Apoptein (E–A-II) Complex of Human Plasma Lipoproteins

II. RECEPTOR BINDING ACTIVITY OF A HIGH DENSITY LIPOPROTEIN SUBFRACTION MODULATED BY THE APO(E–A-II) COMPLEX*

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Normal human high density lipoproteins (HDL) of the \( d = 1.063 \) to 1.125 ultracentrifugal fraction can be separated by Geon-Pevikon block electrophoresis into two subclasses, HDL-I and HDL-II. HDL-I, characterized by the presence of the E apoprotein and the apo(E–A-II) complex along with the A-I and A-II apoproteins, accounted for most, if not all, of the high affinity binding of the human HDL \( d = 1.063 \) to 1.21) to the low density lipoprotein (LDL) receptors on normal human fibroblasts. By contrast, the HDL-II, the major subclass of the \( d = 1.063 \) to 1.125 fraction, lacked the E apoprotein and the apo(E–A-II) complex and exhibited no significant binding activity. Likewise, the HDL\(_d\) \( d = 1.125 \) to 1.21) did not bind to the LDL receptor. In competitive binding studies using iodinated LDL, the high affinity binding of HDL-I correlated directly with the content of the E apoprotein. Furthermore, it was observed that treatment of the HDL-I with disulfide reducing agents resulted in a marked enhancement of the binding activity of the HDL-I, a response which was correlated with the appearance of increased amounts of the E apoprotein. The amount of E apoprotein in the HDL-I was increased by reduction and alkylation (mercaptoethanol/iodoacetamide) of the apo(E–A-II) complex, which resulted in conversion of this complex to its E and A-II apoprotein subunits. Moreover, stimulation of intracellular cholesterol esterification paralleled the enhanced binding activity of the reduced and alkylated HDL-I. The HDL-I, biologically active with normal human fibroblasts, had no effect on the cellular metabolism of fibroblasts from a receptor-negative homozygous type II hypercholesterolemic patient. Even after reduction and alkylation, neither HDL\(_a\) \( d = 1.125 \) to 1.21) nor the HDL-II subclass of the \( d = 1.063 \) to 1.125 fraction exhibited receptor binding activity. Both of these lipoproteins lack the apo(E–A-II) complex. Furthermore, reduction and alkylation did not change the binding activity of LDL. Previously, 1,2-cyclohexanedione was shown to modify the arginyl residues of the B and E apoproteins, thereby selectively blocking the high affinity receptor binding activity of lipoproteins which contained either of these apoproteins (LDL and HDL\(_d\), respectively). Likewise, treatment of HDL-I with this reagent abolished the biological activity of these apo-E-containing lipoproteins. These studies show that the HDL-I subclass accounts for the binding activity of human HDL and that the activity of this subclass can be enhanced by conversion of the inactive apo(E–A-II) complex of HDL-I to the biologically active E apoprotein by reduction of the mixed disulfide. Whether or not this interconversion occurs \textit{in vivo} and operates as a modulator of HDL binding to the LDL receptors remains to be determined.

The extent of high affinity binding to the low density lipoprotein (LDL) cell surface receptors of fibroblasts by specific plasma lipoproteins is determined by the apoprotein content. Lipoproteins that contain either the B or the arginine-rich (apo-E) apoprotein bind to a common cell surface receptor and regulate intracellular cholesterol metabolism (1–3). Selective modification of the amino acid arginine in the B and E apoproteins abolishes the ability of the lipoproteins to bind to the cell surface receptors, which indicates that binding occurs specifically with apoproteins B and E (3). Lipoproteins which bind to the high affinity receptor sites include the apo-B-containing low density lipoproteins (LDL) of man, the dog, and the swine (2, 4, 5) and the apo-E-containing lipoproteins induced by cholesterol feeding of dogs and swine (HDL\(_d\)) (2, 6, 7). The typical high density lipoproteins (HDL\(_d\); \( d > 1.063 \)) obtained from human plasma by ultracentrifugation have only a limited capacity to bind to the receptors (8). Likewise, swine HDL \( d = 1.095 \) to 1.21) are much less active than LDL or HDL\(_d\) with respect to high affinity cell surface receptor binding, and most, if not all, of the binding activity can be correlated with the presence of a minor, but potent, subfraction of the HDL which contains the E apoprotein (9). The potency of this HDL subfraction results from the 10- to 100-fold enhanced binding activity of the E apoprotein as compared to the apo-B-containing HDL (9). The identification of this active subfraction of swine HDL led to the present study which was designed to fractionate human HDL and to characterize the binding activity of these lipoproteins.

