

From: *Chemical Modification of Proteins*. Gary E. Means and Robert E. Feeney. Holden-Day, Inc. San Francisco, 1971 pp. 228-229

THIOETHER GROUPS

The thioether groups of methionine side chains are weakly nucleophilic, remain un-ionized from pH 1 to 14, and, like more typical hydrophobic side chains, usually have little access to the aqueous environment. Their resistance to protonation is in contrast to other nucleophilic groups in proteins and provides a basis for their selective substitution. The reactivity of methionine also increases in many proteins due to partial unfolding at low pH values, where most other groups become relatively unreactive. For proteins lacking sulfhydryl groups, reactions with a haloacetate or with hydrogen peroxide can be used to selectively modify methionine side chains.

Carboxymethylation. The carboxymethylation of methionine residues of pancreatic ribonuclease has been described by Neumann et al. (1962):

To a solution of ribonuclease (6 mg/ml) was added an equal volume of a solution of iodoacetic acid (6 mg/ml). The pH was immediately adjusted to the desired value with 1 M HCl and the solution incubated at 40°C. At pH 2.7, most of the enzyme was converted to inactive di-, tri-, and tetraalkylated species after 3 hours. To terminate the reaction, the solution was poured onto a 0.9 x 5-cm bed of Amberlite IRC-50 pre-equilibrated with 5% acetic acid and eluted with 1-1 glacial acetic acid-water.

The reaction of iodoacetate with isocitrate dehydrogenase occurs under considerably milder conditions (Colman, 1968). When the enzyme (2 mg/ml) at pH 5.5 in 0.2 M sodium acetate buffer and 30°C was treated with 1.35×10^{-2} M iodoacetate, 80% of the dehydrogenase and 60% of the reductase activity was lost in 90 minutes. Reaction was stopped by gel filtration (Sephadex G-25) at 5°C in 0.1 M Tris (pH 7.2). The loss of activity appears to result from the alkylation of one methionine residues.

Reaction with Hydrogen Peroxide. The following procedure has been used for the modification of chymotrypsin with hydrogen peroxide (Schachter and Dixon, 1964):

Chymotrypsin (4.4 mg/ml) at 30°C in 0.0005 M EDTA at pH 3.2 (maintained by adding 0.001 M perchloric acid as required) is adjusted to 0.38 M H₂O₂ with 30% H₂O₂ and allowed to react for 10 to 240 minutes. The reaction is stopped by adding the reaction solution to a solution containing a small amount of catalase. Only one of the two methionines reacted during this period and, although this one is only three residues removed from the active-site serine, its modification effected only a slight decrease in apparent catalytic activity.

Oxidation of the methionine adjacent to the active site of subtilisin (Carlsberg) can be accomplished at pH 8.8 (Stauffer and Etson, 1969). A 1% solution of the enzyme in 0.1 M borate buffer was treated with 10 µl of 30% H₂O₂ per milliliter of enzyme solution to give 0.1 M H₂O₂ and incubated at room temperature. The loss of apparent catalytic activity began to level off after 30 minutes, after which the reaction was stopped by the addition of 1 µl/ml catalase. The apparent loss in activity was due to a decrease in the rate of catalysis of all the molecules and not to complete inactivation of part of the molecules. Only one of the five methionine residues reacted.

REFERENCES

- Neumann, N.P., S. Moore, and W.H. Stein (1962): *Biochemistry*, **1**, 68.
Colman, R.F. (1968): *J. Biol. Chem.*, **243**, 2454.
Schachter, H., and G.H. Dixon (1964): *J. Biol. Chem.*, **239**, 813.
Stauffer, C.E., and D. Etson (1969): *J. Biol. Chem.*, **244**, 5333.