Proteolysis of Human Serum β-Lipoprotein*

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It has been well known for some time that many native proteins are attacked only extremely slowly by proteolytic enzymes, or often not at all (1-6). The seeming resistance of human serum lipoproteins toward proteolysis therefore appeared to be a common phenomenon. Recently, interactions between β-lipoproteins and polyanions, in particular amylopectin sulfate, have been studied (7, 8), and this work has led to the development of improved techniques for the purification (9) and the characterization (10, 11) of human serum β-lipoprotein. The availability of these new techniques and the awareness of the importance of our increasing understanding of the lipoprotein-polyanion interactions for the elucidation of lipoprotein structure appeared to warrant a new approach to the problem of proteolysis of human serum β-lipoprotein; the results of this study are presented herein.

EXPERIMENTAL PROCEDURE

β-Lipoprotein—Pooled, fresh human serum (1,700 ml) was obtained from the chemical laboratory of a diagnostic hospital. The serum was free of oxalate, citrate, or heparin and contained 0.5 to 7 mg of β-lipoprotein per ml, according to nephelometric analysis (see below). Upon addition of 300 ml of 1% aqueous solution of sulfated cornstarch (potassium salt, containing 15.9 to 17.2% sulfur), the β-lipoprotein precipitated and was collected by centrifugation at 3,000 r.p.m. The lipoprotein-polyanion precipitate was dissolved by the addition of 45 ml of 18% NaCl solution, and the density of the resulting turbid solution was adjusted to 1.063 by the addition of water. This solution was subjected to ultracentrifugal flotation for 22 hours at 40,000 r.p.m. in density gradient tubes (7.5 ml of the lipoprotein solution layered beneath 4 ml of an NaCl solution of density 1.005) in the No. 40 rotor of a Spinco model L ultracentrifuge. Fractions were withdrawn from each tube through the center opening in the tube cap by means of a syringe with a 3-inch-long needle, cut straight at the tip. A pink layer, visible to the eye about 1.5 inches from the top, was withdrawn first (3.5 to 3.6 ml per tube). Then the layer above (1.8 to 1.9 ml per tube) and finally the top layer (1.3 to 1.4 ml per tube) were collected. A floating, fat “membrane” was combined with the top layer after removal of the tube cap. Table I indicates the amount of lipoprotein recovered in each fraction, and the densities at which these fractions accumulated in the ultracentrifugal field.

For the purpose of the present studies, only the third layer was purified further by fractional precipitation. This fraction (40 ml) was diluted with an equal volume of water, and 2 ml of a 1% aqueous amylopectin sulfate solution (potassium salt, 14.6 to 16.4% sulfur) were added. The resulting precipitate was collected by centrifugation at 37,000 x g and was dissolved in 1 ml of 18% NaCl solution; the supernatant solution was kept for further addition of amylopectin sulfate. Lipoprotein was precipitated from its redissolved complex with polyanion by the addition of 19 ml of 0.02 M NaH2PO4 solution. This step served to remove other nonprecipitated, adhering protein. The precipitate was collected by centrifugation and dissolved in 18% NaCl solution (0.5 ml), and the polyanion was removed by precipitation with barium acetate (1 ml of a 10% solution for each 3 ml of 1% amylopectin sulfate solution used). Excess barium was eliminated by the addition of sodium sulfate (1 ml of a 0.5 M solution for each milliliter of barium acetate solution used). The β-lipoprotein in the remaining solution (total volume, 6 to 7 ml) was stabilized by the addition of 0.3 ml of a 0.2% aqueous solution of hydroquinone. Further fractions of β-lipoprotein were obtained in the same way by the addition of gradually increasing amounts of amylopectin sulfate to the appropriate supernatant; 15 subfractions of β-lipoprotein were thus prepared with the use of a total of 45 ml of 1% amylopectin sulfate solution.

