DIMERIZATION OF SERUM ALBUMIN ON EXTRACTION WITH AN ORGANIC SOLVENT*

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It has been known for some time that serum albumin is associated with a small amount of fatty acid that is not removed by repeated recrystallization (Kendall, 1941, Cohn et al. 1947). Extraction of bovine serum albumin with methanol to remove the fatty acid was shown to result in the appearance of about 30% of a faster sedimenting component, presumably a dimer (Cohn et al. 1947). We have now found that this dimeric constituent can be reconverted to the monomeric form by treatment of the extracted albumin with cysteine. This results in a preparation free of lipid, having the same physical characteristics as the original. In addition, evidence is presented to show that this dimerization reaction differs from those previously described (King et al. 1960; Williams and Foster, 1960) in which a thiol-disulfide exchange or the oxidative union of two sulfhydryl groups was believed to be involved.

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Experimental

Crystalline bovine serum albumin[1], as well as human serum albumin[2], were extracted with a chloroform-methanol mixture (3:1 by volume) in the dry crystalline form at 4°C. Approximately 200 ml. of solvent was used per gm. of albumin. The mixture was then filtered through a sintered glass filter and the residue extracted again with an equal volume of chloroform-methanol for an additional hour. The residue from the second extraction was dried overnight in a vacuum desiccator.

The chloroform-methanol extracted albumin was dissolved in a 0.01 M cysteine solution in acetate buffer pH 4.5 and allowed to stand at 4°C overnight. The solution was then dialyzed against acetate buffer pH 4.5 until free of cysteine. It was further dialyzed against distilled water and finally lyophilized.

The p-mercuribenzoate derivative was prepared by mixing 25 mg. of p-chloromercuribenzoate (PCMB) dissolved in 5 ml. 0.02 M ammonium hydroxide with 10 ml. of a 10% solution of albumin in acetate buffer pH 4.6, ionic strength 0.1.

Moving boundary electrophoresis studies were made with a Spinco, Model H electrophoresis-diffusion instrument in phosphate buffer at pH 7.0, ionic strength 0.1. Ultracentrifugal analysis was carried out in a Spinco, Model E ultracentrifuge. The titratable sulfhydryl groups were determined by the spectrophotometric method of Boyer (1954). The percentage of dimer was estimated by planimetry on magnified projections of the ultracentrifuge patterns. The fatty acid content of the albumin pre-

[1] Armour (Lt Number T 68412).

[2] Nutritional Biochemicals Corp. (Lt Number 1453).

parations was determined by the method of Dole (1956).

Results and Discussion

Extraction with chloroform-methanol resulted in the complete removal of the lipid associated with serum albumin. This is indicated by the fact that no further lipid could be extracted even after denaturation with 6N HCl or with 8M urea.

It can be seen from Figure 1 (A and B), that removal of the lipid resulted in the appearance of a component with a greater sedimentation rate than that of the original crystalline serum albumin. This component, which constituted approximately 30% of the total material, had a sedimentation constant similar to that of an albumin dimer, $S_{20,w} = 6.41S$. The sedimentation constant was calculated according to the method presented by Taylor (1952) and agreed very well with that of the mercaptoalbumin dimer defined by Hughes (1949). In some instances there appeared a small amount of fast-sedimenting third component. This was notably true with human serum albumin though it also occurred with bovine serum albumin, especially when the albumin was extracted at room temperature. The dimer could not be split by varying the ionic strength or the pH of the solution, nor could the reaction be reversed by re-addition of the extracted lipid material to the solution. However, treatment of the delipidated albumin solution with cysteine at pH 4.5 caused a reversal to the monomeric form. It can be seen that the sedimentation constant of the delipidated albumin, after treatment with cysteine was the same as that of the crystalline albumin, Figure 1 (A and C). The electrophoretic mobility was found to be unaffected by the extraction or treatment with cysteine.

It has been observed by Williams and Foster (1960) that the low

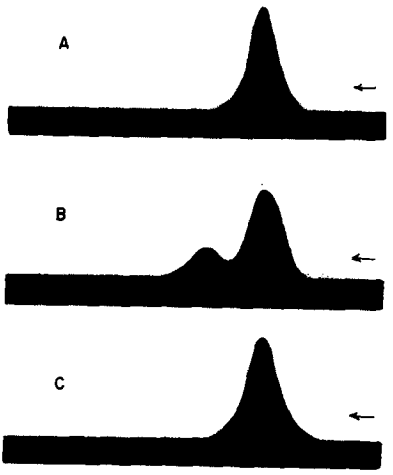

Fig. 1. Ultracentrifugal patterns after 85 min. at 60, 000 R.P.M. A - bovine serum albumin. B - albumin, extracted with chloroform-methanol. C - chloroform-methanol extracted albumin, after treatment with cysteine.

pH treatment of bovine serum albumin resulted in the release of the tightly bound fatty acid and concomitant dimerization. They and Bro et al. (1958) before them attributed this dimerization to the unmasking of aggregation sites by the low pH treatment, followed by thiol-disulfide exchange reactions. On the other hand, King, Yphantis and Craig (1960) found that the countercurrent distribution of plasma albumin in a solvent system consisting of ethanol, 1-propanol, water, and ammonium sulfate resulted in the formation of a mixture of dimer and monomer which was free of fatty acid. They noted that the dimer content could be lowered by treatment with thioglycolate and postulated that the dimer was formed by an oxidative coupling of two molecules of mercaptalbumin.

The following observations indicate that the dimer obtained under

the conditions described above results from a mechanism quite different from either of those reported by Williams and Foster (1960) or King, Yphantis and Craig (1960). It was found that the titratable-SH content of the albumin remained unchanged after extraction with chloroform-methanol, or after treatment of the extracted albumin with cysteine. It was further noticed that extraction of albumin under anaerobic conditions, where air is replaced by purified nitrogen, resulted in the same amount of dimerization as when the extraction was carried out in the presence of air. Finally, masking the -SH groups of albumin by reaction with PCMB, followed by extraction with chloroform-methanol, resulted in the formation of approximately 30% dimer, the same as when the -SH groups are free to react. These observations seem to exclude a simple oxidative coupling or a thiol-disulfide exchange. Further work is in progress in an attempt to elucidate the mechanism of this dimerization.

The extraction and subsequent treatment with cysteine results in a human or bovine albumin which is free of lipid, having the same centrifugal and electrophoretic characteristics as the original. [Goodman (1957) has recently reported the preparation of human serum albumin relatively free of long chain fatty acids] Our preparations have proved valuable in the study of phospholipid binding.

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