Isolation and Amino Acid Composition of Dog Plasma Albumin*

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A need for precise data on the amino acid composition of dog albumin, for the investigation of the rate of albumin synthesis, led to the present study. In preparation of pure dog albumin, it was found that the procedures used for bovine and human albumins were generally applicable, but that certain modifications were required. Likewise, despite a general similarity, differences in amino acid composition are found in comparing dog albumin to that from cows and humans.

EXPERIMENTAL PROCEDURE

Isolation of Dog Plasma Albumin

Materials—Chemicals were of reagent quality with the exception of ethanol, a commercial 95% grade. Ethanol concentrations are expressed as volumes %.

pH—A Cambridge pH meter equipped with a glass electrode was used. Solutions containing ethanol were diluted with 0.02 M sodium chloride to reduce ethanol concentrations to less than 1%. Measurements were performed at or near 25°.

Protein Concentration—A small sample of the albumin-rich Solution V-r (see "Procedure") was dialyzed against a large volume of pH 4.5, ionic strength 0.02 acetate buffer at 2°. Refractive indices of the protein solution and the dialysate were measured with a Bausch and Lomb dipping refractometer at 25°. The absorbancy at 280 mμ of the protein solution was determined, after appropriate dilution, in a Beckman model DU spectrophotometer. On the assumption of a refractive index increment of 0.00186 per g per 100 ml, an absorbancy index of 6.6 per g per 100 ml at 280 mμ was calculated, and then routinely applied in spectrophotometric estimation of protein concentration.

Protein Sulphydryl Groups—A method of Hughes (1), with use of methyl merccuric nitrate as described by Edelhoch et al. (2), was adopted.

Electrophoresis and Ultracentrifugation. The electrophoresis medium was barbital buffer, pH 8.6, ionic strength 0.1. Sedimentation velocity profiles were obtained with a Spinco model E ultracentrifuge.

Procedure—Fresh dog blood was collected in 75 ml of standard acid-citrate-dextrose anticoagulant solution. From this solution, 440 ml of plasma were obtained after centrifugation of the blood at 2°. The plasma was fractionated in accordance with Method 6 of Cohn et al. (3), although it was recognized that adoption of the unmodified fractionation scheme with dog plasma might not yield a Fraction V of optimal purity (4). The Fraction V paste (49 g) was dissolved in 300 ml of water at 0°, and 50 ml of 53% ethanol were added dropwise, with stirring and gradual reduction of temperature to −3°. Some material precipitated under these conditions (3) and was removed by filtration, leaving 490 ml of solution containing 7.1 g of protein (Solution V-r). Paper electrophoresis revealed that an estimated 15 to 20% of the total protein consisted of α-globulin, so that only 5.5 to 6.0 g of albumin were present. The sulphydryl to albumin mole ratio of this solution was 0.5 (assuming a molecular weight of 66,000 for dog albumin). Since, as was confirmed later, the α-globulin contained no sulphydryl groups about half of the albumin was mercaptalbumin. In an attempt to convert non-mercaptalbumin to mercaptalbumin (5), 1.5 moles of cysteine (cysteine to albumin mole ratio was 17) were added, and the solution was kept for a day at −3°, pH 4.8, ionic strength 0.016, and ethanol concentration 10%. After the pH was raised to 5.1 with 2 ml of a sodium bicarbonate solution (6 g/100 ml), chilled ethanol (−15°) was added to a final concentration of 40%. The resulting precipitate was allowed to settle overnight at −5°. It was washed repeatedly with acetate buffer (pH 5.1, ionic strength 0.015) containing 40% ethanol, to ensure complete removal of free cysteine. The precipitate was dissolved in acetate buffer (pH 5.1, ionic strength 0.015) at 2° to yield 70 ml of a solution containing 6.4 g of protein (5.1 to 5.5 g of albumin). This solution still contained ethanol from the paste, and the final ethanol concentration was in the range 15 to 20%. The sulphydryl to albumin mole ratio was 0.7, somewhat higher than that of the original solution before the addition of cysteine. A cysteine to albumin mole ratio of 26 has been found sufficient to effect nearly 70% conversion of human non-mercaptalbumin to mercaptalbumin in a similar procedure;† the approximately 40% conversion achieved here is reasonable in view of the lower concentration of cysteine employed.

After a day or so, it was observed that a white precipitate had formed in the protein solution at 2°. It looked crystalline, although the crystals were small and fragile. Redissolved in

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† Unpublished observation of S. E. Allerton.
ethanol-free buffer, a sample of the precipitate seemed nearly homogeneously in the ultracentrifuge, and sedimented at the rate expected for monomeric albumin. Paper electrophoresis showed that the precipitate also contained a small fraction of the $\alpha_1$-globulin contaminant. The supernatant, greatly enriched in the or2 component, contained no sulfhydryl groups. Crystallization of bovine serum albumin under similar conditions has been noted by Cohn, Hughes, and Weare (6). The dog albumin was further purified by three additional precipitations from acetate buffer (pH 5.1, ionic strength 0.015) containing 20 to 26% ethanol. The sulfhydryl titer of the final redissolved precipitate indicated that monomer and dimer were probably due both to precipitation of monomer with mercury chloride added (1). The ethanol concentration was raised to ethanol. The sulfhydryl titer of the final redissolved precipitate indicated that monomer and dimer were probably due both to precipitation of monomer with mercury chloride added (1). The ethanol concentration was raised to

