Isolation and Characterization of Simple and Complex Lipoproteins Containing Apolipoprotein F from Human Plasma†

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ABSTRACT: Apolipoprotein F (ApoF), one of the minor apolipoproteins in human plasma, has been recently isolated and partially characterized [Olofsson, S. O., McConathy, W. J., & Alaupovic, P. (1978) *Biochemistry 17*, 1032–1036]. In the present work, the interaction of ApoF with other apolipoproteins and lipids in human plasma was studied. By the successive use of immunosorbers specific for ApoF, apolipoprotein A-II (ApoA-II) and apolipoprotein A-I (ApoA-I), three different ApoF-containing lipoproteins were isolated from normolipidemic fasting human plasma. Their apolipoprotein content was determined by double immunodiffusion against monospecific antisera to all known serum apolipoproteins, electroimmunoassay, crossed immunoelectrophoresis, and polyacrylamide gel electrophoresis. Their lipid composition was determined by thin-layer chromatography. The three ApoF-containing lipoproteins were identified as LpF:A-I:A-II (lipoprotein containing ApoF, ApoA-I, and ApoA:II), LpF:A-I (lipoprotein containing ApoF and ApoA-I), and LpF (lipoprotein containing only ApoF). LpF:A-I:A-II was found to contain ApoF, ApoA-I, and ApoA-II in an apparent 2:1:1 molar ratio. Its lipid moiety was characterized by cholesterol ester (45%) and free cholesterol (28%) as the predominant lipids. LpF contained only ApoF, and in its major lipid components were also cholesterol esters (63%) and free cholesterol (21%). It is suggested that ApoF-containing lipoproteins may be involved in transport and/or esterification of cholesterol.

During the last decade, a number of human plasma apolipoproteins have been isolated and characterized to various degrees (Osborne & Brewer, 1977; Alaupovic, 1980). There have been, however, only a few attempts to establish the lipoprotein forms of these apolipoproteins (Kostner & Alaupovic, 1972; Lee & Alaupovic, 1974; McConathy & Alaupovic, 1976). Recently, we have described the isolation from human plasma of a minor apolipoprotein designated apolipoprotein F (ApoF). This apolipoprotein contained all common amino acids except tryptophan and has an acidic isoelectric point of 3.7 and a molecular weight of 28 000 (Olofsson et al., 1978). We have suggested on the basis of immunochemical studies that ApoF forms its own lipoprotein family, occurring predominantly in the ultracentrifugally defined high-density (HDL)[1] and low-density (LDL) lipoproteins (Olofsson et al., 1978).

To further define the lipoprotein nature of ApoF and to determine whether ApoF exists in more than a single lipoprotein form, we have developed a procedure for the isolation of lipoprotein F (LP-F). Results of this study indicate that ApoF occurs in at least three different lipoprotein forms and provide additional evidence for designating ApoF as an apolipoprotein according to the criteria suggested by Alaupovic (1980).

Materials and Methods

Isolation of Apolipoprotein F. Apolipoprotein F was isolated from delipidized HDL by ion-exchange chromatography on carboxymethylcellulose as previously described (Olofsson et al., 1978).

Isolation and Fractionation of Apolipoprotein F Containing Lipoproteins. Fresh human plasma (100 mL) obtained by plasmapheresis from normolipidemic subjects after an overnight fast was run over the anti-ApoF–Sepharose 4B column. The anti-ApoF unretained (a-ApoFU) fraction was concentrated to the original plasma volume and tested for the presence of apolipoproteins by double diffusion analyses with monospecific antisera. The column was then washed with 1500 mL of buffer containing 0.15 M NaCl, 0.05 M Tris-HCl, and 0.01% NaN_3, pH 7.5. The eluates were monitored by the absorption at 280 nm, and the retained fraction (a-ApoFR) was eluted from the column with 30 mL of 3 M NaSCN. The a-ApoFR fraction was then diluted with the equilibration buffer to its original plasma volume (100 mL). Twenty milliliters of the a-ApoFR fraction was used for protein and lipid analyses, while the remainder of the a-ApoFR fraction (80 mL) was run over an immunosorber which contained antibodies to apolipoprotein A-II (a-ApoA-II). The retained fraction (a-ApoA-IIR) was collected in the described fashion and analyzed for lipid and protein composition. The unretained fraction (a-ApoA-IIU) was passed over an immunosorber which contained antibodies to apolipoprotein A-I (a-ApoA-I), and the unretained (a-Apo-IU) as well as the retained (a-ApoA-IR) fractions were collected. The a-ApoA-IU fraction was next run over an immunosorber containing antibodies to ApoF-free plasma. In the latter case, only the unretained fraction was collected, lyophilized, and analyzed for lipid and protein composition.

