POLYPEPTIDE COMPOSITION OF RAT HIGH DENSITY LIPOPROTEIN: CHARACTERIZATION BY SDS-GEL ELECTROPHORESIS

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SUMMARY: Electrophoresis on sodium dodecylsulfate-containing polyacrylamide gels of high density lipoprotein obtained from plasma of sucrose-fed rats reveals a complex pattern of three major bands and several minor, low molecular weight bands. The component present in the greatest amount is a 27,000 molecular weight protein which has aspartic acid or asparagine as N-terminus and alanine as C-terminus, and which is considered comparable to the A-I protein of human high density lipoprotein. The other major rat polypeptides have molecular weights of 46,000 (A-IV) and 35,000 ("arginine-rich"). The "arginine-rich" protein may be identical to a major polypeptide component of rat very low density lipoprotein.

INTRODUCTION

In the past several years the subunit proteins of human HDL have been well characterized in terms of their physical and chemical properties (1,2). Although the rat is widely used as a laboratory model for studying the metabolism of the serum lipoproteins (3,4,5), their subunit proteins have not been studied in such detail. Two earlier reports (6,7) have provided limited and, on some points, conflicting data regarding the number and properties of the rat apoproteins. In these studies polyacrylamide gel electrophoresis of the delipidated proteins in the presence of 8M urea was utilized for analysis of lipoprotein fractions. However, we find that the technique of SDS gel electrophoresis is superior for discerning the subunit composition of the lipoproteins. The SDS method allows us to report new information on the number, molecular weights, and chemical properties of rat HDL[1] subunit proteins and to relate them to the human apolipoproteins.

[1]The following abbreviations will be used in this paper: SDS, sodium dodecylsulfate; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein.

MATERIAL AND METHODS

Preparation of lipoproteins: Male Sprague-Dawley (Holtzmann) rats (370-450 g) were fed a semi-purified diet (60% sucrose, 21% vitamin-free casein, and 5% lard) for 17 days. It has been previously shown that this diet results in increased concentrations of VLDL and to a lesser extent HDL (8). Plasma from these rats was fractionated into density classes by the method of Havel, Eder, and Bragdon (9) and the resultant lipoproteins were delipidated by the procedure of Brown et al (10).

Fractionation of apo-HDL polypeptides: Milligram quantities of the major polypeptide components of apo-HDL were isolated by SDS electrophoresis on 7 x 100mm phosphate gels (11,12) using 10 or 15% acrylamide. Each gel contained 300-400μg of protein. One gel in each series was stained and used as a reference for cutting the remaining gels into the appropriate fractions with a razor blade. Proteins were removed from the gel slices, either by transverse electrophoresis in dialysis tubing or diffusion from the crushed gel into a 0.1% SDS solution.

N- and C-terminal amino acid analysis: Amino terminal amino acids were determined using the dansylation procedure of Weiner, Platt and Weber (13). Carboxyl-terminal amino acids were cleaved by digestion of the peptide with carboxypeptidase A (Worthington) and the released amino acids were measured in the Beckman 120C Amino Acid Analyzer. Total amino acid composition data were obtained after hydrolysis with 5.6N HCl for 22 hours at 105°.

RESULTS AND DISCUSSION

Electrophoresis of rat apo-HDL on gels containing SDS (Fig. 1, gels 1,2) revealed three major proteins with molecular weights ranging from 25-50,000 and lower molecular weight components of 8-12,000, the C-apolipoproteins which are also present in VLDL (14); there was also a small amount of material with molecular weights of 60-70,000. These results contrast with the findings of Bersot et al (6), who found only two major components in addition to the "C" proteins by gel filtration or polyacrylamide gel electrophoresis in 8M urea. Koga et al (7), using similar methods, found four fractions in the higher molecu-

