STUDIES OF THE COMPOSITION AND STRUCTURE OF PLASMA LIPOPROTEINS. *C*- AND *N*-TERMINAL AMINO ACIDS OF THE TWO NONIDENTICAL POLYPEPTIDES OF HUMAN PLASMA APOLIPOPROTEIN A

Gerhard KOSTNER* and Petar ALAUPOVIC
Cardiovascular Section, Oklahoma Medical Research Foundation and Department of Biochemistry, University of Oklahoma School of Medicine, Oklahoma City, Oklahoma 73104, USA

Received 29 April 1971

1. Introduction

Although several authors suggested that the protein moiety of human plasma high density lipoproteins (HDL) (1.063–1.21 g/ml) consisted of nonidentical subunits [1–3], Shore and Shore [4] were first to separate from this protein two polypeptides of similar molecular weight but different amino acid composition and electrophoretic mobilities on polyacrylamide gel. Characterized by threonine and glutamine as *C*-terminal amino acids, these polypeptides have been referred to as 'R-Thr' and 'R-Glu', respectively [4]. Although the *N*-terminal amino acid analysis of these polypeptides has not yet been reported, previous studies indicated aspartic acid as the sole *N*-terminal amino acid of the intact protein moiety [5]. The occurrence of two nonidentical polypeptides in HDL has been confirmed independently by two groups of investigators [6, 7].

We have recently shown [8] that lipoprotein A (LP-A) represents the major lipoprotein family in HDL and that its protein moiety, apolipoprotein A (ApoA), consists of a major and a minor polypeptide characterized by amino acid composition and electrophoretic behavior similar, if not identical, to those of 'R-Thr' and 'R-Glu' polypeptides. In this report we present the results of terminal amino acid analysis of ApoA polypeptides. Contrary to the reports in the literature, both immunologically-homogeneous polypeptides were found to contain glutamine as the *C*-terminal amino acid. The major polypeptide was characterized by aspartic acid as the *N*-terminal amino acid, but failure of the minor polypeptide to react with dansyl chloride or dinitrofluorobenzene indicated that its *N*-terminal amino acid was blocked.

2. Experimental procedure

2.1. *Isolation of human plasma HDL*

Lipoproteins were isolated from the plasma samples of healthy young men and women who had fasted overnight. The VLDL and LDL were removed by precipitation with 4% sodium phosphotungstate and 2 M $MgCl_2$ according to the procedure by Burstein and Morfin [9]. The density of the filtrate was adjusted to 1.21 g/ml by adding solid NaBr and the solution was centrifuged in the No. 50 rotor of the Spinco Model L-2 ultracentrifuge at 140,000 *g* for 30 hr, at 5°. The top layer (1.5–2.0 cm below the solution surface) was removed by a tube-slicing technique and washed by recentrifugation under identical conditions. After washing the top layer, consisting of HDL, was dialyzed against distilled water and lyophilized.

2.2. *Isolation of ApoA polypeptides*

The lyophilized HDL preparation was delipidized by five successive extractions with ethanol-diethyl ether (3:1, v/v, 5 × 50 ml solvent mixture/200 mg HDL) followed by two extractions with diethyl ether

* G. Kostner was a postdoctoral fellow of the Cardiovascular Section, Oklahoma Medical Research Foundation. Present address: Institut für Physiologische Chemie, Universität Graz, Graz, Austria.

North-Holland Publishing Company – Amsterdam

(2 X 50 ml/200 mg HDL) at 4°. The initial step in the fractionation of ApoA polypeptides was carried out according to a modified procedure of Rudman et al. [7]. A sample of totally delipidized HDL was dissolved in 1 M acetic acid (100 mg/6 ml) and placed on a column (200 X 2.5 cm) of Sephadex G-75. The column was eluted with 1 M acetic acid at a flow rate of 40 ml/hr. Fractions of 6.0 ml were collected and the protein was monitored by the absorbance at 280 nm. Gel filtration resulted in separation of three peaks; first two peaks (ApoA-I and ApoA-II) consisted of two main ApoA polypeptides, while the third peak comprised the ApoC polypeptides. Each fraction was rechromatographed under identical conditions. The ApoA-I and ApoA-II polypeptide fractions were purified by column chromatography on DEAE-cellulose. The DEAE-cellulose (Cellex D, 0.39 meq/g capacity, Biorad, Richmond, Calif.) was washed successively with 1 M HCl, 1 M NaOH and distilled water, and equilibrated with 0.005 M phosphate buffer (pH 7.2) containing 8 M urea. Each polypeptide fraction was placed separately on the column (30 X 1.4 cm) and eluted with a linear gradient from 0.005 M to 0.06 M NaCl. Electrophoretically- and immunologically-homogeneous fractions ApoA-I and ApoA-II were combined, dialyzed against distilled water and lyophilized.

