Title
AMINO ACID COMPOSITION OF THE PROTEINS OF THE SERUM LIPOPROTEINS

Author
Shore, Bernard.

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AMINO ACID COMPOSITION OF THE PROTEINS OF THE SERUM LIPOPROTEINS*

Bernard Shore+

Donner Laboratory and Radiation Laboratory
University of California, Berkeley, California

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Human serum contains a lipoprotein spectrum of a very wide range of molecular weights and hydrated densities, i.e., a series of substances containing various lipids in combination with protein.[1,2,3] The major factor responsible for the density variance (from less than 1.0 up to 1.145 g/ml) is the difference in the lipid-protein ratio from one species to another. Interest in the lipoproteins has been heightened by recent researches which have demonstrated a correlation among certain serum lipoprotein classes, atherosclerosis, and various diseases characterized by defects in lipoprotein metabolism.[4,5] In this communication we wish to present paper-chromatographic data on the amino acid composition of the protein moieties of the lipoproteins of S_f 6** (hydrated density 1.035 g/ml), S_f 13 (hydrated density 1.015 g/ml), S_f 4–20 (hydrated density 1.006 to 1.04 g/ml), S_f 20–400 (hydrated density 1.006 to about 0.96 g/ml), high density (containing molecules of hydrated density 1.075 and 1.145 g/ml), and 1.145 g/ml hydrated density.

Lipoproteins were isolated from the serum of presumably normal, fasting humans and the purity was estimated by analytical ultracentrifugal techniques described previously.[1,2] They were delipidized by previously published methods,[6] and washed with water until free of salt. Protein residues were then hydrolyzed and chromatographed by the procedure of

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+ This work was done in collaboration with Virgie G. Shore, Virus Laboratory, University of California.

** By the terminology S_f A, where A is a number, we mean lipoproteins of flotation rate A S_f units, where 1 S_f unit = 10^{-13} cm/sec/dyne/g, in a medium of d_4^{26} 1.0630 g/ml achieved by addition of sodium chloride to the lipoprotein solution.

Levy and Chung.[7] The R_f values of the amino acids of the hydrolysates were compared with those of known amino acids run simultaneously. Superposition of chromatograms indicated that the same amino acids (valine, lysine, aspartic acid, glutamic acid, arginine, histidine, glycine, alanine, tyrosine, leucine, phenylalanine, proline, and cysteine) are found in the proteins of the previously mentioned lipoproteins. The cysteine was present as a decomposition product of the acid hydrolysis. Its presence was corroborated by the sulfur content, 1% of the protein (sulfur analysis by Microanalytical Laboratory, Chemistry Department, University of California). The presence of proline, histidine, and arginine was verified by specific color tests.[8] Histidine was detected by ninhydrin and by the Pauly reagent[8] only when protein samples greater than 0.5 mg were chromatographed. In one case, with 1 mg of S_f 6 protein, a faint spot for methionine was detected with ninhydrin. Its presence was not confirmed by the platinic iodide test.[8] Quantitative amino acid estimation was carried out by measurement in the Beckman Model DU spectrophotometer of the fluorescence of amino acid–sugar complexes developed by spraying the chromatograms with a xylose–sodium bisulfite spray.[9] The intensity of fluorescence is proportional to the amount of amino acid present. If equal weights of protein are analyzed, and if the intensities of the spots after chromatographic separation are identical, one can conclude that the proteins are quantitatively composed of the same amino acids. By this procedure we were unable to distinguish any quantitative differences among the previously mentioned proteins. We were able also to demonstrate the identity of the proteins of lipoproteins of comparable S_f rate and hydrated density from different donors. Typical data are presented in Table I; the values given are averages of duplicate or triplicate determinations. All the proteins analyzed contained between 13.5% and 14% nitrogen by Nesslerization.

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ABSTRACT

Paper chromatographic studies on the amino acid composition of the protein moieties of the $S_f 6$ (hydrated density 1.035 g/ml), $S_f 13$ (hydrated density 1.015 g/ml), $S_f 4-20$ (hydrated density 1.006 to 1.04 g/ml), $S_f 20-400$ (hydrated density 1.006 to about 0.96 g/ml), high-density (containing molecules of hydrated density 1.075 and 1.145 g/ml), and 1.145 g/ml hydrated density lipoproteins of serum indicate the proteins to be of quantitatively identical amino acid composition, both with respect to type and percentage of amino acid present. These amino acids are valine, lysine, aspartic acid, glutamic acid, arginine, histidine, glycine, alanine, tyrosine, leucine, phenylalanine, proline, and cysteine. Methinoine may also be present, but in less percentage than the other amino acids.

Table I

Fluorescence Intensities of Xylose Derivatives of Amino Acids from 0.10-mg Protein Samples of Various Lipoproteins

Amino Acid Spot	S_f 6	1.145 g/ml hydrated density	S_f 13	S_f 4–20	1.075 and 1.145 g/ml hydrated density	S_f 20–400
Lysine, Cysteine	87	84	85	83	78	85
Aspartic acid, Glycine	57	56	57	54	53	56
Glutamic acid	61	62	62	59	57	60
Alanine	47	50	49	50	49	52
Tyrosine	20	18	20	20	18	19
Valine	34	35	35	32	33	32
Phenylalanine	29	30	30	29	28	28
Leucine	38	34	38	36	36	37

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