<table>
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<tr>
<th>Amino acid composition of dog plasma albumin as determined with use of ion exchange columns</th>
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<tbody>
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<td><strong>Amino acid</strong></td>
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<tr>
<td>Glycine</td>
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<td>Alanine</td>
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<td>Aspartic acid</td>
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<td>Glutamic acid</td>
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<td>Amide NH$_2$</td>
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| Total | 100.49 ± 4.21 | 98.85 | 97.19 | 588-594 |

| Mean residue weight | 111,4-112.2 |

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$^a$ Deviation from the mean of two analyses.
$^b$ Value of the 72-hour hydrolysate.
$^c$ Value extrapolated to zero.
$^d$ Estimated on intact protein (14).
$^e$ Estimated as cysteic acid.
$^f$ Value not included in the total.

*We are indebted to Miss Nancy A. Wilson for this analysis.
For molecular weight determination, 0.057 ml quantities of the same protein solution and solvent were employed in a sedimentation equilibrium experiment (9). A speed of 12,590 r.p.m. was selected and temperature was maintained at 25.0°C. The optical components of the ultracentrifuge were adjusted for the production of Rayleigh interference fringes (10), which were recorded on Eastman Kodak spectroscopic II-G plates. An interference filter, type H-1 of Band Atomic, Inc., with a transmission maximum at 5,461 A, was mounted in front of the plate holder. From the final stable fringe distribution, a value of \( M(1 - \eta) \) equal to 18,300 was calculated. The density \( \rho \) cannot be calculated. If \( \eta = 0.730 \), \( M = 67,800 \); if \( \eta = 0.740 \), \( M = 70,400 \).

Organic analysis of an albumin sample equilibrated for 24 hours with room air, gave: \( \text{H}_2\text{O}, 8.707\% \) of moist protein; \( \text{ash}, 16.597\% \) of dry protein; \( \text{N}, 16.597\% \) of dry protein; \( S, 1.85\% \) of dry protein.

Amino Acid Analyses—Samples of albumin (2 to 3 mg) were weighed out at the same time as were those for organic analysis. They were hydrolyzed in glass-distilled, constant boiling HCl for 24 and 72 hours according to Hirs, Stein, and Moore (11). A separate sample was oxidized with performic acid as described by Hirs (12) and similarly hydrolyzed. The amino acid composition of each sample was determined according to Spackman, Stein, and Moore (13) with a Spinco model 120 amino acid analyzer. The tryptophan content of two samples of albumin was estimated by the procedure of Spies and Chambers (14). The results are presented in Table I.

The content of five amino acids was determined independently by isotope dilution. Weighed amounts of \(^{13}C\)-glucose, \(^{19}C\)-alanine, \(^{13}C\)-serine, \(^{14}C\)-aspartic acid, and \(^{14}C\)-glutamic acid were added to weighed albumin samples. These were hydrolyzed by refluxing, under nitrogen, for 24 hours, in glass-distilled, constant boiling HCl. The hydrolysates were taken to dryness and the five added amino acids separated on Dowex 50 columns and reisolated (15). The carrier and reisolated amino acids were counted in stainless steel planchets in a windowless gas flow counter. The observed counts were corrected with a self absorption curve constructed according to Hendler (18). The results are shown in Table II.

**DISCUSSION**

The results of the sedimentation equilibrium experiment suggest that the molecular weight of dog plasma albumin is probably greater than 66,000. Uncertainty of data for the anhydrous partial specific volume of human and bovine, as well as dog plasma albumin, limits the precision to which molecular weights may be assigned. However, it appears that the differences in molecular weights of albumins from the three species are less than the uncertainty in their determination. For these reasons, a molecular weight of 66,000 has been retained for calculation of the number of amino acid residues per molecule.

Although we have no data for dog \( \alpha \)-globulins, in general, the tryptophan content of globulins ranges from 4 to 10 times as high as that of dog albumin (17, 18). If this is indeed the case for dog globulins, then the presence of 2% \( \alpha \)-globulins in the albumin preparation would account for the high value of 1.2 residues of tryptophan per molecule found, as compared to the expected value of 1.

The values found by isotope dilution agree, within experimental error, with those obtained from the amino acid analyzer. This is of particular importance with respect to serine since it tends to confirm the validity of the extrapolation to zero time. The results reported here are in poor agreement with earlier, partial reports (18, 19).

Comparison of the amino acid composition of dog plasma albumin with that of bovine and human albumins (17), shows the former to be high with respect to glycine, alanine, tyrosine, and glutamic acid, and low with respect to histidine, threonine, and isoleucine.

**SUMMARY**

Dog plasma albumin has been prepared 98% pure and examined by electrophoresis and in the ultracentrifuge. The amino acid composition of the albumin has been determined by ion exchange chromatography, and, in the case of five amino acids, by isotope dilution.

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**REFERENCES**

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