The physical and chemical properties of these fractions will be reported in a separate publication. All subfractions resemble each other in most respects; they differ from the corresponding parent lipoprotein solution in that they all are free from detectable amounts of albumin or other serum proteins (see electrophoretic patterns in Fig. 1). There was no detectable difference in effects of proteolytic digestion between the various subfractions.

Proteolytic Enzymes—Trypsin, twice crystallized; α-chymotrypsin, three times crystallized; papain, twice crystallized; and pepsin, twice crystallized, were purchased from the Worthington Biochemical Corporation.

Methods

Assay of β-Lipoprotein—The nephelometric method described earlier (10, 11) was employed. For the determination of all purified lipoprotein fractions, 0.3 ml of a 0.01% solution of amylopectin sulfate (potassium salt, 16.6% sulfur) was used as the reagent, in the presence of 0.1 ml of 0.1 M CaCl2 solution; 0.16 ml of 0.1% amylopectin sulfate, in the absence of added CaCl2, served as the reagent for the determination of β-lipoprotein in whole serum.

Thin Layer Chromatography—This was performed on Silica gel G layers of 250-μ thickness. The plates were developed to a
height of 15 cm in a solvent of chloroform-methanol-17% ammonia (2:2:1, v/v) in the first dimension (or by one-dimensional chromatography), and in a solvent of phenol-water (3:1, w/v) in the second dimension, according to Fahmy et al. (14). Spots of amino acids or peptides were made visible by spraying with ninhydrin.

**Paper Electrophoretic Analyses**—A Beckman-Spinco model R apparatus was used, with Veronal-citrate buffer, pH 8.6, ionic strength 0.16. Paper strips were stained with Ponceau 2R for protein, and with oil red O for lipids. The stained bands were converted into line tracings by an Analytrol recording densitometer.

**Isolation of Lipoprotein after Proteolytic Digestion**—Amylopectin sulfate was added to the digest in order to precipitate the remaining lipoprotein; this was done in the same manner as described above for the subfractionation of β-lipoprotein, except that the amount of polyanion added was sufficient to precipitate the whole lipoprotein present in a single fraction. The lipoprotein-polyanion complex was then treated as indicated in the subfractionation procedure.

**Chemical Analyses of β-Lipoprotein**—Total protein was determined by the method of Lowry et al. (15), with crystalline bovine serum albumin serving as reference protein, and total cholesterol and esterified cholesterol according to Schoenheimer and Sperry (16); cholesterol esters were computed by multiplication of the values of esterified cholesterol by 1.67. Phospholipids were measured by extracting the lipid-soluble phosphorus according to Bloor (17) and determining the phosphorus by the procedure of Fiske and SubbaRow (18). Total phospholipids were calculated by multiplying the value of lipid phosphorus by 25.0. Triglycerides were determined by the method of Van Handel and Zilversmit (19).

**Ultracentrifugal Flotation Rates of β-Lipoproteins after Proteolytic Digestion**—These rates were measured in the No. 40 rotor of a Spinco model L ultracentrifuge. Amounts of 5.0 ml of digested β-lipoprotein, or of the same lipoprotein before digestion, in NaCl solution of 1.005 density were layered beneath 9.5 ml of NaCl solution of 1.005 density. After centrifugation at 40,000 r.p.m. for 22 hours, 18 layers of 0.6 to 0.7 ml were with-

**Isolation of Peptides**—The peptides cleaved off during proteolytic digestion of the β-lipoprotein were isolated by precipitation of the remaining lipoprotein with amyllopectin sulfate (see third step of lipoprotein purification) and subsequent dialysis of the supernatant solution against repeated changes of water in Visking dialysis tubing of 0.008-inch wall thickness. The combined dialysates were concentrated by freeze drying. Incubation of an aliquot of the concentrate with casein for 24 hours and measurement of the absorption at 280 μM of the trichloroacetic acid-soluble material showed that considerably less than 1% of the proteolytic enzyme was present in the dialysate. Over 90% of the peptides liberated during proteolysis had passed across the dialysis membrane, as indicated by comparison of the absorption at 280 μM of the trichloroacetic acid-soluble part of the solution remaining inside the dialysis bag with that of the combined dialysates.