As reported in the preceding paper (10), when the human plasma ultracentrifugal density fraction \( d = 1.063 \) to 1.125 is subfractionated by preparative Geon-Pevikon block electrophoresis, it is possible to obtain two \( a \)-migrating lipoproteins, referred to as HDL-I and HDL-II. The HDL-I migrate more slowly and are larger (124 ± 24 Å) than the HDL-II (80 to 100 Å), but the major difference between these two lipoproteins is

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in their apoprotein content. Whereas the HDL-II contain primarily the A-I and A-II apoproteins, the HDL-I contain, in addition to the A-I and A-II apoproteins, a variable amount of apo-E and a prominent M, = 46,000 protein. Disulfide reduction of the M, = 46,000 apoprotein results in the formation of two subunits identified as the E and A-II apoproteins (10). This M, = 46,000 molecular weight protein is referred to as the apo(E—A-II) complex. In the present study, the binding activities of the HDL-I before and after disulfide reduction are compared to determine whether conversion of the apo(E—A-II) complex to apo-E results in enhanced binding of the HDL-I to the cell surface receptors and subsequent regulation of intracellular cholesterol metabolism.

EXPERIMENTAL PROCEDURES

Materials—Fetal calf serum, trypsin/EDTA solution, potassium penicillin G, streptomycin sulfate, Dulbecco's modified Eagle's medium (DME medium) (Catalogue No. H-21), and Dulbecco's phosphate-buffered saline (Cat. No. K-13) were purchased from GBICO. Petri dishes (60 x 15 mm) and tissue culture flasks (75 cm²) were obtained from Falcon Plastics. Sodium 125I was obtained from Calbiochem. Other analytical grade reagents were purchased from Fisher Scientific.

Plasma Lipoproteins—Human LDL (d = 1.02 to 1.05) were isolated from the plasma of a fasted male subject by sequential ultracentrifugation at 59,000 rpm for 18 h in a 60 Ti rotor (Beckman Instruments). LDL were used for up to 4 weeks after iodination but were redialyzed against 0.15 M NaCl, 0.01% EDTA, pH 7, at 4°C. The cholesterol content of the lipoprotein-deficient serum did not affect LDL binding as measured by direct binding studies with 125I-LDL.

Lipoprotein Modifications—The lipoproteins were modified with 1,2-cyclohexanedione as previously described (9). Reduction and alkylation of LDL, HDL-II, and HDL-I were performed as described in the preceding paper (10). LDL and HDL-II, HDL-I, and HDL were isolated and characterized as described in the preceding paper (10). Human lipoprotein-deficient serum was prepared by centrifugation at d = 1.215 at 59,000 rpm for 48 h (2). The cholesterol content of the lipoprotein-deficient serum, determined by gas-liquid chromatography, was less than 2 mg/ml.

Lipoprotein Modifications—The lipoproteins were modified with 1,2-cyclohexanedione as previously described (3). Reduction and alkylation of LDL-I, LDL-II, and HDL were performed as described in the preceding paper (10). LDL was reduced and alkylated by treating the lipoproteins (3 mg of protein) in 0.2 M phosphate buffer, pH 8 (final volume, 1.4 ml), for 4 h with 3 ml (38.4 μmol) of β-mercaptoethanol at 25°C. Alkylation of the β-mercaptoethanol-treated LDL was performed for 30 min at 0°C in the dark by addition of 10 mg of iodoacetamide (Sigma). The reaction mixture was then dialyzed against 0.15 M NaCl, 0.01% EDTA, pH 7, at 4°C.

Iodination—The iodine monochloride method as modified by Bilheimer et al. (11) was used to iodinate the human LDL. The 125I-LDL were exhaustively dialyzed against 0.15 M NaCl, 0.01% EDTA, pH 7, and sterilized by filtration through a 0.45 μm Millipore filter. The sterile LDL were used for up to 4 weeks after iodination but were preheat treated the day before each experiment. Less than 5% of the total radioactivity was extractable into chloroform/methanol (2:1; v/v).