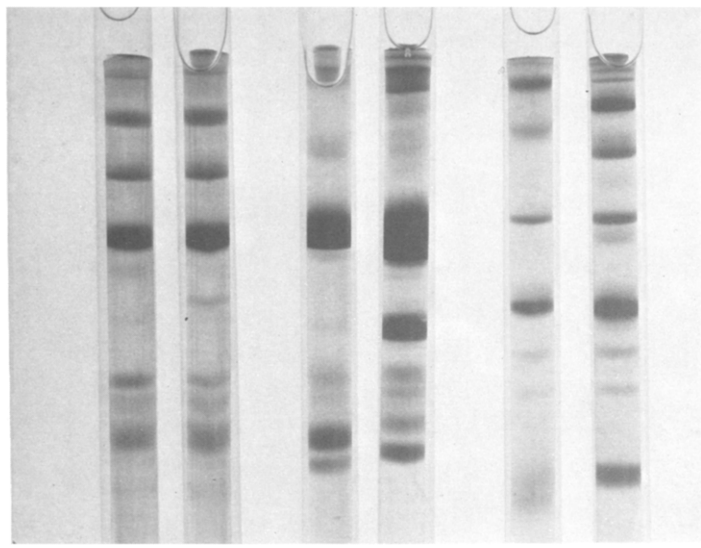

Fig. 1. Electrophoresis on 15% acrylamide gels containing SDS. Samples: gels 1 and 2, rat apo-HDL; gels 3 and 4, human apo-HDL; gels 5 and 6, bovine serum albumin, ovalbumin, concanavalin A, myoglobin, chymotrypsin and insulin. Samples applied to gels 1, 3 and 5 were preincubated with mercaptoethanol and the others were not.

lar weight range, one of which was shown to be an undissociated aggregate of the other fractions. They report molecular weights 30 to 65% higher than we have found. The probable explanation of this discrepancy is that the Stokes' radii of the standards used by Koga et al (bovine serum albumin, ovalbumin, and chymotrypsinogen) are smaller in the absence of mercaptoethanol due to the constraints of intrachain disulfide bonds present in these proteins. This would result in proteins without disulfide bonds appearing larger relative to such standards. Such behavior by the proteins ovalbumin and serum albumin is illustrated by gels 5 and 6 of Fig. 1.

The distribution of proteins as judged by densitometric scanning of gels stained with Coomassie brilliant blue is given in Table 1. Although staining is not a reliable guide to weight percentages, it is clear that a majority of the apo-HDL protein is found in the 27,000 molecular weight component. We believe that this protein corresponds to the A-I protein found in human HDL (15);

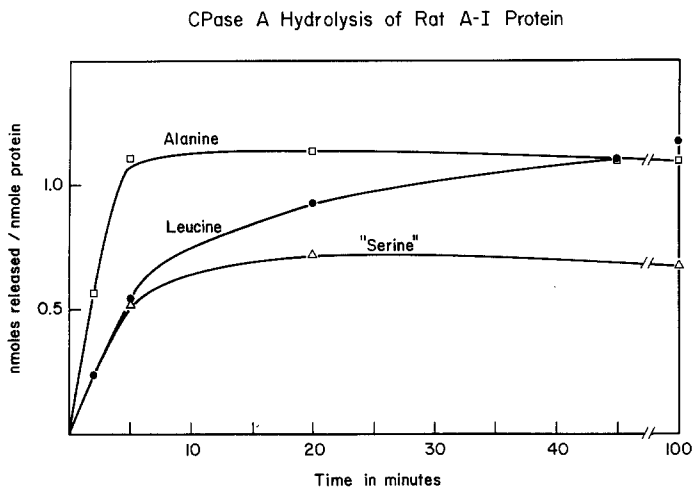

Fig. 2. Enzymatic hydrolysis of rat A-I protein by carboxypeptidase A at a substrate to enzyme ratio of 25:1 (w/w). "Serine" could also be glutamine or asparagine, since they coelute with serine on the amino acid analyzer.

it is the major component of rat HDL and has a molecular weight similar to that of the human protein. This is the only protein which appears common to HDL in all species studied thus far (16,17).