For the purpose of comparing the amino acid composition of these peptides with that of the parent β-lipoprotein, the detached peptides were hydrolyzed with 20% HCl for 22 hours at 110°. A sample of undigested β-lipoprotein, after removal of lipids according to Scamu, Lewis, and Bumpus (20), was hydrolyzed in the same manner and served as a control.

**RESULTS**

The tendency of β-lipoprotein to precipitate with polyanions, as measured nephelometrically at high dilutions, indicates whether the β-lipoprotein is present in its native or in an altered configuration. Treatment of β-lipoprotein with weak acid or alkali, exposure to elevated temperatures, or freeze-drying were found to decrease significantly the nephelometric index (see Table II).

In contrast to the marked effect of the chemical and physical means mentioned, proteolytic enzymes were observed not to

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**Fig. 1.** Typical paper electrophoresis patterns of a preparation of human serum β-lipoprotein used for the proteolytic digestions. The tracings of the intensities of lipid and protein stains are superimposed, and the origin of migration is marked by a dashed line. Slight intensities of both lipid and protein stains between the origin and lipoprotein peak are due to saturation of the paper strip with lipoprotein. This was shown by changing the conditions of electrophoresis, i.e., by using a horizontal instead of a vertical setup and by applying the protein at slightly different spots. The distance between origin and lipoprotein peak could thus be increased or decreased, while a simultaneous control experiment with whole human serum exhibited a corresponding shift of the whole gamma of serum protein. The peak of the preparation of isolated lipoprotein always appeared at the same position between the α- and β-globulins of the whole serum. The area of light lipid and protein stains of the isolated lipoprotein always covered the area between origin and lipoprotein peak.

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**TABLE I**

<table>
<thead>
<tr>
<th>Layers</th>
<th>β-Lipoprotein concentrationa</th>
<th>Total β-lipoprotein per layerb</th>
<th>Density of layerc</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (top)</td>
<td>84.0 mg/ml</td>
<td>1375 mg (12.5%)</td>
<td>1.000</td>
</tr>
<tr>
<td>2</td>
<td>30.6 mg/ml</td>
<td>880 mg (6.2%)</td>
<td>1.022</td>
</tr>
<tr>
<td>3</td>
<td>100.0 mg/ml</td>
<td>4900 mg (30.0%)</td>
<td>1.103</td>
</tr>
<tr>
<td>4 (bottom)</td>
<td>Discarded</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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a According to nephelometric analysis.

b Percentage recoveries from original serum are given in parentheses.

c Measured after the layer had been removed from the tube.
decrease the nephelometric index of β-lipoprotein, and were found in some instances even to increase this characteristic slightly (see Table III, Lines 1 to 4). There was no increase in the light scattering of the β-lipoprotein solutions during exposure to the proteolytic enzymes, as measured simultaneously with the nephelometric index. That β-lipoprotein does not contain, or behave as, an inhibitor of the proteolytic enzymes used was shown by the observation that casein, added to the reaction mixture, was completely broken down to trichloroacetic acid-soluble peptides, while the nephelometric index of β-lipoprotein remained unchanged (Table III, Lines 5 to 7). Even the combined action of the three proteolytic enzymes had no effect on the nephelometric index of β-lipoprotein (Table III, Line 8).