Immunological Methods. Double diffusion analyses and immunoelectrophoresis in 1% agar were performed as previously described (Alaupovic et al., 1972). Preparation of apolipoproteins A-I, A-II, C-I, C-II, C-III, D, and E and their corresponding antisera has been previously described (Alaupovic et al., 1972; McConathy & Alaupovic, 1976; Curry et al., 1976b).

Quantitative determination of apolipoproteins A-I and A-II in plasma and lipoprotein fractions was done by the electroimmunoassay developed by Curry et al. (1976a). Concentrations of ApoF were also measured by electroimmunoassay (E. Koren, W. J. McConathy, and P. Alaupovic, unpublished experiments). Crossed immunoelectrophoresis of lipoproteins in plasma and lipoprotein fractions was performed against anti-ApoF by the method of Laurell (1965). The rocket and

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[1] Abbreviations: LDL, low-density lipoproteins (d = 1.006–1.063 g/mL); HDL, high-density lipoproteins (d = 1.063–1.21 g/mL); Tris, tris(hydroxymethyl)aminomethane.

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peak areas were measured by using a Hewlett Packard system consisting of an HP9874A digitizer interfaced to an HP9815 S desktop computer (Hewlett Packard, Fort Collins, CO) with a program developed by Weech (1981).

Antiserum to ApoF (anti-ApoF) was prepared in rabbits as previously described (Olofsson et al., 1978). In addition, anti-ApoF was prepared by injecting a goat intraperitoneally with 0.5 mg of ApoF emulsified in Freund's complete adjuvant. After three additional injections of 0.1 mg of ApoF, blood was drawn from the jugular vein, and precipitating antibodies were obtained. The antiserum was passed over the ApoF-free plasma–Sepharose 4B column to assure monospecificity of the anti-ApoF.

Antiserum to ApoF-free plasma was prepared by injecting white New Zealand rabbits intraperitoneally with an aliquot of ApoF-free plasma (1 mg of protein) emulsified in Freund's complete adjuvant. Following three additional weekly injections, blood was drawn by cardiac puncture. Antiserum to ApoF-free plasma gave a positive reaction with all the major plasma proteins but showed no reaction with ApoF on double immunodiffusion analyses.

Immunosorbers. One hundred milliliters of goat antiserum to ApoF was utilized for the isolation of the IgG antibody containing fraction. A saturated ammonium sulfate solution was added to the antiserum at 4 °C to give 33% saturation. The IgG precipitate was collected, washed 3 times with 33% saturated ammonium sulfate solution, dissolved, and dialyzed extensively against 0.02 M K_2HPO_4 (pH 8.0). The IgG fraction was then chromatographed on a DEAE-Sepharose column (Bio-Rad, Richmond, CA) equilibrated with 0.02 M K_2HPO_4 (pH 8.0) to remove plasma proteins other then IgG. The purified IgG fraction was further dialyzed against 0.1 M K_2HPO_4 (pH 6.5) and coupled to CNBr-activated Sepharose 4B-CL at pH 6.5 as described by Cuatrecasas (1970). A ratio of 15 mg of protein/mL of activated Sepharose 4B was utilized. So that any unreacted groups would be blocked, the immunosorber was incubated for 2 h with 0.1 M ethanolamine (pH 8.0) and then washed extensively with the equilibration buffer (0.15 M NaCl, 0.05 M Tris-HCl, and 0.1% NaN_3, pH 7.5). Material bound to anti-ApoF–Sepharose 4B was eluted with 3 M NaSCN and equilibration buffer. Chromatography was performed on a column (60 × 2.5 cm) made of immunosorber and Sephadex G-25 as described previously (McConathy & Alaupovic, 1976) to avoid prolonged interaction of the dissociating agent (3 M NaSCN) with lipoproteins eluted from the immunosorber. By this method, lipoproteins are separated from NaSCN in the G-25 layer immediately after being dissociated from the immunosorber. Immunosorbers with antibodies to ApoA-I, ApoA-II, and ApoF-free plasma were prepared and used in the same fashion.

