, lipid analyses,[3] and immunochemistry[8, 9] have been described. For purposes of solubilization, 3–4 mg of delipidated β-lipoprotein were incubated for 16 hr at 37° with 0.13 *M* tris(hydroxymethyl)aminomethane buffer, pH 8.3 (0.01% EDTA) which contained 60 mM sodium decyl sulfate. The concentration of detergent was then decreased by dialysis against the above buffer system containing only 0.1–0.2 mM sodium decyl sulfate. β-Apoprotein, thus prepared, contained no cholesterol or triglyceride detectable by thin-layer chromatography (1 to 1.5% phospholipid by chemical analysis), formed precipitin lines of immunological identity with native β-lipoprotein, and retained activity with rabbit antisera[9] to native β-lipoprotein.

Results.—Optical rotatory dispersion: β-Lipoprotein exhibited a negative Cotton effect at 232 mμ with $[m']$ of -6000 to -8300 for different preparations (Fig. 1). There was an apparent slight shoulder from 215 to 220 mμ. Succin-
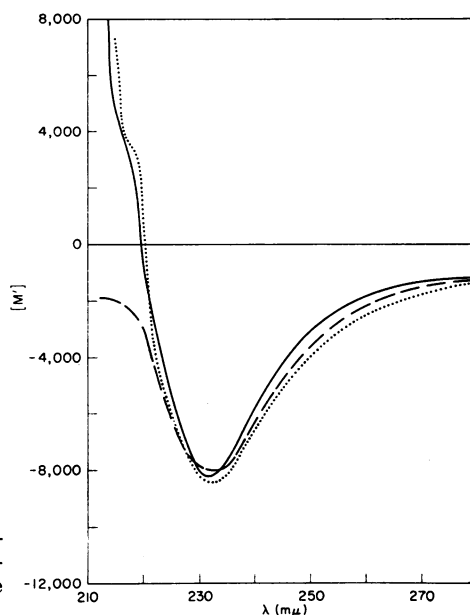

FIG. 1.—Optical rotatory dispersion of β-lipoprotein ($\cdots$), β-apoprotein (——), and β-lipoprotein plus 20 mM sodium decyl sulfate (- - -).

ylation of β-lipoprotein did not significantly alter the spectrum. β-Apoprotein had a similar dispersion with $[m']$ of -8100 (Fig. 1). The effect of the addition of 20 mM sodium decyl sulfate to β-lipoprotein, succinylated β-lipoprotein, or β-apoprotein was evident especially below 230 mμ where the strong positive rotations normally present were abolished (Fig. 1). A Drude plot of $[\alpha]$ λ^2 against $[\alpha]$ for β-lipoprotein was complex and did not yield a straight-line junction. When the dispersion values of β-lipoprotein were plotted by the method of Moffitt and Yang ($[m']$ $(\lambda^2 - \lambda_0^2)$ against $(\lambda^2 - \lambda_0^2)^{-1}$) with $\lambda_0 = 212$ mμ, a reasonably straight-line fit was obtained between 300 and 425 mμ, but not above or below these wavelengths. The b_0 values from the slopes of the lines between 300 and 400 mμ varied from 0 to -100 for different preparations.

Circular dichroism: Both β-lipoprotein and β-apoprotein had spectra similar to that of the β-form of poly-L-lysine (β-poly-L-lysine) with a single negative trough at 216–218 mμ (Figs. 2 and 3). This spectrum has been described as characteristic of β-structure.[10] $[\theta']_{217}$ were $-12,400$ and $-8,600$ for β-lipopro-
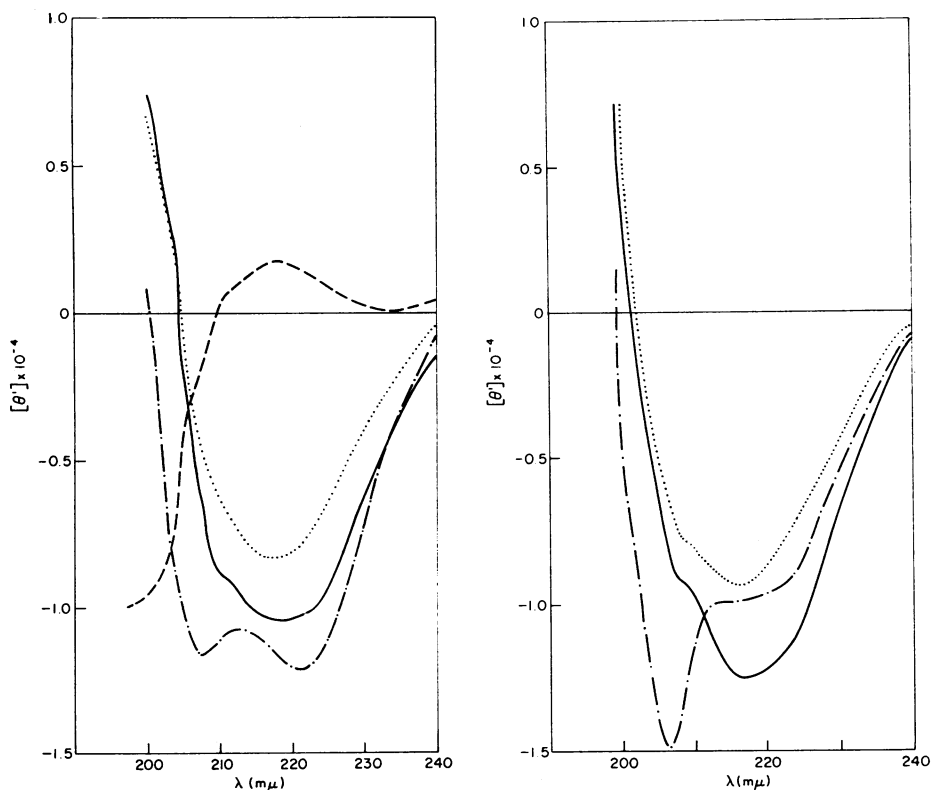
(*Left*) Fig. 2.—Circular dichroic spectra of β-lipoprotein (——), the β-form of poly-L-lysine (· · ·), the α-helical form of poly-L-lysine (—·—), and the disordered form of poly-L-lysine (- - -).

