obvious from the fact that a common method of drying cysteine (and its hydrochloride) is by warming in a stream of nitrogen.

If internal rearrangement does occur in cysteine then solution and recrystallisation might be expected to restore the original state. A sample of cysteine hydrochloride which had changed to give 2.1 times its theoretical –SH content was dissolved in distilled water, concentrated *in vacuo* and precipitated with conc. HCl. The precipitate was filtered off and divided into two lots. One was dried in a stream of warm nitrogen and on titration gave an –SH content of 1.3 times the theoretical. The second lot was dried by successive washes of acetone and finally ether. This lot on titration gave a theoretical –SH content. Left in the dark at a normal room temperature (not over 22°) this particular sample has not varied from its theoretical –SH content during 8 weeks.

The evidence given above is strongly suggestive that anomalous cysteine titrations are the result of internal rearrangements of the molecule leading to availability of amino or carboxyl group for reaction. Certainly, the evidence indicates the necessity for the avoidance of heat during the final stages of preparation or storage of cysteine or its hydrochloride.

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[1] L. A. AE. SLUYTERMAN, *Biochim. Biophys. Acta*, 25 (1957) 402.
[2] W. STAIB AND E. TURBA, *Biochem. Z.*, 327 (1956) 475.
[3] G. CALCUTT AND D. DOXEY, *Exptl. Cell Research*, 17 (1959) 542.

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Reduction of protein disulfide bonds by sodium borohydride

Although a number of substances have been used for the reduction of disulfide bonds in proteins there still exists a need for more satisfactory means of reducing such disulfides quantitatively[1]. MOORE *et al.*[2] described the use of $NaBH_4$ to reduce quantitatively disulfides in a number of proteins in 8 *M* urea. This paper describes the use of $NaBH_4$ for the quantitative reduction of disulfide bonds in proteins in aqueous solution in the presence and absence of the denaturant, sodium dodecyl sulfate.

Reagents and proteins were of commercial origin except whale myoglobin which was isolated from muscle tissues by ammonium sulfate precipitation as described previously[3]. The extent of the reduction was determined by measuring sulfhydryl concentration by amperometric titration[3,4]. All titrations were conducted in a nitrogen atmosphere; reduced glutathione served as a standard. Titration values were corrected by a blank value (10 % or less of total) obtained by titrating a solution containing $NaBH_4$, buffer and other additives, but no disulfide-containing material. The titrations were readily reproducible and the standard titration value was always within 5 % of theory.

Reduction of the proteins and other materials was achieved by combining solutions of the reactants (0.10–2.00 μmoles protein + 1 ml of a 25 mg/ml freshly

Abbreviation: SDS, sodium dodecyl sulfate.

prepared solution of $NaBH_4$, total volume 5 ml) in a small beaker. When SDS was included in the mixture it was added from a 5 % stock solution to give a final concentration of 0.5 %. A minute amount of Dow Antifoam was added and the mixture incubated under a gentle stream of nitrogen in a water bath at the desired temperature. Under these conditions the reduced proteins remained soluble. At the end of the incubation period the material was washed into the titration vessel which was being flushed with nitrogen. Buffer and other components of the titration mixture were added, and the titration started after an equilibration period of about 5 min. The excess $NaBH_4$ was destroyed by the addition of 2 ml acetone[5].

Results are shown in Table I. Values are averages of at least duplicate determinations and have been rounded to the nearest 5 % titratable –SH. Control reductions done on oxidized glutathione, cystine, and lipoic acid gave theoretical values. Whale myoglobin, which has no sulfhydryl or disulfide, served as a negative control for the reduction and showed the expected zero titration. Since aqueous solutions of $NaBH_4$ are alkaline, the titrations of proteins treated with SDS at pH 9.0 were also included as controls.