Plasma free of ApoF was prepared by runing 20 mL of normal human plasma over the anti-ApoF–Sepharose 4B column 3 times. In the final ApoF-free plasma, no ApoF was detected by double immunodiffusion or immunoelectrophoresis. By these techniques, all other apolipoproteins and major plasma proteins were found in apparently unchanged concentrations. This material was utilized for the preparation of an immunosorber containing ApoF-free plasma proteins and described immunization.

Thin-Layer Chromatography. All the lipoprotein fractions were lyophilized and delipidized by chloroform/methanol (2:1 v/v) as described previously (McConathy & Alaupovic, 1976). The extracts were concentrated under nitrogen and chromatographed on silica gel plates (G1500, Schleicher & Schuell, Keene, NH; and LK5D, 80A, Whatman, Clifton, NJ), using either petroleum ether/glacial acetic acid/methanol (75:20:5 v/v/v) or chloroform/methanol/water (64:25:4 v/v/v) as the developing solvent systems. Before lipid chromatography, the plates were prerun with chloroform/methanol (2:1 v/v) and hexane. Lipid spots were visualized by a chromium/H_2SO_4 charring method (Privett & Blank, 1962), and their intensities were measured by a scanning densitometer (Helena Laboratories, Beaumont, TX).

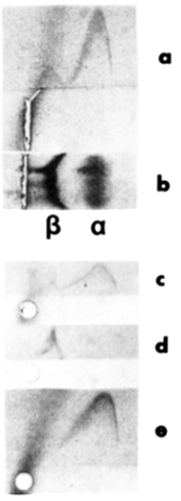

FIGURE 1: Crossed immunoelectrophoresis of whole plasma (pattern a), mixture of ApoF-containing lipoproteins (a-ApoFR) (pattern c), LP-F:A-I:A-II lipoprotein complex (pattern e), and lipoprotein F (pattern d) against an antiserum to ApoF. Agarose gel electrophoresis of plasma lipoproteins stained with Sudan black B is shown on pattern b. $\beta = \beta_1$-globulin mobility, $\alpha = \alpha_1$-globulin mobility.

Electrophoresis. Basic (Davis, 1964) polyacrylamide electrophoresis was carried out in 7% acrylamide gels in the presence of 8 M urea. Gels were stained with Coomassie Brilliant Blue (Chrambach et al., 1967).

Results

Immunochemical Characterization of Apolipoprotein F Containing Lipoproteins in Whole Plasma. Crossed immunoelectrophoresis of normolipidemic fresh fasting plasma samples against antiserum to ApoF showed two ApoF peaks corresponding to α_1- and β-lipoproteins (Figure 1a). Measurement of the areas of the respective peaks revealed that about 86% of ApoF migrated with α_1- and 14% with β-lipoproteins. Double immunodiffusion analyses of plasma samples freshly drawn from normolipidemic subjects showed reactions of partial identity between ApoF and apolipoproteins A-I and A-II. This finding indicated that in normal plasma at least a portion of ApoF is associated with ApoA-I and ApoA-II. On the other hand, ApoF gave nonidentity reactions with ApoB, ApoC-I, ApoC-II, ApoC-III, ApoD, and ApoE, indicating a lack of interaction between ApoF and these apolipoproteins which is in agreement with our previous report (Olofsson et al., 1978).