(*Right*) Fig. 3.   Circular dichroic spectra of β-lipoprotein (——), β-apoprotein (· · ·), and β-lipoprotein plus 20 mM sodium decyl sulfate (—··—).

tein and β-apoprotein, respectively.   Definite negative troughs at 208 and 222 mμ which have been associated with helical structure,[10] as demonstrated by the helical form of poly-L-lysine (Fig. 2), were not observed.   However, an apparent shoulder was noted in the spectra of β-lipoprotein and β-apoprotein at 208 to 210 mμ.   The noise-to-signal ratio increased considerably below 210 mμ, but this slight shoulder may have reflected a small helical content.   A similar small negative deflection at 208 mμ was recorded in the tracing of α-crystallin, another protein with reported β-structure (see Fig. 3 of ref. 11).

The spectra of β-lipoprotein or β-apoprotein were significantly altered by the addition of 20 mM sodium decyl sulfate (Fig. 3).   The negative Cotton effect at 216–218 mμ was reduced, and a deep negative trough appeared at 205–208 mμ. In the presence of 7 *M* urea, β-apoprotein no longer exhibited a negative trough at 216–218 mμ.

Infrared spectroscopy: β-Lipoprotein had an asymmetric peak with a definite maximum at 1617–1620 cm^{-1} (corrected) in D_2O (Fig. 4).   The exact position of the maximum varied from 1617 to 1625 for different preparations of β-lipoprotein.

Small shoulders were probably present at 1640 and 1650 cm^{-1}, the two frequencies assigned respectively to random structure and α-helix.[10, 11]   A definite, reproducible shoulder was always seen at 1680–1690 cm^{-1}.   For comparison with α-helical and β-structures, spectra were recorded for myoglobin (Mann) and the β-form of poly-L-lysine, respectively (Fig. 4).   These spectra showed maxima
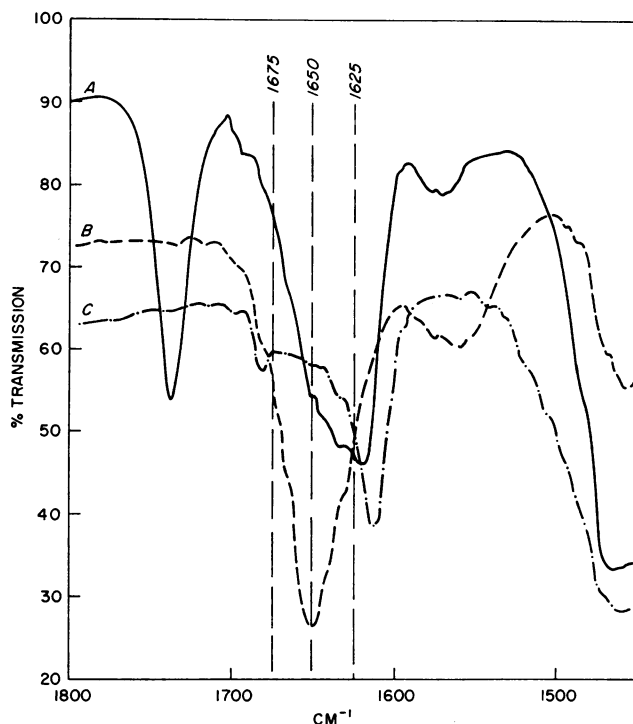

Fig. 4.—Infrared spectra of β-lipoprotein, 18 mg/ml (———) myoglobin, 20 mg/ml (- - -), and the β-form of poly-L-lysine, 6 mg/ml (–·–).

at 1650 cm^{-1} for the α-helix and at 1610 and 1680 cm^{-1} for β-structure with antiparallel chains consonant with other reports.[12–14]   When 100 mM sodium decyl sulfate was added, the spectrum of β-lipoprotein was markedly changed. The peak at 1617–1620 cm^{-1} and the shoulder at 1680 cm^{-1} were abolished and replaced by a more symmetrical peak with a maximum at 1640–1650 cm^{-1}. Although we have not yet obtained preparations of β-apoprotein at sufficient concentrations to obtain completely satisfactory infrared spectra, measurements with dilute solutions indicated that the maximum at 1617–1620 cm^{-1} was retained and that there was a relative increase in the size of the peak between 1630 and 1640 cm^{-1}.   Addition of sodium decyl sulfate to the β-apoprotein produced similar changes.   The sharp maximum at 1735 to 1740 cm^{-1} with β-lipoprotein, due to ester linkages in the lipids of the lipoprotein, was not observed with preparations of β-apoprotein.

