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The free fatty acids bound to human serum albumin*

ABRAHAM SAIFER and LAWRENCE GOLDMAN

Department of Physical Chemistry, Isaac Albert Research Institute of the Jewish Chronic Disease Hospital, Brooklyn 3, New York

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SUMMARY

The fatty acid composition of human serum albumin (Cohn Fraction V) was determined by gas-liquid chromatography. Forty-three peaks were observed; 26 of these were identified by various methods. Eight of the identified peaks constituted about 90% of the total free fatty acids, and 65% of the total were unsaturated acids. The patterns of fatty acids bound to albumin were essentially the same as those found in plasma. However, quantitative differences were observed in the percentage of 18 carbon acids in the free fatty acids of human serum albumin samples as compared to plasma. Possible explanations to account for these differences are discussed.

The free fatty acids (FFA) present in human blood plasma represent a transport form of lipid readily available as substrate for energy production (1). FFA exist in plasma primarily as anions and are "free" only in the sense of not being bound by covalent linkages. Less than 0.01% are actually free in solution. Although a small percentage of FFA is normally bound to lipoproteins, the entity of greatest biological significance is the albumin-fatty acid complex (2). One of the main physiological roles of the serum albumins is to serve as a transport vehicle for many kinds of anions, including FFA. In previous publications from this laboratory, the anomalous behavior of human serum albumin in the Tiselius electrophoresis apparatus (3), and the effect of bound lipids on the electrophoretic patterns, have been investigated at acid pH (4). Human serum albumin prepared by low temperature-ethanol fractionation (Cohn Fraction V) gives a single electrophoretic boundary in barbital buffer, pH 8.6, 0.10 ionic strength (μ), but multiple boundaries in the acid pH region. Removal of lipid from the albumin preparation by ether (5), or isooctane (6) treatment at low temperature, yields samples which give a single ascending boundary in both 0.10 μ acetate (pH 4.0) and barbital (pH 8.6) buffer systems.

This paper deals with the composition of the fatty acids removed from human serum albumin by denaturation of the protein and by low temperature-ether treatment of albumin solutions. Results obtained for FFA bound to human serum albumin were compared with those found by Dole *et al.* (7) for human plasma.

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EXPERIMENTAL

Isolation of Free Fatty Acids from Albumin. The human serum albumin employed in this procedure was supplied in the form of a 25% solution (Squibb Fraction V) containing 0.04 M acetyl tryptophanate as a stabilizer.[1] The absence of cholesterol and its esters, triglycerides, and lipid phosphates was verified by silicic-acid chromatography (8). For the gas-liquid chromatographic study of the FFA bound to albumin, the protein was subjected to the denaturation-extraction procedure of Cohn *et al.* (9). This was necessary because of the small amount of bound lipid, which cannot be completely removed without denaturing the protein (10). Solvent was removed from the extract by low temperature vacuum distillation and the residue extracted with petroleum ether.

Methylation and Gas-Liquid Chromatography of Esters. For gas-liquid chromatography, the acids were methylated directly on an anion exchange resin, IRA-400, as described by Hornstein *et al.* (11). The methyl esters were chromatographed at 197° on a nonpolar stationary phase, Apiezon L, and then on ethylene glycol adipate, a polar stationary phase. A Pye Argon

[1] This albumin was supplied gratis by James H. Pert, Research Director, American National Red Cross, Washington, D. C.

Chromatograph, employing the Sr 90 ionization chamber of Lovelock (12) as a sensing device, was used. Acids were identified by five methods: addition of known components, comparisons with the retention timetables published by the Rockefeller Institute group (13), peak reversal on Apiezon and ethylene glycol adipate columns, the linear relationship between the logarithm of the retention time and the number of carbon atoms in a homologous series, and by microbromination. Quantitation was accomplished by measurement of the areas under the peaks with a planimeter.

RESULTS

Gas-liquid chromatography revealed the presence of at least 43 different acids, of which 26 were identified. Eight acids constituted about 90% of the total: oleic 33; palmitic 25; linoleic 20; arachidonic 5; palmitoleic 3; and myristic, stearic, and 20:3, each about 1.5% of the total. Approximately 66% of the total fatty acids were unsaturated, about 1% were odd-numbered, and less than 1% C_{10} or shorter. Branched-chain and polyunsaturated fatty acids appeared to be present in trace amounts. Table 1 represents the percentage by weight of the fatty acids of albumin found in all samples tested, in comparison with those reported by Dole *et al.* (7) for human blood plasma. The "double bond index" (7) shown in Table 1 is a measure of unsaturation, calculated as $\Sigma(n_i w_i/m_i)$, where n_i, is the number of double bonds in the fatty acid "i," m_i is the molecular weight, and w_i the per cent by weight of the fatty acid in the given material.

TABLE 1. THE FREE FATTY ACID COMPOSITION OF HUMAN SERUM ALBUMIN (FRACTION V) IN COMPARISON WITH THOSE PRESENT IN HUMAN BLOOD PLASMA

Free Fatty Acids* in Albumin		Free Fatty Acids in Plasma†
	per cent	*per cent*
Saturated		
12:0	<1	<1
13:0	<1	
14:0	1.5	1.7
15:0	<1	
16:0	24.7	23.2
17:0	<1	
18:0	1.5	12.9
20:0	<1	
Monounsaturated		
14:1	<1	<1
16:1	3.1	2.4
18:1	33.1	28.9
18:1‡		3.7
Diunsaturated		
18:2	20.0	14.5
Polyunsaturated		
18:3	<1	
20:3	1.4	1.3
20:4	5.0	4.7
20:5	<1	
Double bond index	36.0	28.0

* The system of nomenclature used in this table is the same as that listed in reference 13.

† Values given are those reported by Dole (7).

‡ Isomers.

DISCUSSION

The compositions of the FFA removed by denaturation of albumin shown in Table 1 are similar to those reported by Dole *et al.* (7) for the FFA of human plasma. The differences obtained reside in the per cent composition of 18:0, and in the degree of unsaturation. The nature of the materials which were methylated may in part account for the differences noted in the composition of the FFA in human serum albumin, as compared to human plasma. Trout *et al.* (14) have suggested that some phospholipid is probably extracted and titrated in the Dole method (15). If this is so, gas-liquid chromatography of the methyl esters recovered by Dole's method may represent not only those acids bound to albumin, but also the fatty acids constituting part of the plasma phospholipid fraction as well.

Gas-liquid chromatography of the methylated FFA, removed by low temperature-ether treatment of albumin solution, showed patterns similar to those obtained by denaturation of the protein. An essential difference involved the 16:0 and 18:0 components which were present to about the same extent, i.e., 29% in the case of the ether-treated albumin. This is not unexpected, since extraction at low temperature does not remove all the lipid (or FFA) from albumin. Oleate, exhibiting the greatest affinity for albumin (2), would be more completely removed from albumin only after denaturation.

The relatively stronger binding affinity of unsaturated FFA, such as oleate, to albumin, as compared to saturated acids, e.g., stearate, as found by Goodman (2), has been confirmed by means of moving boundary electrophoresis at acid pH (4). Experimental work now in progress employs a combination of electrophoretic and gas-liquid chromatographic procedures for

determining the binding affinities of various FFA to albumin.

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