It was noted, however, that small amounts of trichloroacetic acid-soluble material were released from the lipoprotein during exposure to trypsin, α-chymotrypsin, or papain (see last column in Table III, Lines 1 to 4). Thin layer chromatography of β-lipoprotein, treated with either one of these enzymes, revealed the liberation of ninhydrin-staining substances as a consequence of the proteolytic action (see Fig. 2), indicating that the trichloroacetic acid-soluble material consists of low molecular weight peptides or amino acids. The release of peptides from β-lipoprotein was a progressive reaction, as seen from the data in Fig. 3. Although the slope of the straight line in this chart, where

### Table II

**Decrease of nephelometric index of β-lipoprotein by exposure to acid, alkali, elevated temperatures, or freeze-drying**

<table>
<thead>
<tr>
<th>Exposure of β-lipoprotein* for 2 hours to</th>
<th>Nephelometric index*</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>Temperature</td>
</tr>
<tr>
<td>7.05</td>
<td>20°</td>
</tr>
<tr>
<td>3.0</td>
<td>20</td>
</tr>
<tr>
<td>12.5</td>
<td>20</td>
</tr>
<tr>
<td>7.05</td>
<td>47</td>
</tr>
<tr>
<td>7.05</td>
<td>56</td>
</tr>
</tbody>
</table>

* Concentration, 30 mg per ml.

### Table III

**Influence of proteolytic enzymes on β-lipoprotein**

<table>
<thead>
<tr>
<th>Line</th>
<th>Composition of reaction mixture*</th>
<th>After incubation at 35° for 20 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β-Lipoprotein</td>
<td>Casein</td>
</tr>
<tr>
<td>1</td>
<td>900</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>900</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>900</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>900</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>900</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>900</td>
<td>10</td>
</tr>
<tr>
<td>7</td>
<td>900</td>
<td>10</td>
</tr>
<tr>
<td>8</td>
<td>900</td>
<td>10</td>
</tr>
<tr>
<td>9</td>
<td>4500</td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td>4500</td>
<td>10</td>
</tr>
</tbody>
</table>

* In 0.1 M Tris buffer, pH 7.6.

* Lipoprotein peptide.

* In the presence of 0.055 M cysteine.

* Increase in light scattering of a 0.2-ml reaction mixture in 25 ml of Tris buffer, pH 8.6, upon addition of 30 μg of amylopectin sulfate and 1 mg of CaCl₂, expressed in Nephelos units.

* No visible precipitate formed with trichloroacetic acid; optical density was measured after 1:4 dilution.

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**Fig. 2.** One-dimensional, thin layer chromatography of β-lipoprotein (10 mg per ml of Tris buffer) without and after 19 hours of exposure at 35° to various proteolytic enzymes (0.67 mg of enzyme per ml). The spots were stained with ninhydrin: I, with trypsin at pH 7.6; 2, with α-chymotrypsin at pH 7.6; 3, with papain at pH 6.5; 4, with papain at pH 7.95 in the presence of 0.05 M KCN; 5, 6, and 7, lipoprotein controls without enzyme at pH 7.6, 6.5, and 7.95 (with KCN), respectively. The following served as controls without lipoprotein: 8, glycyl-L-tyrosine; 9, L-aspartic acid; 10, L-lysine.

**Fig. 3.** Increase of trichloroacetic acid-soluble material (measurement of optical density at 280 μm) during incubation at 35° of β-lipoprotein (54 mg per ml) with trypsin (0.125 mg per ml) in Veronal citrate buffer, pH 8.6 (○). Optical densities were measured on the filtrates of mixtures of 0.1-ml aliquots with 2.25 ml of 5% trichloroacetic acid and 1.4 ml of 0.85% sodium chloride. In the control experiment (△), β-lipoprotein was incubated without added trypsin.
time is plotted on a logarithmic scale, seemed to indicate that the proteolytic digestion of \( \beta \)-lipoprotein would reach completion, the reaction came to a standstill for all practical purposes when about 20% of the lipoprotein peptide had been converted into trichloroacetic acid-soluble peptides, i.e. after 48 to 72 hours under the conditions described.

In order to ascertain that the digested protein actually was a part of the lipoprotein itself and was not a contaminating protein accompanying the lipoprotein, the electrophoretic patterns of the \( \beta \)-lipoprotein preparation before proteolytic digestion were carefully examined and were found to be free from any protein-staining material with an electrophoretic mobility different from that of the lipid-staining substances (see Fig. 1). The mobility of the digested lipoprotein was between those of human serum \( \beta \)- and \( \alpha \)-globulins (see also Fig. 4).

