Correction of COOH-terminal Amino Acids of Human Plasma Very Low Density Apolipoproteins

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SUMMARY

The COOH-terminal amino acids of the low molecular weight apoproteins of human plasma very low density lipoprotein have been reinvestigated. Previously these were designated by their apparent COOH-terminal amino acids as alanine apolipoprotein (apoLP-Ala), valine apolipoprotein (apoLP-Val), and glutamic acid apolipoprotein (apoLP-Glu). Alanine has been confirmed as the COOH terminus of apoLP-Ala. Serine rather than valine is the COOH-terminal amino acid of the polypeptide previously termed apoLP-Val. Hydrazinolysis releases both glutamic acid and valine in a molar ratio of 3:1 from the DEAE-cellulose fraction previously designated apoLP-Glu.

The delipidated very low density lipoproteins of human plasma have been shown to contain a minimum of five different apolipoproteins (1-4). Earlier reports from this laboratory have described in detail their isolation and characterization (1-3). Three small polypeptides with molecular weights in the range of 7,000 to 10,000 have been found to constitute about 50% of the total VLD protein. As a temporary form of nomenclature, they have been designated by their COOH-terminal residues as apoLP-Val, apoLP-Glu, and apoLP-Ala. Improved techniques for their isolation in quantity (2, 4) have permitted a reinvestigation of their COOH-terminal amino acids. Two significant errors in previous data have been revealed.

VLD apolipoproteins were obtained from patients with types I and V hyperlipoproteinemias. Lipoproteins from these subjects do not differ qualitatively from those of normal subjects (6, 7). The techniques of isolation, delipidation, solubilization, gel and ion exchange chromatography, and Ouchterlony double diffusion in agarose have previously been described (1, 2, 4). The abbreviations used are: VLD lipoproteins, very low density lipoproteins of d < 1.006 g per ml; apoLP-Val, valine apolipoprotein; apoLP-Glu, glutamic acid apolipoprotein; apoLP-Ala, alanine apolipoprotein.

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All polypeptide fractions chosen for analysis migrated as single bands on polyacrylamide gel electrophoresis (Fig. 1) (8). The technique described by Potts et al. (9) was used for carboxypeptidase A digestion and hydrazinolysis was performed according to the method of Akabori, Ohno, and Narita (10). The sources of equipment and materials used were identified elsewhere (1-3). The free amino acids liberated by both techniques were identified, and the amounts of protein reacted were determined on a Beckman model 121 amino acid analyzer.

Four preparations of D-1 from three patients were evaluated. The amino acid composition (Table I) did not differ significantly from that previously reported (1, 3) and the fraction gave a single precipitin line against specific antisera. D-1 did not react with antisera prepared against the major apolipoproteins of high or low density lipoproteins. Carboxypeptidase A liberated no amino acids from D-1 even in incubation media containing 100 mM decyl sulfate or 4 M urea. When hydrazinolysis was performed on D-1, serine was the only free amino acid recovered in significant quantity (Table II). Valine had been described as the COOH-terminal amino acid of this polypeptide (1). High voltage paper electrophoresis was used in that study to identify the amino acids liberated. In the system used (11) valine and serine migrate to contiguous places on the chromatogram.

COOH-terminal residues were determined on three preparations of D-2 from different donors. They all appeared homog-
### Table I