Characterization of Apolipoprotein F Containing Lipoproteins Isolated by Immunosorbers. Using the anti-ApoF immunosorber, it was possible to isolate a mixture of ApoF-containing lipoproteins (a-ApoFR). Virtually all the ApoF-containing lipoproteins from 100 mL of normal fresh plasma were removed by a single run over the anti-ApoF immunosorber. The unretained fraction showed no reaction with antiserum to ApoF while all the other apolipoproteins were present as shown by double diffusion analyses. Crossed immunoelectrophoretic patterns with antiserum to ApoF showed no difference between the a-ApoFR fraction (Figure 1c) and whole plasma. In addition to ApoF, only small but constant

Table I: Apolipoprotein Composition of ApoF-Containing Lipoproteins Determined by Electroimmunoassay

apolipoproteins	starting whole plasma (mg/100 mL)	mixture of ApoF-containing lipoproteins (a-ApoFR) (mg/100 mL)	LP-F:A-I:A-II lipoprotein complex (a-ApoA-IIR) (mg/100 mL)	lipoprotein F (a-ApoF-free U) (mg/100 mL)
ApoA-I	130 ± 14[a] (3)	0.58 ± 0.11 (3)	0.48 ± 0.04 (2) (1.7)[b]	<0.01
ApoA-II	67 ± 11 (3)	0.35 ± 0.09 (3)	0.33 ± 0.04 (2) (1.9)[b]	<0.01
ApoF	1.8 ± 0.2 (3)	1.20 ± 0.15 (3)	0.89 ± 0.18 (2) (3.3)[b]	0.35 ± 0.07 (2)

[a] Mean ± standard deviation. Number of different plasmas or lipoprotein preparations analyzed is indicated in parentheses. [b] Moles of apolipoprotein × 10^{-5}.

Table II: Lipid Composition of ApoF-Containing Lipoproteins Determined by Thin-Layer Chromatography

lipids	% total lipids: mixture of Apo-F-containing lipoproteins (a-ApoFR)	LP-F:A-I:A-II lipoprotein complex (a-ApoA-IIR)	lipoprotein F (a-ApoF-free U)
cholesterol esters	44.06 ± 3.15[a] (3)	44.52 ± 3.60 (2)	63.27 ± 3.20 (2)
triglycerides	6.23 ± 0.70 (3)	10.89 ± 1.52 (2)	5.24 ± 0.95 (2)
cholesterol	21.29 ± 1.26 (3)	27.67 ± 1.89 (2)	21.09 ± 0.63 (2)
phospholipids	29.40 ± 0.79 (3)	13.12 ± 3.59 (2)	11.50 ± 2.47 (2)

[a] Mean ± standard deviation. Number of different plasmas or lipoprotein preparations analyzed is indicated in parentheses.

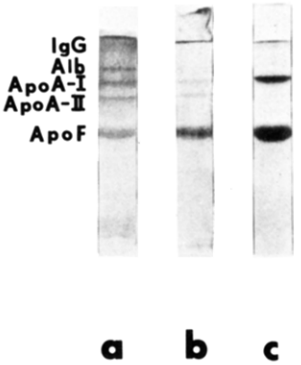

FIGURE 2: Polyacrylamide gel electrophoresis of a-ApoFR fraction (pattern a), LP-F:A-I:A-II lipoprotein complex (pattern b), and LP-F:A-I lipoprotein complex (pattern c). IgG = immunoglobulin G, Alb = albumin.

amounts of ApoA-I and ApoA-II (Table I) were always found in the a-ApoFR fraction (Figure 2a). Traces of IgG and albumin were also usually present in a-ApoFR (Figure 2a). The lipid moiety of a-ApoFR consisted of all the major plasma lipids with esterified cholesterol comprising almost half of the total lipid content (Table II). Negative-staining electron microscopy of the a-ApoFR fraction revealed spherical particles of different sizes. Analysis of 300 particles showed the following size distribution: 67.3% of the particles had a diameter between 70 and 100 Å; 27% between 100 and 200 Å; 4.5% between 200 and 300 Å; 0.9% between 300 and 500 Å; and 0.3% between 500 and 800 Å.

a-ApoA-IIR Fraction (LP-F:A-I:A-II Lipoprotein Complex). The immunochemical characterization of a-ApoFR indicated the possible presence of several forms of lipoprotein F (LP-F). One of these forms, the LP-F:A-I:A-II association complex, was isolated by affinity chromatography of a-ApoFR on anti-ApoA-II immunosorber. This complex lipoprotein consisted of apolipoproteins F, A-I, and A-II (Figures 2b and 3) in an apparent molar ratio of 2:1:1 (Table I). ApoF showed

FIGURE 3: Double diffusion analysis of the LP-F:A-I:A-II lipoprotein complex (a-ApoA-IIR) against antisera to apolipoproteins A-I, A-II, and F and albumin (Alb). a = antiserum.