TABLE I

$NaBH_4$ REDUCTION OF DISULFIDES IN PROTEINS

*Protein and treatment**	*Percentage** of total –SH titratable after:*			
	5 min	*15 min*	*30 min*	*60 min*
Ribonuclease				
+ SDS, 40°			0	
+ SDS, pH 9.0, 40°			0	
+ BH_4^-, 40°			35	
+ BH_4^-, 45°			80	
+ BH_4^-, 50°			100	
+ BH_4^- + SDS, 40°	15	15	65	80
+ BH_4^- + SDS, 45°	45	75	95	100
Pepsin				
+ SDS, 40°			0	0
+ SDS, pH 9.0, 40°			0	
+ BH_4^-, 40°		35	95	95
+ BH_4^- + SDS, 40°	10	45	95	100
Ovalbumin				
+ SDS, 40°		15		20
+ SDS, pH 9.0, 40°				10
+ BH_4^-, 40°				40
+ BH_4^- + SDS, 40°	40	60	75	100
Bovine plasma albumin				
+ SDS, 50°			0	
+ SDS, pH 9.0, 50°			0	
+ BH_4^-, 50°	30	75	100	
+ BH_4^-, 40°			85	100
+ BH_4^- + SDS, 40°			see text	

* Treatment refers to conditions of incubation of proteins with SDS and/or BH_4^- at indicated temperature.

** Percentage total –SH based on total disulfides/mole of: ribonuclease, 4 per mol. wt. of 13,700; pepsin, 6 per 34,400; ovalbumin, 1 per 45,000 (plus 4–SH); bovine plasma albumin, 17 per 69,000 (plus 1–SH).

The results indicate that $NaBH_4$ can be used to quantitatively reduce disulfides in several proteins in aqueous solutions under relatively mild conditions. Generally, slightly milder conditions could be used for the reduction in the presence of SDS. The only difficulty with apparent reoxidation in the presence of the detergent was in the case of bovine plasma albumin where quantitative results could not be repeatedly obtained with systems containing SDS. However in this case complete reduction was obtained by treatment with $NaBH_4$ alone.

The use of $NaBH_4$ appears to have distinct advantages over other reagents commonly used for disulfide reduction in proteins: thioglycolic acid, mercaptoethanol and sulfite. Thioglycolic acid and mercaptoethanol, as any other thiols, have the disadvantage of making difficult the determination of the extent of the reduction. Additionally it has been reported recently by WHITE[6] that thiolation by polythioglycolides is a major side reaction during thioglycolate reduction of disulfide bonds in ribonuclease. The reaction of sulfite gives one sulfhydryl plus one S-sulfonate per disulfide, whereas $NaBH_4$ treatment yields two sulfhydryls. The extent of reduction by sulfite can be followed titrimetrically, but it is difficult to determine when there has been complete conversion of each half-cystine to S-sulfonate. Furthermore the reaction with sulfite also must be conducted in 8 *M* urea solutions to achieve completion[7,8].

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[1] R. CECIL AND J. R. MCPHEE, *Advances in Protein Chem.*, 14 (1959) 271.
[2] S. MOORE, R. D. COLE, H. G. GUNDLACH AND W. H. STEIN, *Intern. Congr. Biochem. 4th Meeting, Symposium No. 8, Vienna, 1958*, Pergamon Press, New York.
[3] A. M. DOLLAR, W. D. BROWN AND H. S. OLCOTT, *Biochem. Biophys. Research Communs.*, 1 (1959) 276.
[4] R. E. BENESCH, H. A. LARDY AND R. BENESCH, *J. Biol. Chem.*, 216 (1955) 663.
[5] H. C. BROWN, E. J. MEAD AND R. C. SUBBA RAO, *J. Am. Chem. Soc.*, 77 (1955) 6209.
[6] F. H. WHITE, JR., *J. Biol. Chem.*, 235 (1960) 383.
[7] J. L. BAILEY AND R. D. COLE, *J. Biol. Chem.*, 234 (1959) 1733.
[8] J. R. CARTER, *J. Biol. Chem.*, 234 (1959) 1705.

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Behavior of some selenium compounds in transmethylation

CANTONI AND MUDD[1] have shown that the selenium analogue of methionine is converted to "active selenomethionine" (Se-adenosylselenomethionine) at a rate which is similar to that at which "active methionine" is formed under identical conditions, and that when this Se-adenosylselenomethionine is incubated with guanidinoacetic acid and creatine methylpherase from pig liver, creatine is formed in excellent yield by transmethylation.

BREMER AND GREENBERG[2] have described a system in which choline biosynthesis is observed when [*Me*-^{14}C]S-adenosylmethionine is incubated with the microsome