Cells in Culture—Normal human fibroblasts from a prepubescent specimen of a healthy infant or receptor-negative skin fibroblasts from a homozygous type II hyperlipoproteinemic patient were grown routinely in Dulbecco's modified Eagle's medium (DME medium) supplemented with 10% fetal calf serum, 3.7 mg/ml of NaN3, 100 units of penicillin G, and 100 μg/ml of streptomycin sulfate. The human fibroblasts (GM-2014) were obtained from NGMS genetic mutant repository. The cultures were maintained in a humidified atmosphere of 5 to 8% CO2 in air at 37°C. A solution of 0.5% trypsin, 0.02% EDTA in Hanks' solution was used to harvest the confluent monolayers. The cells were grown in 75-cm² flasks for cell maintenance or in 60-mm Petri dishes (10 cells/dish) for use in experiments. The Petri dishes were washed twice 48 h before the experiment with DME medium containing 5% (v/v) lipoprotein-deficient serum, and 2 ml of DME medium containing 10% lipoprotein-deficient serum was added. The experiments were begun on Day 7 after the cells had grown for 48 h in media containing 10% human lipoprotein-deficient serum. All cultures were routinely tested for and found free of mycoplasma contamination (12).

Asays for Binding, Internalization, and Degradation—The procedures of Goldstein and Brown (8, 13) with minor modifications (2) were used for binding and degradation assays performed at 37°C and binding studies at 4°C (14). In several studies, HDL-I were reduced with β-mercaptoethanol (10) and, to maintain the apoproteins in a reduced state, mercaptoethanol was added to the media to a final concentration of 0.05%. It was necessary to exclude the lipoprotein-deficient serum during incubation with 125I-LDL, since the proteins in the serum reacted with the mercaptoethanol, thus allowing the interchain disulfide bonds of the E and A-II apoproteins to re-form, with subsequent inactivation of the HDL-I. To estimate the 125I-LDL binding to Petri dishes in experiments where lipoprotein-deficient serum was not used, blank Petri dishes were incubated at 37°C with 10% d > 1.21 HDL with no added LDL. After the media containing the lipoprotein-deficient serum was removed, 2 μg/ml of 125I-LDL protein was added to all dishes and the blank plates were carried through the binding assay procedure. The average radioactivity associated with the blank dishes was subtracted from the total value obtained for the experimental dishes to provide the corrected net counts per min. We have determined that the addition of as much as 0.5% mercaptoethanol to the culture media devoid of lipoprotein-deficient serum did not affect LDL binding as measured by direct binding studies with 125I-LDL.

Assay for Incorporation of [14C]Oleate into Cholesteryl Ester—The incorporation of [14C]oleate into cholesteryl ester by the cell monolayers was measured as previously described (15). The [14C]oleate was complexed to albumin as described by Van Harken et al. (16). On Day 5, media containing lipoprotein-deficient serum was added to the cells for 24 h. The sterile lipoproteins were then added to 2 ml of fresh 10% lipoprotein-deficient media in the amounts indicated. After 15 h, 20 μl of 10 mM [14C]oleate bound to albumin was added; the monolayers were harvested 2 h later. After aliquots were taken for protein determination, [12H]cholesterol was added as an internal standard, the cells were extracted with chloroform/methanol (2:1; v/v), and the cholesteryl 14C-ester content was determined following isolation by thin layer chromatography.

RESULTS

The HDL-I, a subfraction of the d = 1.063 to 1.215 ultracentrifugal fraction isolated by preparative block electrophoresis, have been shown to differ from the major component of this ultracentrifugal fraction (referred to as HDL-II) with respect to apoprotein content (10). As shown in Fig. 1, the HDL-I contained the A-I, A-II, and C apoproteins, a variable amount of apo-E, and a prominent M, = 46,000 protein (referred to as the apo(E—A-II) complex). Disulfide reduction of the (E—A-II) apoprotein resulted in the formation of two subunits identified as apo-E and apo-A-II (10). By comparison, the HDL-I like HDL (d = 1.125 to 1.21), contained primarily the A-I and A-II apoproteins and little, if any, of the apo(E—A-II) complex or the E apoprotein as determined by polyacrylamide gel electrophoresis (Fig. 1).