FIGURE 4: Double diffusion analysis of the LP-F:A-I lipoprotein complex (a-ApoA-IR) against antisera to apolipoproteins A-I, A-II, and F. a = antiserum.

reactions of complete identity with both ApoA-I and ApoA-II on double immunodiffusion (Figure 3) and α-lipoprotein mobility on crossed immunoelectrophoresis against antiserum to ApoF (Figure 1e). The lipid moiety of LP-F:A-II:A-I consisted of all the major plasma lipids with esterified cholesterol as the predominating lipid component (Table II). On the basis of the ApoF distribution among the ApoF-containing lipoproteins (Table I), approximately 70% of ApoF in the a-ApoFR fraction is associated in an LP-F:A-I:A-II complex.

a-ApoA-IR Fraction (LP-F:A-I Lipoprotein Complex). After chromatography of a-ApoFR on the anti-ApoA-II immunosorber, the unretained fraction (a-ApoA-IIU) was chromatographed on an anti-ApoA-I immunosorber. The retained fraction (a-ApoA-IR) gave on double diffusion analysis a reaction of identity between ApoF and ApoA-I (Figure 4). The ApoA-II was absent (Figure 4, bottom well, and Figure 2c). The isolated LP-F:A-I lipoprotein complex was a minor ApoF-containing lipoprotein.

Lipoprotein F (LP-F). In the final step of this fractionation procedure, the a-ApoA-IU fraction was run over immunosorber with antibodies to ApoF-free plasma to remove all the nonlipoprotein components. The unretained fraction was shown by double diffusion, electroimmunoassay, and basic polyacrylamide gel electrophoresis only to contain ApoF (Figure 5 and Table I). The determination of the lipid composition showed the prevalence of cholesterol esters (Table II). These results show that the final fraction consisted of only lipoprotein F (LP-F). On crossed immunoelectrophoresis against anti-ApoF, lipoprotein F showed β-lipoprotein mobility (Figure 1d).

Discussion

Our previous studies suggested that ApoF occurs in human plasma as a distinct lipoprotein called LP-F (Olofsson et al.,

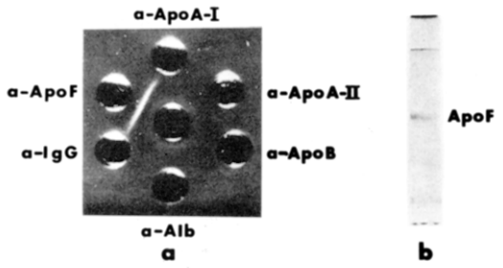

FIGURE 5: Double diffusion analysis of lipoprotein F (central well) against antisera to apolipoproteins A-I, A-II, B, and F and immunoglobulin G (IgG) (pattern a). Polyacrylamide gel electrophoresis of lipoprotein F (pattern b).

1978). According to the lipoprotein family concept (Alaupovic, 1980), the simplest lipoprotein form of ApoF would consist of ApoF and lipids, whereas a complex or associated form of ApoF would be comprised of ApoF, other apolipoproteins, and lipids.

