Fractionation of Serum Albumin on Sephadex

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It has been known for a long time that serum albumin on standing changes in such a way that more and more of the albumin aggregates to form dimers and higher polymers. Recently it has been shown that these albumins may be separated by so called gel filtration through columns of Sephadex G-150[1]. In this way monodisperse solutions of monomer and of dimer may be obtained. However, the elution curve for the monomer is always skewed on the front, indicating some inhomogeneity of this component, whereas the curve for the dimer is quite symmetrical. In Tris buffers at pH $\sim$ 8 this asymetry has been found to be caused by the presence of at least three chemically different monomers: A, B and C, where B and C are mainly responsible for the front of the curve and A for the rear. The A molecule (mercaptalbumin) has one free $-$SH group, B has a masked $-$SH group, whereas no $-$SH group can be detected in C. In fresh solutions of serum albumin the monomer contains only a very small amount of C. Monomers prepared from old albumin solutions consist almost entirely of the C component. Monomers from fresh solutions of bovine serum albumin (BSA) consist of 2/3 A and 1/3 B molecules. On aging A is transformed faster than B to C molecules. Monomer A can be oxidized to dimer and this may after isolation be reduced to mercaptalbumin (A).

If the separation is carried out in an acid medium (*e.g.* 0.1 M acetic acid in 0.1 M NaCl, pH $\sim$ 3) the elution curve for the monomer is much broader and more skewed on the front than at pH 8. From ultracentrifugations it is found that fractions from the front of the elution curve sediment much slower (s_{20} = 2.9 S) than those from the rear. The sedimentation diagrams from the rear fractions show a main peak with s_{20} = 3.3 S in addition to some apparently unaltered BSA monomer.

At pH 4 a smaller fraction of the molecules have undergone a configurational change and fractions from the rear of the elution peak show normal sedimentation properties, whereas those from the front show retarded sedimentation. This is in qualitative agreement with results from zone electrophoresis on polyacrylamide gels[2].

As the molecular weight of BSA is the same at pH 3 and 8 the experiments show that gel filtration on Sephadex can be used for the separation of proteins on the basis of molecular configuration as well as of molecular weight.

This work is part of an investigation supported by grants from the *Swedish Natural Science Research Council.*

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On the Arrangement of Phosphoamino Acids in a Part of α-Casein

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Recently [1,2], the isolation of a phosphopeptide from a trypsin hydrolysate of bovine α-casein was described. The composition of this peptide was determined [2,3], and by titration its seven phosphate groups were found to exist as O-monophosphate esters linked to serine and threonine residues [3].

Pepsin hydrolysates of this peptide have been fractionated on Dowex 1$-$X2 columns. Paper electrophoresis, N-terminal group analysis and quantitative amino acid determination on resulting peptide fractions indicate that pepsin splits the peptide into four main fragments:

Asp(Asp,ThrP,$SerP_2$,Glu_3,Gly,Ileu)
Glu(Asp,$SerP_4$,Glu_3,Pro,Ala,Val_2,$Ileu_2$,Lys)
Glu(Ala,Met)
Asp(Glu_2,Ileu,Met,Lys)

The amino acids of these smaller peptides comprise together those originally present.

At this point the investigation conforms to the presence of local aggregations of phosphoamino acids in α-casein [4]. In the part studied there are at least two such sequences with a possible number of three and four consecutive phosphoamino acid residues.

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