\( \beta \)-Lipoprotein was treated with trypsin or \( \alpha \)-chymotrypsin for 48 hours in the way described above (see legend to Fig. 3) and was isolated by precipitation with amylopectin sulfate; it thus was freed from the proteolytic enzymes and from the peptides detached from it as the consequence of proteolytic action. It was found that the total protein content of the resulting lipoprotein was about one-fifth less than that of the parent compound (see Table IV), while the composition of the correspondingly increased lipid moiety was essentially unchanged (see ratios of lipid components in Table IV).

Physical and physicochemical properties of the lipoproteins exhaustively treated with proteolytic enzymes were distinctly different from those of the corresponding parent substances. Whereas aqueous solutions of the latter can be kept at 5-10° for several months without evident signs of deterioration (no changes in turbidity or nephelometric index), the stability of lipoprotein preparations treated with trypsin or chymotrypsin and subsequent reprecipitation by amylopectin sulfate was diminished considerably: their nephelometric index decreased markedly during storage within a few days, the turbidity of their solutions increased sharply, and the material eventually precipitated. The modified lipoprotein-polyanion complexes dissolved much more readily in an excess of polyanion than the corresponding complexes of the untreated lipoproteins; i.e. a smaller excess of polyanion was required for dissolution. When the modified lipoproteins were not reprecipitated with polyanions, their stability was much higher than after reprecipitation, but somewhat lower than before treatment with proteolytic enzymes. All studies on electrophoretic mobilities and ultracentrifugal flotation rates of the modified lipoproteins were carried out, therefore, directly on the digestion mixtures without prior removal of proteolytic enzymes and low molecular weight peptides by polyanionic reprecipitation.

Electrophoretic mobilities of \( \beta \)-lipoproteins were found to be slightly accelerated by tryptic or chymotryptic action (see Fig. 4), but never to exceed or even to reach that of human serum \( \alpha \)-globulin. In some cases, however, when the \( \beta \)-lipoprotein

\[ \text{Composition of } \beta \text{-lipoprotein before and after proteolytic digestion} \]

<table>
<thead>
<tr>
<th>Component</th>
<th>Before enzyme digestion</th>
<th>After digestion with Trpyn</th>
<th>Chymo-trypsin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol esters (CE)</td>
<td>30.8 %</td>
<td>39.2 %</td>
<td>37.2 %</td>
</tr>
<tr>
<td>Unesterified cholesterol</td>
<td>10.4 %</td>
<td>11.05 %</td>
<td>12.55 %</td>
</tr>
<tr>
<td>Phospholipids (PL)</td>
<td>18.8 %</td>
<td>20.7 %</td>
<td>20.5 %</td>
</tr>
<tr>
<td>Triglycerides (TG)</td>
<td>8.85 %</td>
<td>9.35 %</td>
<td>9.95 %</td>
</tr>
<tr>
<td>Protein</td>
<td>25.2 %</td>
<td>19.6 %</td>
<td>19.8 %</td>
</tr>
<tr>
<td>TC:PL</td>
<td>1.72</td>
<td>1.07</td>
<td>1.70</td>
</tr>
<tr>
<td>CE:TC</td>
<td>0.679</td>
<td>0.080</td>
<td>0.640</td>
</tr>
<tr>
<td>PL:TG</td>
<td>2.13</td>
<td>2.22</td>
<td>2.06</td>
</tr>
</tbody>
</table>

* TC, total cholesterol.
The present results suggest that the proteolysis of β-lipoprotein consists in the breakdown of all of the protein moiety of some lipoprotein particles, while the peptide of other particles.