Results of the present study clearly show that in normolipidemic fasting human plasma ApoF exists in several lipoprotein forms, i.e., as a simple lipoprotein, LP-F, and as complex lipoproteins, LP-F:A-I and LP-F:A-I:A-II. This conclusion is based on the following evidence. Immunodiffusion of fresh whole plasma against antisera to all known apolipoproteins showed reactions of partial identity between lipoproteins containing ApoF and ApoA-I as well as between ApoF and ApoA-II. However, nonidentity reactions were found between ApoF and all other apolipoproteins. The anti-ApoF–Sepharose retained no other plasma apolipoproteins except ApoF, ApoA-I, and ApoA-II. The lipoprotein nature of the a-ApoFR fraction was confirmed by the determination of its chemical composition, electron microscopy, and electrophoretic mobility. Chemical analyses revealed the presence of all major plasma lipids. Crossed immunoelectrophoresis of a-ApoFR showed two ApoF peaks having mobilities similar to those of α_1- and β_1-lipoproteins in whole plasma (Figure 1c). Electron microscopy of a-ApoFR demonstrated spherical particles. Their size distribution is compatible with the distribution of ApoF in density classes (Olofsson et al., 1978) and with the electrophoretic mobility of a-ApoFR. On the basis of these data, we have concluded that the a-ApoFR fraction is a mixture of ApoF-containing lipoproteins apparently unchanged by the isolation procedure. This lipoprotein mixture was fractionated by the use of anti-A-II–Sepharose, anti-A-I–Sepharose, and antibodies to ApoF-free plasma coupled to Sepharose. Anti-A-II immunosorber retained the LP-F:A-I:A-II complex, which was shown to have a unique apolipoprotein and lipid composition. It contained apolipoproteins F, A-I, and A-II in an apparent 2:1:1 molar ratio (Table I). All three apolipoproteins seemed to be present on the same lipoprotein particle, since ApoF showed reactions of complete identity with both ApoA-I and ApoA-II (Figure 3). LP-F:A-I:A-II had an α_1-lipoprotein mobility on crossed immunoelectrophoresis (Figure 1e), and its lipid moiety consisted of all the major plasma lipids. However, the unique compositional feature of this complex lipoprotein is that free and esterified cholesterol accounts for 72% of the total lipids. LP-F:A-I also exists in normolipidemic plasma, since the anti-A-I immunosorber retained some ApoF and ApoA-I even after the LP-F:A-I:A-II had been completely removed from the mixture of ApoF-containing lipoproteins. Double diffusion analysis and basic polyacrylamide gel electrophoresis of a-ApoA-IR showed a reaction of identity between ApoF and ApoA-I as well as a complete absence of ApoA-II (Figure 4, bottom well, and Figure 2c). It was estimated on the basis of the ApoF distribution among ApoF-containing lipoproteins (Table I) that less than 10% of the total ApoF is present in the form of LP-F:A-I. Approximately 20–30% of ApoF was recovered in the free LP-F form which only contained ApoF (Figure 5, Table I). Again, compositional analyses indicated that free cholesterol accounted for 21% and esterified cholesterol for 63% of the total lipids (Table II). LP-F seems to be present mainly in the β-lipoprotein fraction (Figure 1d).

Since most of the ApoF is found within HDL (Olofsson et al., 1978), the present identification of distinct ApoF lipoproteins provides additional evidence for the lipoprotein heterogeneity of this density class. There are already several reports describing the compositional diversity of lipoprotein particles within the HDL region (Borut & Aladjem, 1971; Alaupovic et al., 1972; Kostner & Alaupovic, 1972; Olofsson et al., 1975; Kostner & Holasek, 1977; Weisgraber & Mahley, 1978; Suenram et al., 1979). These include lipoproteins which only contain ApoA-I and ApoA-II (LP-A) (Albers & Aladjem, 1971; Kostner & Alaupovic, 1972; Olofsson et al., 1975; Norfeldt et al., 1981), ApoA-I (LP-A-I) (Albers & Aladjem, 1971; Olofsson et al., 1975; Wille, 1978; Ayrault-Jarrier et al., 1980; Norfeldt et al., 1981), ApoB (LP-B) (Kostner & Alaupovic, 1972; Kostner, 1972), ApoC (LP-C) (Kostner & Alaupovic, 1972), ApoD (LP-D) (Olofsson & Gustafson, 1974; McConathy & Alaupovic, 1976), and ApoE (LP-E) (Curry et al., 1976b). Results of this study indicate that approximately 0.5% of the plasma ApoA-I and ApoA-II are associated with ApoF. The identification of LP-F:A-I:A-II and LP-F:A-I raises the question as to whether these complex lipoproteins are generated in the plasma compartment by a direct interaction between LP-F and LP-A and LP-A-I, respectively, or by some other metabolic events. It would appear from the distribution and composition of ApoF-containing lipoproteins that complex ApoF lipoproteins are distinct lipoprotein particles rather than simple associations between LP-F and LP-A or LP-A-I. It should also be emphasized that ApoF only forms complex lipoprotein particles with ApoA-I and ApoA-II. This finding suggests a possible functional link between these three apolipoproteins. In view of the already established role of ApoA-I as an activator of lecithin/cholesterol acyltransferase (Fielding et al., 1972) and the high content of cholesterol and cholesterol esters in LP-F and LP-F:A-I:A-II, we suggest that ApoF-containing lipoproteins may be involved in cholesterol transport and/or esterification.

