

DETERMINATION OF DISULFIDE BONDS
IN 8.2 S GLOBULIN OF COTTON SEEDS

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The disulfide bonds in 8.2 S globulin [1, 2] were determined by titrating the SH groups with a solution of methylmercury nitrate after the reduction of the disulfide bonds of the protein with mercaptoethanol [3, 4] at room temperature. Approximately 10 mg of the protein was dissolved in 1 ml of a 2% solution of sodium dodecyl sulfate (in phosphate buffer, pH 7), and mercaptoethanol was added to a concentration of 4 M (0.3 ml of 95% mercaptoethanol); a current of nitrogen was passed through, and the reaction mixture was left for 1 and 24 h. The reduced protein was precipitated (after 1 and 24 h) with ethanol and a 10% solution of trichloroacetic acid. The protein precipitate was centrifuged at 3000-4000 rpm for 5 min. The protein in the centrifuge tube was washed (in 10-ml portions) with the precipitants until the supernatant liquid ceased to give a positive reaction with sodium nitroprusside. The reduced protein was dissolved in 4 ml of an 8 M solution of guanidine chloride (after reduction the solubility of the protein had greatly decreased) in 0.067 M phosphate buffer with pH 7, 10 mg of solid indicator (a mixture of sodium nitroprusside and Na₂CO₃, 1:2 by weight) was added, and the SH groups were titrated with a solution of methylmercury nitrate until the color of the solution changed from red-purple to straw color (Table 1). The concentration of the methylmercury nitrate was determined by means of a standard solution of thioglycolic acid. (1 · 10⁻⁵ mole of SH groups corresponds to 1.234 ml of methylmercury nitrate solution.)

It can be seen from the table that the 8.2 S globulin contains 14 moles of -SH groups or 7 moles of -S-S- bonds per mole of 8.2 S protein. The native globulin and globulin denatured in 8 M guanidine chloride and in a 2% solution of DDS in phosphate buffer, pH 7, do not give a positive reaction for -SH groups with the solid indicator.

TABLE 1

Weight of protein (8.2 S), mg	Reduction of the -S-S- bonds of the protein, h	Amt of CH ₃ HgNO ₃ , ml	Moles of -SH groups in the sample, 10 ⁻⁶	-SH groups in 10 ⁵ g of protein	-S-S- bonds in 10 ⁵ g of protein	Precipitants
10,2	1	1,82	1,48	14	7	96% - 96% ethanol
10,7	24	2	1,62	15,1	7,5	
10,2	1	1,75	1,42	14,4	7	10% - 10% sol TCA
Average		1,86	1,5	14,5	7,17	

Note. The protein was treated with a 2% solution of DDS in phosphate buffer.

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