| Amino acid | D-1 (apoLP-Val) | D-2 (apoLP-Glu) | D-3, D-4 (apoLP-Ala) |
|------------|----------------|----------------|---------------------|
|            | mmoles/100,000 g protein | mmoles/100,000 g protein | mmoles/100,000 g protein |
| Asp        | 82 (58-83) | 61 (60-62) | 87 (86-88) |
| Thr        | 42 (40-44) | 99 (96-100) | 59 (59-60) |
| Ser        | 163 (103-104) | 98 (97-100) | 23 (22-23) |
| Glu        | 145 (144-147) | 164 (161-166) | 124 (125-126) |
| Pro        | 10 (7-11) | 40 (39-41) | 17 (15-20) |
| Gly        | 19 (18-22) | 25 | 36 (33-39) |
| Ala        | 47 (47-48) | 73 (72-74) | 120 (119-122) |
| Val        | 51 (50-52) | 44 (44-47) | 71 (69-74) |
| Met        | 16 | 22 (21-23) | 24 (23-26) |
| Ile        | 44 (44-45) | 10 | 0 |
| Leu        | 38 | 64 (63-66) | 63 (61-68) |
| Tyr        | 1 | 54 (52-56) | 23 (22-23) |
| Phe        | 45 (45-47) | 22 (21-22) | 46 (44-47) |
| Lys        | 135 (135-137) | 69 (67-71) | 69 (67-71) |
| His        | 0 | 0 | 0 |
| Arg        | 47 (45-48) | 11 (11-12) | 22 (21-23) |

### Table II

**Hydrazinolysis of the VLD apolipoproteins**

Values shown are the means (and ranges) of three analyses for D-1 and two analyses each for D-3 and D-4.

| DEAE-fraction | Amount of protein analyzed | Amino acids recovered |
|---------------|---------------------------|----------------------|
|               | mg | µ moles/mg |
| D-1           | 370-530 | Serine: 74 (72-76) |
|               |     | Glutamic acid: 2 (0-5) |
|               |     | Glycine: 1 (0-4) |
| D-2           | 400-600 | Glutamic acid: 53 (50-58) |
|               |     | Valine: 17 (17-18) |
|               |     | Serine: 4 (3-5) |
|               |     | Alanine: 2 (1-2) |
|               |     | Glycine: 1 (1-2) |
| D-3, D-4      | 250-420 | Alanine: 18 (10-90) |
|               |     | Serine: 11 (0-26) |
|               |     | Methionine: 2 (1-4) |
|               |     | Proline: 2 (0-4) |

Analysis on polyacrylamide gel electrophoresis. The amino acid composition of this DEAE-cellulose fraction does not differ significantly from that reported earlier (5), even when entirely different techniques of isolation were employed (12). However, antibodies raised against this fraction invariably gave two lines of reaction against all D-2 preparations. Carboxypeptidase A digestion at pH 8.0 released no amino acids from D-2 after 24 hours of incubation. Experiments at lower pH levels were not attempted. Hydrazinolysis (Table II) released significant quantities of both glutamic acid and valine in a consistent molar ratio of 3:1. Smaller quantities of serine, alanine, and glycine were also recovered, but their significance is uncertain since their amino acid hydrasides are particularly labile in aqueous solution (13). The data suggest that D-2 may contain at least two polypeptides having COOH-terminal glutamic acid and valine, respectively. Rechromatography of D-2 in 6 M urea, however, did not alter the amino acid composition, end group analysis, or migration as a single band on polyacrylamide gel electrophoresis.

D-3 and D-4 have been shown to differ only in their content of sialic acid (3). They gave single precipitin lines against specific antisera and the four fractions analyzed had the expected amino acid composition (1, 3, 4, 12) (Table I). After carboxypeptidase digestion of D-3 and D-4, the time-release curve was essentially identical with that reported by Brown et al. (1), establishing alanine as the COOH-terminal amino acid of these polypeptides. Hydrazinolysis of D-3 and D-4 repeatedly produced very poor recoveries of alanine and highly variable amounts of serine (Table II). The reason for this is unclear and no recovery data from other laboratories are available for comparison.

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### Reconstitution of the Third Site of Oxidative Phosphorylation*

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***SUMMARY***

A complex was reconstituted with hydrophobic proteins from bovine heart mitochondrial membranes, cytochrome c, cytochrome oxidase, phospholipids, and coupling factors. These vesicular structures catalyzed oxidative phosphorylation with reduced N-methylphenazinium methyl sulfate as substrate.

It was shown recently (1) that a preparation of hydrophobic proteins from bovine heart mitochondria combined with phospholipids and coupling factors yielded vesicles which catalyzed an oligomycin-sensitive ATP exchange. These vesicles also

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