2.3. *Immunochemical and analytical methods*

The polypeptides were studied by double diffusion and immunoelectrophoresis in 1% agar and by 7.5% polyacrylamide gel electrophoresis according to the procedures described previously [10]. Rabbit antihuman sera HDL_3, LP-C and albumin were prepared and tested as previously described [11].

Analysis of *C*-terminal amino acids by hydrazinolysis and carboxypeptidases A and B, and of *N*-terminal amino acids by dansyl chloride and dinitrophenylation were carried out according to the procedures described in earlier papers [10, 12]. Amino acid analysis was performed on a Beckman Model 120C analyzer according to an accelerated automatic procedure on spherical resins [11].

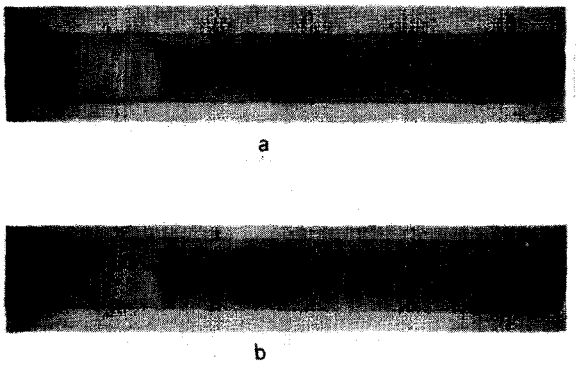

Fig. 1. Polyacrylamide gel electrophoresis of ApoA-I (b) and ApoA-II (a). The anode is to the right.

3. Results and discussion

3.1. *Characterization of ApoA polypeptides*

Both ApoA polypeptides gave single protein-staining bands on 7.5% polyacrylamide gel electrophoresis (fig. 1). However, the migration rate of ApoA-I was slower than that of ApoA-II. Each polypeptide exhibited a single immunoprecipitin line with antibodies to HDL_3. In contrast to the ApoC peptides eluted as the third major peak from the Sephadex column, they gave a negative reaction with antibodies to LP-C. When tested simultaneously with antibodies to HDL_3, the immunoprecipitin lines of ApoA polypeptides showed a non-identity reaction. The amino acid composition of ApoA-I was characterized by the absence of isoleucine and halfcystine and that of ApoA-II by the absence of histidine, arginine and tryptophan. We concluded from these results that the ApoA-I corresponded to the 'R-Thr' peptide [4], fraction III [6] or fraction II peptide [7], and the ApoA-II to 'R-Glu' [4], fraction IV [6] or fraction III peptide [7].

3.2. *C-terminal amino acids*

Hydrazinolysis of ApoA-I peptide performed at four different reaction times (10, 20, 40 and 70 hr) resulted always in detection of only trace amounts of serine, glycine, threonine and alanine (0.05–0.1 mole per 22 X 10^3 g of peptide*). In a simultaneous control experiment, hydrazinolysis of glucagon yielded

* Sedimentation equilibrium studies of ApoA polypeptides indicated that the average value for the molecular weight of ApoA-I was 22,000 and that of ApoA-II 12,500 (G. Kostner and P. Alaupovic, unpublished results).

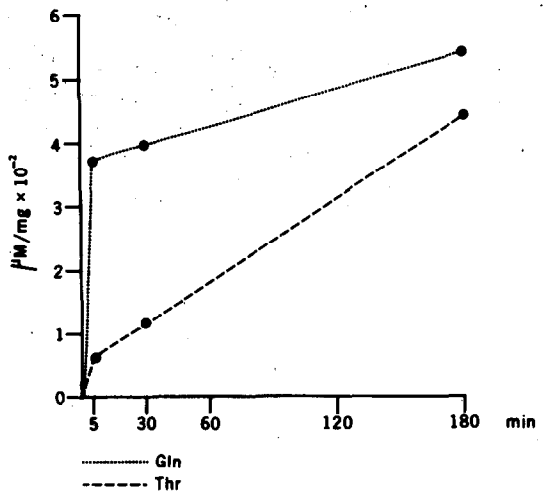

Fig. 2. Release of amino acids from ApoA-I polypeptide with carboxypeptidases A and B at 23°.

