PARTIAL AMINO ACID SEQUENCE OF THREE NEW APOLIPOPROTEINS ISOLATED FROM HUMAN HIGH DENSITY LIPOPROTEINS

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1. Introduction

We have found that plasma HDL of patients fed exclusively by intravenous perfusions of 10% glucose contain 6 new apoproteins [1]. These unusual apoproteins shared a peculiar amino acid composition characterized by a low content in threonine and valine and a high content in alanine, glycine and arginine, a higher pI (5.0–8.0) than the main apoproteins C and mol. wt ~10 000. This report summarizes the results of our investigation on the partial sequence of 3 new human apoproteins.

2. Materials and methods

2.1. *Source and isolation of apoproteins*

HDL obtained from patients receiving glucose perfusions intravenously was isolated by preparative ultracentrifugation at salt densities of 1.063–1.21 g/ml [2] and delipidated [3]. Fraction V was isolated by gel filtration on Sephacryl S-200 in 6 M urea [1] and then fractionated by DEAE-ion exchange column chromatography [4]. Apoproteins SV-D2 (Sephacryl fraction V, DEAE fraction 2) and SV-D4 and SV-D5 were assayed for purity by techniques including polyacrylamide gel electrophoresis, amino acid analysis and isoelectric focusing. These procedures were as in [5–7].

2.2. *Phenylisothiocyanate degradations*

Apoproteins SV-D2, D4 and D5 were sequenced by automated Edman degradation on 0.1–0.2 μmol purified proteins in a Beckman 850 C sequencer using a 0.33 M Quadrol buffer system and single acid cleavage. Repetitive yields over the first 20 steps were >95%. Phenylthiohydantoin amino acids were identified by high pressure liquid [8] and thin-layer chromatography [9].

Table 1
Amino acid composition of three new polypeptides from HDL

	SV-D2	SV-D4	SV-D5
Lys	5	5	8
His	3	3	2
Arg	10	10	7
Asp[b]	12	12	11
Thr[a]	1	<1	2
Ser[a]	7	9	7
Glu[b]	9	10	11
Pro	4	4	4
Gly	12	11	10
Ala	15	15	13
Val	1	1	3
Met	2	2	2
Ile	3	3	2
Leu	4	3	5
Tyr	5	5	5
Phe	6	7	7
Trp	3	4	3
Predicted no. residues	88	102	104
Est. mol. wt[c]	9870	11 000	11 700

[a] Corrected for loss during hydrolysis
[b] The value represents the sum of aspartic acid and asparagine or glutamic acid and glutamine
[c] Individual values were estimated by calculating the minimal weight from the amino acid composition by the method in [10]

SV-D2 NH_2-Ser-Phe-Phe-Ser-Phe-Leu-Gly-Glu-Ala-Phe-Asp-Gly-Ala-Arg-Asp

SV-D4 NH_2-Arg-Ser-Phe-Phe-Ser-Phe-Leu-Gly-Glu-Ala-Phe-Asp-Gly-Ala-Arg-Asp

SV-D5 NH_2-Ser-Phe-Phe-Ser-Phe-Leu-Gly-Glu-Ala-Phe-Asp-Gly-Ala-Arg-Asp

and

NH_2-Phe-Phe-Ser-Phe-Leu-Gly-Glu-Ala-Phe-Asp-Gly-Ala-Arg-Asp

Fig.1. Partial amino acid sequence of apoproteins SV-D2, SV-D4 and SV-D5 of HDL.

3. Results

3.1. *Amino acid composition*

Amino acid composition of 3 apoproteins of HDL fraction V is given in table 1. Minor differences exist between SV-D2, D4 and D5 in lysine and arginine content.

3.2. *Partial sequence and N-terminal amino acid*

The first 16 amino acids of the 3 apoproteins are exactly the same except for the first N-terminal. The results are given in fig.1. SV-D5 is heterogeneous and two sequences are present.

4. Discussion

All 3 apoproteins were eluted in fraction V of Sephacryl chromatography, had est. mol. wt 9 000–11 000, comparable amino acid composition but different pI values and different N-terminal amino acids.

The amino acid sequence as well as the amino acid composition differed completely from those of known apoproteins [11–15]. The sequence studies on the terminal portion (16 residues) of these unusual proteins revealed a striking similarity and suggested that the 3 polypeptides may derive from each other and are probably proteolytic cleavage products of the larger apoprotein. However small differences in molecular weight and pI might be explained by differences in carbohydrate content. The presence of sialic acid must be determined.

This study is a first characterization of 3 new apoproteins. For the most abundant of those (SV-D4 and D5) a complete amino acid sequence is in course to determine if the other 60 amino acids of the chain differ or not.

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