After hydrolysis of the peptides in the dialysate, their amino acid composition was analyzed by two-dimensional, thin layer chromatography. Thirteen to fourteen different amino acids could be discerned in each of the three hydrolysates examined, i.e. from (a) undigested, undialyzed lipoprotein; (b) the dialysate of trypsin-digested lipoprotein; and (c) the dialysate of chymotrypsin-digested lipoprotein (see Figs. 6 and 7). The spots occurred in identical positions and in proportionate intensities. It is obvious, therefore, that the peptides cleaved from β-lipoprotein by either trypsin or chymotrypsin had an amino acid composition very similar to that of the parent lipoprotein. Chromatography of the dialysates prior to their acid hydrolysis did not yield ninhydrin-stainable spots corresponding to individual amino acids. There were no detectable amounts of ninhydrin-staining material in the dialysate of β-lipoprotein samples that were not treated with proteolytic enzymes.

**DISCUSSION**

The human serum β-lipoprotein used in the present work is a highly purified preparation of the complex mixture of proteins frequently referred to as low density lipoprotein of flotation rates Sf 0 to 20 (21). Its hydrated density and its composition of protein, cholesterol (free and esters), phospholipids, and triglycerides agree with those measured on preparations of low density lipoprotein with Sf 0 to 20 (22–24). Proteolytic digestion of this protein is extremely slow, as compared with that of casein, and comes to a virtual standstill when about one-fifth of the lipoprotein peptide has been broken down to low molecular peptides which are no longer attached to the lipoprotein. This behavior is reminiscent of that of many native globular proteins and may be due to a possible coiling of the peptide chains of β-lipoprotein in a manner analogous to that suggested as the cause of the resistance toward proteolytic enzymes of the native forms of hemoglobin, ovalbumin, serum albumin, pseudoglobulin, and others (4, 5).

Whether or not the peptide chains in the β-lipoprotein particles are actually coiled cannot be decided from our results or from those of other workers. It is generally accepted, however, that the protein moiety in low density lipoprotein is located at the surface of the nearly spherical lipoprotein particles (23, 24). According to Oncley et al. (22, 23), there is an insufficient amount of protein to cover the whole surface of the lipid particles completely, and Vandenhoevel (25) demonstrated that the protein covers the particles uniformly. If it is assumed that the peptide chains are partially or totally uncoiled in order to spread evenly over the surface of the lipid particles, it would then probably be a phenomenon different from the resistance of native globular proteins against these enzymes. If, on the other hand, β-lipoproteins resist proteolytic attack for the same reason as do many native proteins, i.e. because of a marked coiling of their peptide chains, it would then appear that the surface layer of β-lipoproteins is shared by peptide and some other material, most likely phospholipid. A significant portion of the phospholipids must indeed be exposed, and the various types of interactions between β-lipoprotein and polyanions would appear to depend on the presence of this portion of phospholipid.

Two-dimensional thin layer chromatography of the peptides cleaved from β-lipoprotein by trypsin and chymotrypsin, as well as the dialysates of these enzymes, was performed. The peptides were separated by polyanionic precipitation of the latter and by dialysis. The dialysate was found to be free from proteolytic activity.

There was a considerable increase in the flotation rates, i.e. a decrease in the hydrated densities of the lipoproteins, resulting from their trypsin or chymotryptic digestion (see Fig. 5). The hydrated density of the untreated control was between 1.035 and 1.04; it decreased to values between 1.02 and 1.025 after exposure to trypsin, and to a range from 1.01 to 1.025 after incubation with α-chymotrypsin.

The peptides released from β-lipoprotein by proteolytic action were separated from the enzyme and from the remaining lipoprotein by polyanionic precipitation of the latter and by dialysis. The dialysate was found to be free from proteolytic activity.

A clear distinction is made between a protein behaving on electrophoresis like a γ-globulin and a protein immobilized at the origin because it has precipitated from solution. The latter exhibits a sharp band of stain with clearly visible contours and remains always at the origin, even when electro-osmotic flow or any other secondary flow movement occurring during electrophoresis displaces the γ-globulin from the origin. A γ-globulin is characterized by a much broader zone of stain which, in many instances, is shifted to either side of the origin by electro-osmotic or other flow movements.