The ability of the various HDL subfractions (HDL-I, HDL-II, and HDL) to compete with iodinated human LDL for binding, internalization, and degradation by normal human fibroblasts was compared by competitive assays performed at 37°C. As shown in Fig. 2, human LDL displaced approximately 50% of the 125I-LDL at a concentration of 20 to 25 μg/ml of LDL protein, whereas the HDL (d = 1.125 to 1.21) displaced less than 10% of the 125I-LDL from the high affinity receptors at a concentration of 100 μg/ml. The HDL-I used in this particular study were also incapable of displacing a significant amount of the receptor-bound LDL, while other preparations of HDL-I possessed significant binding activity (see below). The ability of the HDL-I to displace the 125I-LDL appeared to correlate directly with the amount of E apoprotein present. Moreover, following reduction and alkylation, binding activities of all the HDL-I preparations were markedly increased and, as shown in Fig. 2, the activated HDL-I used in this study displaced approximately 55% of the 125I-LDL at a concentration of 100 μg/ml of protein. Reduction and alkylation of LDL and HDL, did not alter their binding activity.

2 Unpublished data.
reduced and alkylated HDL-I appeared to correlate with the major apoproteins were the (E-A-II), A-I, and A-II apoproteins. Following reduction and alkylation, the apo(E-A-II) complex disappeared and the E and monomeric A-II apoproteins increased. These were the only significant changes in either the physical or chemical properties of the HDL-I (see below). Therefore, the increase in binding activity of the reduced and alkylated HDL-I appeared to correlate with the appearance of the E apoprotein, an apoprotein which has previously been shown to be a necessary determinant for HDL binding to the high affinity cell surface receptors (2, 9). The lack of significant displacement of $^{125}$I-LDL by the untreated HDL-I indicated that prior to reduction the apo(E-A-II) complex did not possess binding activity. The chemical compositions (Table I) and particle size and morphology by negative staining electron microscopy (Fig. 3) were identical for reduced and alkylated and for native HDL-I. The reduced and alkylated HDL-I migrated as a sharp α-band on paper electrophoresis but slightly slower than the untreated HDL-I. The relative decrease in negativity may have resulted from alkylation of free sulphhydryl groups.

To establish further that binding of HDL-I was enhanced by disulfide reduction of the apolipoproteins, competitive binding assays were performed at 4°C. Such assays measure cell surface receptor binding without significant internalization of the bound lipoproteins (14). As shown in Fig. 4A, reduction and alkylation of HDL-I significantly increased displacement of $^{125}$I-LDL as compared with the untreated HDL-I. The HDL-I used in this particular study possessed binding activity prior to reduction and alkylation and this activity appeared to correlate with the presence of the E apoprotein prior to treatment with the reducing agent. The reactivity of the LDL and HDL₃ are shown for comparison (Fig. 4A).

When reduction by β-mercaptoethanol of the apo(E-A-II) complex of HDL-I to its apo-E and apo-A-II components was not followed by alkylation of the free sulphhydryl groups, the apo(E-A-II) complex spontaneously re-formed after removal of the reducing agent by dialysis. Therefore, to stabilize the conversion of the (E-A-II) to apo-E in the HDL-I to be used in the fibroblast binding studies (Figs. 2 and 4A), the reduced lipoproteins were alkylated prior to removal of the reducing agent. An alternate approach was to perform binding studies at 4°C in the presence of 0.05% mercaptoethanol in media which did not contain lipoprotein-deficient serum. The HDL-I either reduced or reduced and alkylated reacted identically with respect to the displacement of the $^{125}$I-LDL from the receptor sites (Fig. 4B). In addition to confirming the data obtained with the reduced and alkylated HDL-I, the results obtained with reduced HDL-I which had not been alkylated indicated that the alkylation did not influence the enhanced activity of the lipoprotein.

Studies were undertaken to clarify the mechanism of HDL-I reactivity with the cell surface receptors by modification of the HDL-I arginyl residues with cyclohexanone. Previously, we have demonstrated that selective modification of the arginyl residues of the apoproteins of human LDL and canine HDL₃ with 1,2-cyclohexanone resulted in abolishment of the ability of human LDL to compete with HDL-I (20) and HDL₃ (21) for binding and degradation by normal human fibroblasts. On Day 7, the media was replaced with 2 ml of media containing 10% human lipoprotein-deficient serum, 5 µg/ml of $^{125}$I-LDL (69 cpm/ng of protein), and the unlabelled lipoproteins at the concentrations indicated. The cells were incubated at 37°C for 5 h. Untreated HDL-I, reduced and alkylated HDL-I, untreated LDL, reduced LDL, and untreated HDL₃ were labeled as indicated. Untreated HDL-I, reduced and alkylated HDL-I, untreated LDL, reduced LDL, and untreated HDL₃ were labeled as indicated.

