REDUCTION OF PROTEIN DISULFIDE BONDS BY SODIUM HYDRIDE IN DIMETHYL SULFOXIDE

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A variety of procedures have been developed to reduce disulfide bonds in proteins to sulfhydryl groups (Bailey, 1962; Cecil, 1963). None of these methods, however, involve the reducing agents, such as sodium hydride, which can exist only in aprotic solvents. During a recent study of anionic graft polymerization of methyl acrylate to protein functional groups in dimethyl sulfoxide (DMSO) in the presence of sodium hydride (Krull and Friedman, 1967a), it became evident that the sodium hydride was reducing disulfide bonds in wheat gluten proteins to free sulfhydryl groups.

This communication describes a system for the reduction of disulfide bonds in proteins under aprotic conditions. Rates of reduction were followed by alkylation of the generated sulfhydryl groups with acrylonitrile, isolation of the modified proteins by dialysis, and amperometric titration of the remaining disulfide and sulfhydryl groups. These results indicate that sodium hydride in DMSO will reduce protein disulfides to sulfhydryl groups with minimal side reactions.

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MATERIALS AND METHODS

Bovine serum albumin (BSA), crystallized, and lysozyme (egg white) were obtained from Pentex[2]; sodium hydride in mineral oil from Metal Hydrides; and dimethyl sulfoxide (DMSO) from Baker. The latter was dried over calcium hydride and distilled under reduced pressure.

The following is a typical reduction procedure: 2 g of bovine serum albumin were placed in a flask and dried in a vacuum oven for 24 hours at 100°C. The protein was kept under an atmosphere of dry nitrogen, 200 ml of previously dried DMSO were added, and the mixture was stirred magnetically. After the protein was well dispersed, sodium hydride was added in quantities corresponding to molar ratios of 1 to 5 of the 1/2 cystine residues present in the protein (2 g bovine serum albumin contain 0.980 mMole 1/2 cystine residues) and allowed to react for up to 4 hours. The sulfhydryl groups were then alkylated by pouring the reaction mixture into aqueous acrylonitrile solution (10 mole excess of acrylonitrile to sulfhydryl) and stirring for 30 minutes. The alkylation was quenched by addition of glacial acetic acid (proton source); the reaction mixture was dialyzed againt H_2O until free of DMSO and then lyophylized. An ion-exchange procedure may be used to remove DMSO from amino acids and peptides (Krull and Friedman, 1967b).

Disulfide and sulfhydryl groups in the proteins were determined by amperometric titrations according to the modified micro method of Benesch and Benesch (1948; Benesch et al., 1955; Rothfus, 1966). The accuracy of these titrations is estimated to be ±3%.

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[2]The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

RESULTS AND DISCUSSION

The reduction of protein disulfide bonds by sodium hydride in DMSO was examined as a function of concentration and time (Table I).

TABLE I

Effect of Sodium Hydride Concentration and Time on Disulfide Reduction in Bovine Serum Albumin (0.49 mMole 1/2 cystine/g)

	Mole Ratio of NaH to 1/2 Cystine Residues		
Time	1	2	5
min	Residual 1/2 Cystine in Millimoles/gram BSA		
5	0.44	0.08	0.03
15	--	--	0.04
30	0.37	0.06	0.04
60	--	--	0.02
120	0.32	0.04	0.00
240	--	--	0.01

The molar ratio of NaH to 1/2 cystine residues should exceed 2 to 1 to achieve complete reduction of the disulfide bonds of bovine serum albumin. Since the reduction is dependent on the concentration of sodium hydride, suggested convenient conditions for complete reduction is treatment of a 1% protein solution in DMSO with a 5 to 1 molar ratio of sodium hydride to 1/2 cystine residues for 1 hour. Optimum conditions may be a function of the accessibility of the disulfie bonds in specific proteins.

Hydrolysis of peptide bonds or of glutamine, or asparagine amide groups has been shown previously not to occur in this system (Krull and Friedman, 1967a). This conclusion is further substantiated by the absence of nitrogen (Kjeldahl) in the dialysis water, concentrated under reduced pressure, of protein samples which were allowed to reoxidize without alkylation.

Samples of BSA which were reduced with sodium hydride and allowed to reoxidize without alkylation were found to contain 0.46 ± 0.02 millimoles of 1/2 cystine per gram. This result demonstrates that little if any desulfurization occurs during the reduction.

The results of a kinetic study on the reduction of disulfide bonds in lysozyme by sodium hydride in DMSO were similar to those described for bovine serum albumin.

There may be equilibria between sodium hydride, DMSO, and methyl sulfinyl carbanions, as well as proton abstractions from other functional groups (Krull and Friedman, 1967a). Mechanistic considerations suggest formulation of the aprotic reduction of disulfide bonds as follows:

$$PSSP + H^{\ominus} \rightleftharpoons PSH + PS^{\ominus} \quad (1)$$

$$PSH + H^{\ominus} \rightleftharpoons PS^{\ominus} + H_2 \quad (2)$$

$$2PS^{\ominus} + 2HOAc \rightleftharpoons 2PSH + 2OAc^{\ominus} \quad (3)$$

The initial hydride ion cleaves the protein disulfide (PSSP) to PSH and PS^- (equation 1). A second mole of hydride abstracts the proton from the newly formed PSH which produces the second mole of PS^- as well as a mole of H_2 (equation 2). Finally the 2 moles of PS^- are transformed to PSH during acidification with acetic acid (equation 3). Thus, 2 moles of PSH are produced from a protein disulfide group with the utilization of 2 moles of sodium hydride.

The mechanism of reduction by sodium hydride in aprotic media should be analogous to that for sodium borohydride in aqueous media. In contrast, sodium hydride in DMSO appears not to cleave peptide bonds nor to desulfurize the products. This may result from the absence of hydroxide ions in the aprotic medium.

A detailed evaluation of sodium hydride as a reducing agent for disulfide bonds is in progress with a number of proteins and model compounds in several aprotic solvents.

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