A STUDY OF GLOBULIN A

Reduction of the Disulfide Bonds

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We have performed the reductive cleavage of the globulin A from cottonseed, its isolation and partial chemical characteristics having been reported previously [1, 2]. The disulfide bonds were reduced with an excess of mercaptoethanol [3] using various conditions for the denaturation of the protein. The sulfhydryl groups in the reduced protein were determined by titration with a solution of methylmercury nitrate in the presence of sodium nitroprusside [4] and by spectrophotometric titration according to Boyer [5].

A 10-mg sample of the protein was dissolved in 1 ml of 6 M guanidine chloride (pH 7) and a 2% solution of sodium dodecyl sulfate (DDS) at pH 7 and 9.2. Mercaptoethanol was added to the solutions to a concentration of 4 M, a current of nitrogen was passed through them, and they were left under nitrogen for 24 hr. Then the reduced protein was precipitated and washed three times with chilled and acidified ethanol. The washed protein was dissolved in 1 ml of 6 M guanidine chloride (pH 7) and was titrated with a 0.001 M solution of methylmercury nitrate with cooling to 0° C.

When spectrophotometric titration was used to determine the SH groups, the protein was reduced in 8 M urea at pH 7.4 and in a 5% solution of DDS at pH 7.4 and 9.2. The reduced protein was dissolved in 6 M urea (pH 10). For titration, solutions were prepared which contained 3.1–60 μg of p-chloromercuribenzoate (PCMB) in 1 ml. Portions (4 ml) of the PCMB solution were taken, and 1 ml of protein solution was added to each. The solutions were kept in an atmosphere of nitrogen for 90 min and their absorption was measured at 250 mμ on an SF-4-A instrument. The equivalence point was found from the sharp break in the titration curve.

The table gives the results of the determination of the number of S—S groups per mole of protein (the molecular weight of the protein, according to gel filtration on Sephadex G-200, is ≈ 170,000).

Method of determining the SH groups	Treatment of the protein	Number of S–S bonds per mole of protein
Titration with methylmercury nitrate	6 M guanidine chloride in phosphate buffer (pH 7)	2.55
	2% DDS in phosphate buffer (pH 7)	25.5
	2% DDS in tris buffer (pH 9.2)	30.7
Spectrophotometric titration with PCMB	8 M urea in phosphate buffer (pH 7.4)	20.0
	5% DDS in phosphate buffer (pH 7.4)	25.0
	5% DDS in tris buffer (pH 9.2)	31.0

The cleavage of the disulfide bonds with mercaptoethanol in 6 M guanidine chloride was very slight (2.55 bonds per mole of protein). In detergent solution at the same pH the number of reduced bonds increased tenfold. And finally, the most complete reduction of the protein takes place in DDS solution at pH 9.2 (31 S—S bonds per mole of protein).

The number of sulfhydryl groups in the protein was also determined. A solution of the native protein in 1 M NaCl did not give a positive reaction with sodium nitroprusside. The titration of a weighed sample of protein dissolved in 6 M guanidine chloride (pH 7) with a 0.001 M solution of methylmercury nitrate gave 0.93 SH group per mole of protein.

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