### Table I

**Chemical composition of human HDL-I**

<table>
<thead>
<tr>
<th></th>
<th>Untreated</th>
<th>Reduced/alkylated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride</td>
<td>3.1</td>
<td>4.5</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>22.5</td>
<td>20.7</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>33.9</td>
<td>34.6</td>
</tr>
<tr>
<td>Protein</td>
<td>38.5</td>
<td>40.2</td>
</tr>
</tbody>
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*Analyses performed in duplicate.*

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![Graph](image-url)
Fig. 3. An electron micrograph of negatively stained HDL-I after (A) treatment with β-mercaptoethanol and subsequent alkylation. The untreated HDL-I (B) are shown for comparison. × 100,000.

Fig. 4. A, ability of untreated HDL-I (■), reduced and alkylated (R/A) HDL-I (○), HDL₃ (▲), and LDL (○) to compete with 125I-LDL for binding at 4°C in normal human fibroblasts. On Day 7, the media was replaced with 2 ml of media (minus NaHCO₃) containing 10% human lipoprotein-deficient serum, 25 mM Hepes buffer (pH 7.4), 2 µg/ml of 125I-LDL (69 cpm/ng of protein), and the unlabeled lipoproteins at the concentrations indicated. The cells were incubated at 4°C for 2 h. B, ability of untreated HDL-I (●), reduced HDL-I (○), reduced and alkylated HDL-I (■), and LDL (▲) to compete with 125I-LDL for binding at 4°C in normal human fibroblasts. On Day 7, the media was replaced with 2 ml of media without 10% human lipoprotein-deficient serum, 25 mM Hepes buffer (pH 7.4), 2 µg/ml of 125I-LDL (116 cpm/ng), and the unlabeled lipoproteins at the concentrations indicated. The treated HDL-I were applied to the cell monolayers in 0.05% β-mercaptoethanol (final concentration). The cells were incubated at 4°C for 2 h.
with 1,2-cyclohexanedione (CHD), and lipoproteins from which the binding at 4°C to normal human fibroblasts. The conditions were as described in Fig. 4A except that 2.5 µg/ml of 125I-LDL (210 cpm/ng of protein) were used. The cells were incubated at 4°C for 2 h. LDL (4 mg) and reduced and alkylated HDL-I (3 mg) were allowed to react with cyclohexanedione to determine whether the binding activity of the reduced and alkylated HDL-I could be blocked. Abolishment of binding activity would suggest that the mechanism responsible for HDL-I binding to the cell surface receptors was similar to that of LDL and HDL-e, and furthermore, by analogy with the HDL-e, that the binding activity of the HDL-I might be ascribed to the apo-E generated by disulfide reduction of the apo(E—A-II) complex. In the study shown in Fig. 5, the reduced and alkylated HDL-I as well as active in displacing the 125I-LDL as were the unlabeled LDL. Treatment of those HDL-I, as well as the LDL, with cyclohexanedione abolished their ability to displace the 125I-LDL competitively from the receptor sites of the fibroblasts. Most of the binding activity was restored to the HDL-I following regeneration of the arginyl residues by hydroxylamine treatment to remove the cyclohexanedione. By several criteria, this treatment has been shown not to alter the lipoproteins in any unforeseen way (3). Amino acid analyses of the untreated HDL-I and the HDL-I which were modified, compared in Table II, showed that the only amino acid residue modified by the cyclohexanedione treatment was arginine. Other properties of HDL-I including lipid composition and particle morphology by negative staining electron microscopy were unaltered. Similar results have been reported for cyclohexanedione-treated human LDL and canine HDL-e (3).

One of the metabolic activities which parallels binding to the cell surface receptors is the conversion of free to esterified cholesterol. Intracellular cholesteryl ester synthesis has been shown to increase as a result of receptor-mediated uptake of human LDL by fibroblasts (15). While incorporation of [14C]-oleate into the cholesteryl esters of fibroblasts was observed to increase following the addition of LDL protein to the culture media (Fig. 6), the addition of untreated HDL-I had only a minimal effect on cholesteryl ester synthesis (Fig. 6). However, addition of reduced and alkylated HDL-I caused [14C]-oleate incorporation to increase markedly (Fig. 6). For further comparison between LDL and reduced and alkylated HDL-I with respect to cholesteryl ester synthetic activity, Fig. 7 shows the relationship calculated on the basis of the concentra-
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Fig. 7. Incorporation of [14C]oleate into cholesteryl esters as a function of lipoprotein cholesterol concentration (replotted from Fig. 6). Reduced and alkylated (R/A) HDL-I, ○ LDL, ●.