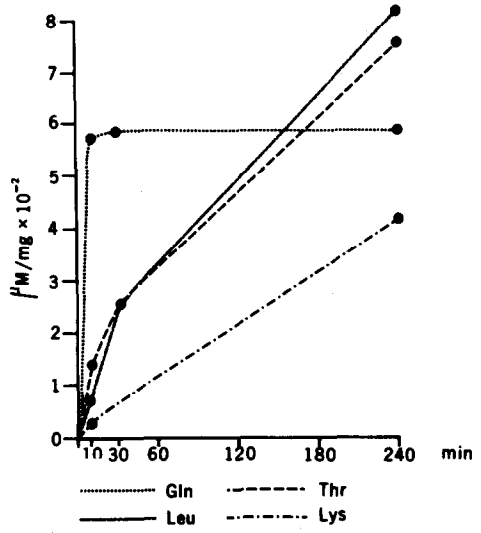

Fig. 3. Release of amino acids from ApoA-I polypeptide with carboxypeptidases A and B at 37°

only threonine in a significant amount (0.52 mole per mole of glucagon), but traces of serine, glycine and alanine were also detected. The absence of half-cystine from the amino acid composition and the negative results of hydrazinolysis experiments suggested the possible presence of either glutamine or asparagine as the *C*-terminal amino acid of ApoA-I. To explore this possibility, the ApoA-I polypeptide was digested with a mixture of carboxypeptidases A and B at 23 and

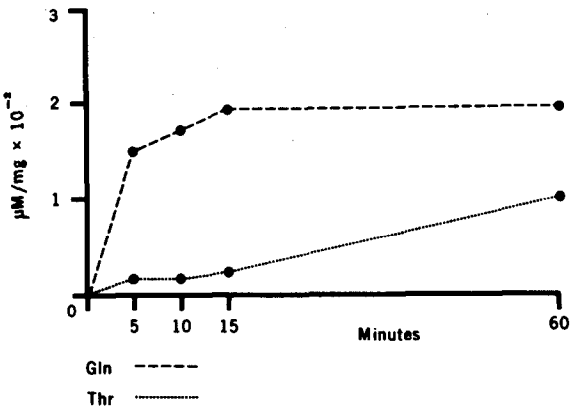

Fig. 4. Release of amino acids from ApoA-I polypeptide with carboxypeptidase A at 23°.

37°. Results of these time studies (figs. 2 and 3) showed that the initial release of glutamine was 4 to 6 times greater than that of threonine. Liberation of glutamine (1.0 mole/17.2 × 10^3 g protein) was almost completed after 30 min at 37°. Digestion of ApoA-I polypeptide by carboxypeptidase A at 23° (fig. 4) proceeded at a slower rate than that achieved by the mixture of both enzymes (fig. 2), but the initial release of glutamine was 15–20 times higher than that of threonine. To verify the sequence of *C*-terminal amino acids, the ApoA-I polypeptide was deaminated according to the procedure by Alexijev et al. [13] and the reaction product was digested by carboxypeptidase A. Although the results of this experiment showed (table 1) an incomplete deamidation (40–42%) of the terminal amino acid, the identification of glutamic

Table 1
The release of amino acids from deaminated ApoA-I by digestion with carboxypeptidase A*.

Amino acids released	μmoles/10^2 mg of peptide	
	30 min	150 min
Glutamine	0.51	0.92
Serine	0.47	0.58
Glutamic acid	0.34	0.68
Threonine	0.28	0.74
Σ (glutamine + glutamic acid)	0.85	1.60

* To increase the rate of hydrolysis of glutamic acid the enzymatic digestion was carried out at pH 6.0 and 37° [14].

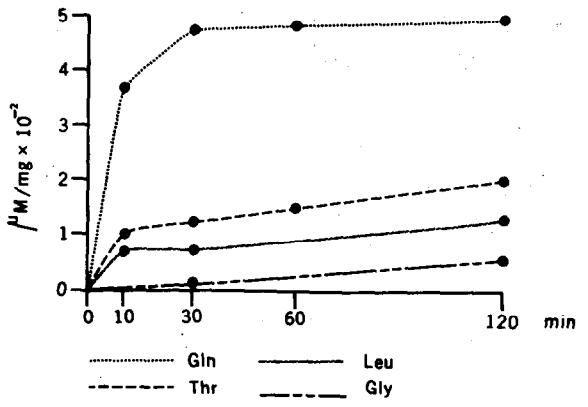

Fig. 5. Release of amino acids from ApoA-I polypeptide with carboxypeptidases A and B at 37°.

acid among the reaction products confirmed the identity and presence of glutamine as the *C*-terminal amino acid. The combined amount of glutamic acid and glutamine released after 30 min was three times as high as that of threonine. We concluded from these studies that glutamine is the *C*-terminal amino acid of ApoA-I polypeptide, followed in sequence by threonine and leucine.