**FIG. 6.** Amino acid patterns (established by two-dimensional, thin layer chromatography) of the peptides cleaved off from human serum β-lipoprotein by the action of trypsin (right). The amino acid patterns of whole β-lipoprotein obtained in a simultaneous experiment (control) are shown on the left.

**FIG. 7.** Same as Fig. 6 except for the enzyme, which was α-chymotrypsin.
remains completely attached to the lipoprotein and probably is not attacked at all. Such a mechanism is the only one compatible with the findings that the amino acid composition of the original lipoprotein was identical with that of the dialysable cleavage products. As to the fate of the deproteinized lipoprotein particles, it can only be stated that they do not become insoluble in water, since the light scattering of the $\beta$-lipoprotein solutions does not increase during exposure to proteolytic enzymes. The lipoprotein remaining after proteolytic digestion has the same lipid composition as the parent compound before proteolysis (ratios in Table IV), but it contains more total lipid and less protein, and its ultracentrifugal flotation patterns and its stability have changed largely. It therefore appears that the deproteinized lipid has been taken up by the lipoprotein particles which have not undergone proteolytic digestion in a manner analogous to the uptake or exchange of cholesterol by lipoprotein in vitro (26). A modified type of lipoprotein with the properties described thus arises (Table IV; Figs. 4 and 5). This modified lipoprotein appears to be resistant to further proteolytic attack.

It should be emphasized that the nephelometric index of $\beta$-lipoprotein, i.e. its tendency to interact with amylopectin sulfate, does not decrease even though one-fifth of the protein has been transformed into low molecular weight, lipid-free peptides. This observation suggests that the protein moiety is not essential for the lipoprotein-polyanion interaction. Since this interaction is believed to be due mainly to polar forces (27, 28), it now appears that some phospholipid must be exposed at the surface of the $\beta$-lipoprotein particles, and that this portion may be responsible for the formation of complexes with polyanions. The protein moiety, however, may also play a role in these interactions and, through weaker bonds, be a determining factor for the various types of interaction (7, 8).

**SUMMARY**

The action of trypsin, $\alpha$-chymotrypsin, or papain on highly purified subfractions of human serum $\beta$-lipoprotein, synonymous with the low density lipoproteins of $S_0$ to 20, has been studied. Prolonged action of the proteolytic enzymes does not decrease the ability of $\beta$ lipoprotein to interact with amylopectin sulfate, as measured by the nephelometric index. At the same time, low molecular weight peptides are detached very slowly from the lipoprotein, but no lipid material becomes water-insoluble. This reaction comes to a virtual stop when 20% of the protein has been degraded. The amino acid composition of the peptides cleaved off by proteolytic action has been found to be very similar to, if not identical with, that of the parent $\beta$-lipoprotein.

Proteolytic degradation of $\beta$-lipoprotein follows, therefore, a mechanism during which the protein moiety of one-fifth of the lipoprotein particles is broken down to small peptides while the lipid moiety of the deproteinized particle is taken up by the remainder of the lipoprotein particles. This results in the formation of a modified type of lipoprotein which is resistant to further proteolytic attack.

The modified lipoprotein was found to contain one-fifth less protein and correspondingly more lipid than the parent compound. Its lipid composition is identical with that of the undergraded lipoprotein with regard to free and esterified cholesterol, phospholipids, and triglycerides. Its ultracentrifugal flotation rate is increased significantly, its electrophoretic mobility at pH 8.6 is only slightly increased, and its stability during storage is markedly decreased. It has retained its ability to interact with polyanions.

It can be concluded that the protein moiety of $\beta$-lipoproteins is not essential for the formation of complexes with polyanions, whereas phospholipid plays an important role in this connection. For this reason and because of insufficient coverage of the $\beta$-lipoprotein particles by protein, which probably occurs in the form of strongly coiled peptide chains, phospholipid must be present in the peripheral protein layer.

**REFERENCES**