Another major component of human apo-HDL, A-II, has been shown to be a disulfide-linked dimer with identical subunits (18). Fig. 1, gels 3 and 4, shows that this protein has a molecular weight of 17,000 in the absence of mercaptoethanol and 8,500 after reduction. By contrast, in rat apo-HDL only a minor component is so affected by reduction. Herbert et al (19) have reported that rat apo-HDL contains the monomeric form of the A-II peptide, analogous to what has been reported in monkey HDL by Edelstein, Lim and Scanu (17). This may be one of the two components which are found in the fastest migrating band on SDS gels (Fig. 1, gels 1,2). It has a molecular weight of approximately 8000 and represents only a small percentage of the total protein.

Rat apo-HDL contains two major components with molecular weights of 46,000 and 35,000; we have designated these A-IV[2] and "arginine-rich", respectively.

[2]The term A-III has been used by Kostner (20) to describe a 19,000 molecular weight minor component of human HDL which might correspond to a minor band seen on some SDS gels of rat HDL.

Table 1 Molecular weights and distribution of rat apo-HDL proteins determined by optical density scanning of 10 stained gels representing several preparations of protein.

Protein	Molecular Weight	% of Total O.D.
A-IV	46,000	10-15%
"arginine-rich"	35,000	10-15%
A-I	27,000	50-60%
"C" Proteins	8-12,000	15-20%

These peptides have not been described in human HDL. All three major components were isolated by preparative SDS gel electrophoresis. Amino acid analyses of these fractions are presented in Table II. None of the proteins show the total absence of any amino acid. By contrast, human A-I is devoid of isoleucine, and human A-II is devoid of histidine or arginine. Our amino acid composition data are generally similar to those of Koga et al (7) for their PII, PIII and PIV fractions which we would expect to be comparable to our A-IV, "arginine-rich", and A-I proteins, respectively. However, their fractions were incompletely separated from each other.

Enough purified A-I peptide was obtained to carry out a determination of N- and C-terminal amino acids. Analysis of the hydrolyzed dansylated protein by thin-layer chromatography on polyamide sheets, indicated an N-terminal aspartic acid or asparagine. A time-course hydrolysis with carboxypeptidase A, shown in Fig. 2, indicated that alanine is at the C-terminus with leucine as the penultimate amino acid. The third amino acid released (labeled "serine" in the figure) could be serine or either glutamine or asparagine, which coelute with serine. As yet, we do not have similar data on the A-III and "arginine-rich" proteins.

The "arginine-rich" protein is so named because of its abnormally high arginine content, compared with other apo-HDL proteins. Its amino acid composi-

Table 2: Amino acid composition of rat HDL proteins expressed as a weight percentage

Amino Acid	A-IV	"arginine-rich"	A-I
Lysine	8.18%	5.79%	9.80%
Histidine	2.28	1.53	2.40
Arginine	5.27	10.9	5.15
Aspartic Acid	11.2	9.50	14.0
Threonine	4.58	4.36	4.40
Serine	4.24	3.47	3.40
Glutamic Acid	19.6	24.3	21.3
Proline	3.92	2.91	2.41
Glycine	2.86	3.03	2.80
Alanine	4.09	5.66	5.16
Cys/2	N.D.	N.D.	N.D.
Valine	6.48	5.54	5.47
Methionine	2.15	3.38	1.40
Isoleucine	3.04	2.41	2.16
Leucine	12.9	12.0	13.5
Tyrosine	2.50	2.06	2.68
Phenylalanine	5.07	2.83	4.12
Tryptophan	N.D.	N.D.	N.D.

N.D. = not determined

tion is similar to one of the VLDL polypeptides studied in our laboratory, which corresponds to the VS-2 fraction of Bersot et al (6). We agree with Koga et al (7) that these two peptides are probably identical. This conclusion is supported by SDS-gels which show both peptides to possess similar, if not identical, molecular weights. Preliminary data indicate that there is also immunochemical identity between these two peptides. We believe that the "arginine-rich" peptide may be the same component which we have found to exist free in the plasma (21) uncomplexed with lipid.

The technique of SDS-gel electrophoresis has thus provided us with a useful method for comparing the rat and human apo-HDL proteins as well as for isolating the three major rat apo-HDL proteins on a preparative scale. An additional advantage of this technique versus others used for the characterization of lipoproteins is that the proteins may be separated without prior delipidation.

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