Whereas hydrazinolysis of ApoA-II polypeptide resulted in detection of only trace amounts of serine, glycine and threonine, the enzymatic digestion with a mixture or individual carboxypeptidases A and B revealed glutamine as the major *C*-terminal amino acid (fig. 5). The initial release of glutamine was four times greater than that of threonine; the release of glutamine with carboxypeptidase A leveled off after 8 to 10 hr (1.0 mole/12.5 X 10^3 g protein). Analysis of the carboxypeptidase A digest (pH 6.0 at 37°) of a partially deamidated polypeptide revealed the presence of glutamic acid. We concluded from these results that ApoA-II contains also glutamine as the *C*-terminal amino acid.

In contrast to the published report [4] suggesting threonine and glutamine as the *C*-terminal amino acids of the two major HDL polypeptides ('R-Thr' and 'R-Glu'), the results of our kinetic experiments with intact and deaminated preparations showed that both these immunochemically-homogeneous ApoA polypeptides contain glutamine as the carboxyl terminus. Although not yet explainable, this discrepancy may be due either to the presence of contaminating peptides, methodological differences or microheterogeneity of ApoA-I polypeptide. It should be pointed out, however, that Shore and Shore [4] also found glutamine to be the most rapidly released amino acid during the separate digestion of HDL protein by carboxypeptidase A and B at 25°.

3.3. *N-Terminal amino acids*

Qualitative *N*-terminal amino acid analysis of ApoA-I polypeptide by dansylation method indicated aspartic acid as the only major *N*-terminus. Dinitrophenylation of ApoA-I resulted in the paper chromatographic detection of a single ether soluble component (in addition to dinitrophenol and dinitroaniline) with the R_f value of DNP-aspartic acid. Hydrolysis of this DNP-derivative with saturated baryte-water [15] yielded aspartic acid as the sole amino acid. The recovery of DNP-aspartic acid was 0.5 mole per 22 X 10^3 g of polypeptide. These results showed clearly that aspartic acid is the *N*-terminal amino acid of ApoA-I polypeptide.

N-Terminal amino acid analyses of ApoA-II by either of these two methods were negative. Since no dansylated or DNP-amino acids could be detected in these experiments, we concluded that *N*-terminal amino acid of ApoA-II was blocked.

Detection of identical *N*- or *C*-terminal amino acids in the consitutive polypeptides of human plasma apolipoproteins argues strongly against the present practice of coding or identifying polypeptides by their terminal amino acids. We propose, therefore, that the major polypeptide (former 'R-Thr') be designated as ApoA-I and the major polypeptide (former 'R-Glu') as ApoA-II. The ApoA-I is characterized by glutamine as the *C*-terminal and by aspartic acid as the *N*-terminal amino acid. The ApoA-II is characterized by glutamine as the *C*-terminal and by, as yet unknown, blocked *N*-terminal amino acid.

Acknowledgements

This study was supported in part by Grants HE-7005 and HE-6221 from the U.S. Public Health Service, and by the Oklahoma Heart Association.

We thank Mrs. Carmen Quiroga for her excellent assistance with the amino acid analyses and Mrs. M. Farmer for her help in the preparation of the manuscript.

References

[1] P. Alaupovic, S.L. Walraven and M.L. Sullivan, Circulation 36, Suppl. II (1967) 2.
[2] L. Cohen and J. Djordjevich, Proc. Soc. Exptl. Biol. Med. 129 (1968) 788.
[3] A. Scanu, W. Reader and C. Edelstein, Biochim. Biophys. Acta 160 (1968) 32.
[4] B. Shore and V. Shore, Biochemistry 7 (1968) 2773.
[5] J. Avigan, R. Redfield and D. Steinberg, Biochim. Biophys. Acta 20 (1956) 557.
[6] A. Scanu, J. Toth, C. Edelstein, S. Koga and E. Stiller, Biochemistry 8 (1969) 3309.
[7] D. Rudman, L.A. Garcia and C.H. Howard, J. Clin. Invest. 49 (1970) 365.
[8] P. Alaupovic, G. Kostner, D.M. Lee, W.J. McConathy and H.N. Magnani, Exposés Ann. Biochem. Med. 31 (1971) in press.
[9] M. Burstein and R. Morfin, Life Sci. 8, Part II (1969) 345.
[10] D. Seidel, P. Alaupovic, R.H. Furman and W.J. McConathy, J. Clin. Invest. 49 (1970) 2396.
[11] D.M. Lee and P. Alaupovic, Biochemistry 9 (1970) 2244.
[12] A. Gustafson, P. Alaupovic and R.H. Furman, Biochemistry 5 (1966) 632.
[13] B. Alexijev, V. Holeysovsky and F. Sorm, Collection Czech. Commun. 29 (1964) 1296.
[14] K. Titani, K. Narita and K. Okunuki, J. Biochem. (Tokyo) 51 (1962) 350.
[15] G.L. Mills, Nature (1950) 403.