Acknowledgments

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Purification and Partial Amino Acid Sequence of Papain-Solubilized Class II Transplantation Antigens†

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ABSTRACT: Papain-solubilized human class II (HLA-DR) antigens have been purified from cadaveric spleens by ion-exchange chromatography, gel chromatography, and immunosorbent purification. The isolated papain-solubilized antigens comprised two subunits with apparent molecular weights of 23 000 and 30 000, respectively. The circular dichroism spectrum for the isolated class II antigens was similar to spectra recorded for HLA-A, -B, and -C antigens, immunoglobulins, and immunoglobulin fragments. Thus, class II antigens contain a considerable amount of β structure. The small subunit (β chain) exhibited extensive charge heterogeneity on two-dimensional isoelectric focusing polyacrylamide gel electrophoresis, whereas the large subunit (α chain) was more homogeneous. The structural heterogeneity of β chains remained after neuraminidase treatment. The NH_2-terminal amino acid sequence of the β chains displayed multiple residues in several positions in accordance with the genetic polymorphism displayed by this chain. The α chain also displayed multiple residues in some positions, suggesting either that some of the genetic polymorphism of the class II antigens may be endowed in this chain or that multiple loci control the expression of several α chains. Papain-solubilized class II antigen subunits were homologous in their amino acid sequences with HLA-DR antigens of defined antigenic specificity as well as with murine I-E/C antigens.

The HLA-D/DR locus of the human major histocompatibility complex (MHC),[1] which was originally defined as the main locus controlling the stimulation in the mixed leucocyte culture reaction, contains genes expressed as polymorphic cell surface glycoproteins (Wernet, 1976). Recently, several groups using monoclonal antibodies have demonstrated that adjacent to the DR locus one or more loci occur that control the expression of cell surface proteins similar in structure to those coded for by the DR locus [see Shackelford et al. (1981) and Accolla et al. (1981)]. Henceforth, this group of antigens, including the DR antigens, will be called class II antigens. The human class II antigens and their murine counterparts, the Ia antigens, have been implicated in a variety of cell recognition phenomena within the immune system, including T-cell–B-cell collaboration (Katz & Benacerraf, 1976), interaction between T suppressor and T helper cells (Miller et al., 1977), and antigen presentation by macrophages (Thomas & Shevach, 1978). These types of antigens consist of two noncovalently associated polypeptides, designated α and β chains, with molecular weights of approximately 35 000 and 29 000, respectively [see Cullen et al. (1976)]. During intracellular transport of newly synthesized α and β chains they are associated with a third invariant chain (Jones et al., 1979; Kvist et al., 1982). Until recently the cell surface expression of the invariant chain was in doubt (Jones et al., 1979; Charron & McDevitt, 1980; Moosic et al., 1980). However, the invariant chain occurs on the cell surface but not in association with α and β chains.[2]

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[1] Abbreviations: MHC, major histocompatibility complex; NaDodSO$_4$, sodium dodecyl sulfate; DEAE, diethylaminoethyl; EDTA, ethylenediaminetetraacetate; DTT, dithiothreitol; CD, circular dichroism; Tris, tris(hydroxymethyl)aminomethane.

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