Discussion. —These results indicate that β-lipoprotein has a significant content of β-structure, disordered, and probably also contains some helical structure. The values of $[m']_{233}$ and $[\theta']_{217}$ are $-6,000$ to $-8,300$ and $-12,400$ as compared to $-6,300$ ($[m']_{233}$) and $-14,400$ ($[\theta']_{217}$) for β-poly-L-lysine.[11] In D_2O, the IR spectrum of the β-conformation has a maximum for the amide I linkage which is well below that shown by a random structure (1640 cm^{-1}) or the α-helix (1650 cm^{-1}). Reported values are 1632 cm^{-1} for β-lactoglobulin,[15] 1628 cm^{-1} for polyglycine I,[12] and 1610 cm^{-1} for β-poly-L-lysine[10] as compared with 1617–1620 cm^{-1} for β-lipoprotein. The appearance and symmetry of the peak with β-lipoprotein is very similar to that recorded for β-lactoglobulin.[15] The shoulder at 1680 cm^{-1} is consistent with the presence of some antiparallel chain type of β-structure.[12, 14] An interpretation of the nonlinear Moffitt-Yang plots below 300 and above 450 mμ is not clear. The values of b_0 for the plot from 300 to 425 mμ, viz., 0 to -100, are similar to those found for α-crystallin[11] and β-poly-L-lysine,[11] and they contrast with the usual value of -650 assigned to a 100 per cent α-helical structure.[16]

The addition of sodium decyl sulfate to β-lipoprotein or β-apoprotein leads to marked shifts in the CD (Figs. 2 and 3) and IR (Fig. 5) spectra and a negative shift of ORD below 230 mμ. These changes are consistent with a transition from β-structure to a more disordered one. We cannot rule out the possibility that sodium decyl sulfate induces some increase in α-helical structure. It seems likely that the conformational changes attributed by other workers to delipidation were possibly caused by the presence of sodium dodecyl sulfate,[6, 7] since the apoprotein retains the β-structure in the absence of detergent (Fig. 3). Similarly, failure to find evidence of β-structure by IR measurements[6] may have been due to the fact that the determinations were performed in the presence of sodium dodecyl sulfate. Great caution must be used in interpreting structural alterations induced by this detergent since it may increase α-helical content,[17] convert helical to β-structure,[10] abolish β- but not helical-structure,[18] or act as
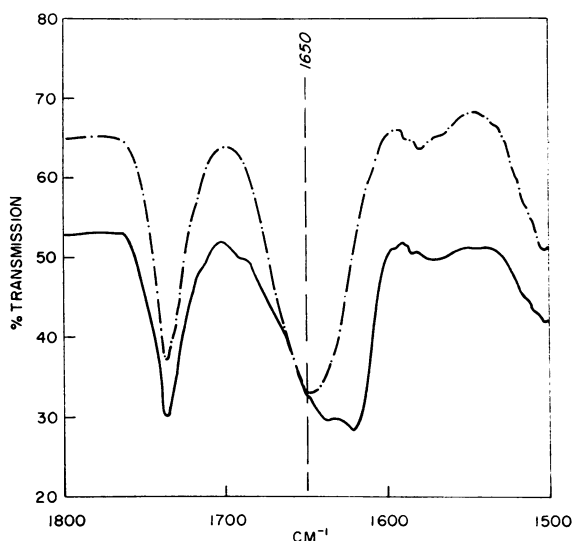

Fig. 5.—Infrared spectra of β-lipoprotein (——) and β-lipoprotein plus 100 mM sodium decyl sulfate (—·—). Protein concentrations were 9 mg/ml.

denaturing agent[19] with different proteins. The small shoulders at 208–210 mμ with CD and at 1650 cm^{-1} with IR measurements may reflect some helical content, but this would seem to be of relatively less quantitative importance than the β-structure.

Summary.—The occurrence of β-structure in β-lipoprotein is indicated by a negative trough at 216–218 mμ in circular dichroism and a maximum at 1617–1620 cm^{-1} in infrared spectroscopic measurements. A shoulder at 1680 cm^{-1} suggests some content of antiparallel chains. The spectra are suggestive of some content of helical or disordered structure, or both. Beta-apoprotein retains the β-configuration although there probably is some increase in disordered structure. The ordered β-structure of β-lipoprotein or β-apoprotein was abolished by the presence of sodium decyl sulfate.

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