The HDL-I subclass of the d = 1.063 to 1.125 fraction and the HDL-II of the d = 1.063 to 1.125 fraction can be subfractionated by Geon-Pevikon preparative electrophoresis into two subclasses referred to as HDL-I and HDL-II (10). The HDL-I subclass, which represents less than 15% of the total lipoprotein in this ultracentrifugal fraction and less than 5% of the total HDL (d = 1.063 to 1.21) protein, appears to account for all of the high affinity cell surface receptor binding activity of the high density lipoproteins in man. In addition to the A-I and A-II apoproteins, HDL-I contain the apo(E—A-II) complex as a major apoprotein constituent and a variable amount of the E apoprotein. Occasionally, it is possible to isolate HDL-I which contain only trace amounts of the E apoprotein but in which the apo(E—A-II) complex is a major constituent. Such HDL-I are incapable of competitively displacing iodinated LDL from the receptors. This suggests that the apo-E component of the apo(E—A-II) complex is masked and unavailable to interact with the receptor. However, following reduction or reduction and alkylation of the HDL-I, the ability of the HDL-I to react with the cell surface receptors is significantly enhanced. This enhanced binding activity is further reflected by an increase in the rate of cholesteryl ester synthesis, a property which has previously been shown to be a consequence of lipoprotein binding, internalization, and degradation (18).

Reduction of the disulfide linkages converts the M, = 46,000 apo(E—A-II) complex to two subunits having molecular weights of 37,000 and 8,500, identified as apo-E and apo-A-II, respectively (18). It is reasonable to suggest that the increased binding reactivity is the result of unmasking the binding site on the apo-E of the apo(E—A-II) complex following treatment of the HDL-I with reducing agents. To substantiate this hypothesis, it was shown that reduction and alkylation of other HDL subfractions (HDL-II, d = 1.063 to 1.25, and HDLs, d = 1.25 to 1.30) or LDL neither increased nor decreased their reactivity with the cell surface receptors. Furthermore, the HDL-II and HDLs fractions, which contain little or no apo(E—A-II) complex or apo-E, are relatively inactive with respect to high affinity receptor binding (displace less than 10% of the 125I-LDL at 100 μg/ml) whether they are reduced and alkylated or not. The most significant difference between the active HDL-I and the inactive HDL-II and HDLs which could account for the enhanced binding is the presence of a reducible apo(E—A-II) complex which gives rise to the apo-E.

It has been shown earlier that the binding activities of both the B apoprotein of LDL and the E apoprotein of canine HDL, can be abolished by 1,2-cyclohexanedione modification of 40% of their arginyl residues (3). This observation suggests not only that the protein itself is the important determinant for binding but also that the mechanism of lipoprotein-receptor interaction is similar for both reactive apolipoproteins. Cyclohexanedione treatment of the reduced and alkylated human HDL-I likewise modifies approximately 40% of the arginyl residues and abolishes binding activity. Furthermore, it is possible to restore most of the binding activity to the HDL-I after regeneration of the arginyl residues by removal of the cyclohexanedione. This strongly suggests that the E apoprotein produced from the apo(E—A-II) complex by reduction and alkylation of the HDL-I is responsible for the binding activity.

A situation somewhat analogous to that described for the apo(E—A-II) to apo-E activation has been described for growth hormone (19). One form of pituitary and plasma growth hormone exists as a dimer composed of M, = 22,000 subunits linked by interchain disulfide bonds. It has been suggested that this dimeric form, largely inactive in promoting...
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growth, serves to modulate the level of active and inactive growth hormone (19). Following reduction of the dimer and carbamidomethylation, the growth-promoting activity of the growth hormone is increased. Likewise, when the apo(E—A-II) complex is converted to its E and A-II apoprotein subunits by reduction of the disulfide bonds, the inactive HDL-I are transformed to active lipoproteins which are bound, internalized, and degraded. It remains to be determined if an interconversion between the apo(E—A-II) complex and the E and A-II apoproteins occurs in vivo and if changes in the ratio between the inactive and active forms of the E apoprotein could serve to modulate lipoprotein interaction with cell surface receptors.

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Apoprotein (E--A-II) complex of human plasma lipoproteins. II. Receptor binding activity of a high density lipoprotein subfraction modulated by the apo(E--A-II) complex.

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