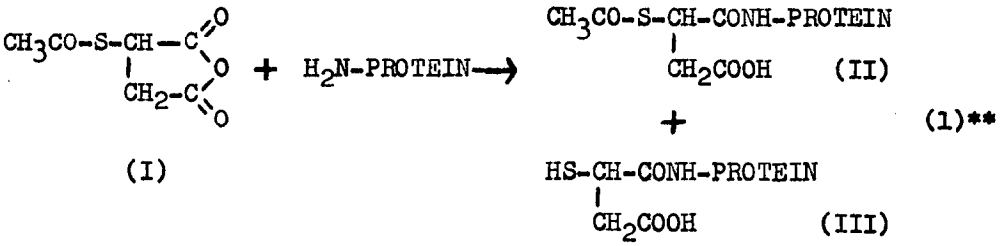
# INTRODUCTION OF SULFHYDRYL GROUPS INTO MACROMOLECULES

Irving M. Klotz and Virginia H. Stryker

Department of Chemistry*
Northwestern University
Evanston, Illinois

Received August 24, 1959

A novel method has been described recently (Klotz and Heiney, 1959) for the introduction of mercaptan and acetylmercaptan groups into proteins based on the reaction:



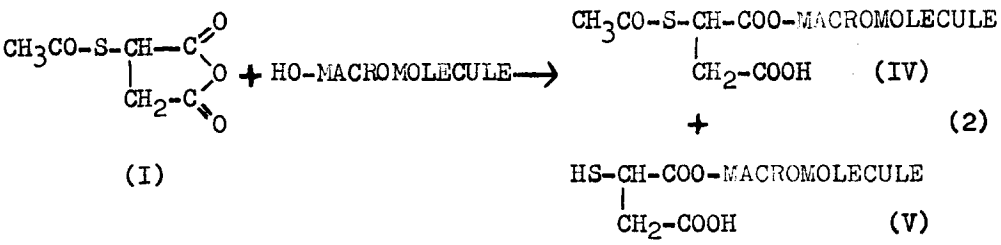
(I) (II) (III) (1)**

In contrast to other methods (Schöberl, 1948; Benesch and Benesch, 1958) for the de novo insertion of thiol groups into proteins, which are based on the use of thioester or thiolactone reagents, that of equation (1) using an anhydride can be extended to macromolecules containing primarily hydroxylic functional groups.

Mercaptosuccinyl polymers are obtained readily by straightforward application of the procedure used with proteins. For example, to a 5% solution of dextran adjusted to pH 8, one adds

* This investigation was assisted by a grant from the National Science Foundation..

** Compound (III) appears during the course of the reaction due to hydrolytic cleavage of acetyl groups from compound (II). This hydrolysis probably occurs in the vicinity of the droplets of NaOH added to maintain the pH. In reaction (1), amide formation has been represented to occur with the upper anhydride bond of compound (I). This choice is based on the relative inductive effects of substituents described in L. P. Hammett, Physical Organic Chemistry, McGraw-Hill Book Co., Inc., New York, 1940, pp. 211-12.




(I)

solid acetylmercaptosuccinic anhydride (I) in small amounts over a period of about one-half hour. The pH is maintained by periodic addition of NaOH to the continuously stirred solution. An atmosphere of nitrogen is maintained above the solution. At the conclusion of the reaction, the solution is passed through Amberlite IRA 400 (in its chloride form) to remove hydrolyzed anhydride which is not coupled to the macromolecule. Thereafter salts are removed either by dialysis or passage through a mixed-bed exchanger, Amberlite MB-1. For several preparations, both procedures for removing salt were followed; the total -SH content in each product was the same. To recover the mercaptosuccinyl polymer as a solid, the desalted solution is lyophilized.

Some typical results with dextran are assembled in Table I. The dextrans used were clinical samples obtained from Abbott Laboratories, that marked -DL having been dialyzed and lyophilized.

The experiments with dextran demonstrated that a polysaccharide as well as a protein could be thiolated. It seemed of interest, therefore, to attempt the reaction with the third major class of biological macromolecules, the nucleic acids. For this purpose a crude commercial yeast nucleic acid was used. The coupling with acetylmercaptosuccinic anhydride proceeded readily. Some results for this thiolated polymer are also shown in Table I.

There is no apparent reason why reaction (2) should be limited to biological macromolecules. Experiments were attempted, therefore, with polyvinylalcohol. Excellent preparations of mer-

captosuccinylated synthetic polymers were obtained with two different samples of commercial polyvinyl alcohols of different intrinsic viscosities, Du Pont Elvanols grade 72-60 and 71-30, respectively (Table I).

Table I

Mercaptosuccinylated Macromolecules

| Macromolecule | Moles (I) Added per $10^5$ Grams Polymer | Moles Introduced per $10^5$ Grams Polymer* | |
|---|---|---|---|
| | | Mercapto-succinyl(V) | Acetylmercapto-succinyl(IV) |
| Dextran | 600 | 22 | 25 |
| Dextran-DL | 150 | 8 | 18 |
| Ribonucleic acid | 300 | 1 | 16 |
| Polyvinylalcohol 72-60 | 600 | 20 | 5 |
| Polyvinylalcohol 72-60 | 300 | 9 | 12 |
| Polyvinylalcohol 71-30 | 300 | 7 | 18 |

* The entries in the third column refer to titration on the desalted preparation. Figures in the fourth column were computed after a similar titration was carried out on a sample exposed to 0.01 M NaOH to remove the $CH_3CO$- linked to the mercapto groups. From this latter titration we subtracted the number of free mercaptan groups originally present.

In all of these preparations, as well as those of proteins, the content of thiol groups was determined primarily by amperometric silver titration (Benesch, Lardy and Benesch, 1955). In several cases a quantitative colorimetric method with a disulfide dye (Klotz, Ayers, Ho, Horowitz and Heiney, 1958) was also used. Except for preparations with high content of -SH, the two methods agreed well. Discrepancies as high as 20% were found at the highest range. Since we are uncertain which method is more reliable in the upper ranges, only the amperometric results are listed in Table I.

For dextran and polyvinylalcohol an ultracentrifugal test was carried out to verify that the thiol group is actually linked to the macromolecule. An azomercurial dye which reacts specific-

ally with mercaptan groups (Horowitz and Klotz, 1956) was added to a 1% (buffered) solution of mercaptosuccinyl polymer, which rapidly solubilized the normally insoluble dye. A portion of this solution was sedimented at 60,000 r.p.m. in a Spinco Model E ultracentrifuge. The schlieren maximum and the color boundary moved together. With mercaptosuccinyl polyvinylalcohol, for example, a sedimentation coefficient of 1.93 S was computed from the schlieren patterns and a value of 2 S $\pm$ 0.5 S was estimated from absorption optics. Furthermore, the supernatant liquid showed no color. Consequently the thiol groups must be attached to these macromolecules in solution. Similar experiments were not carried out with the nucleic acid derivative, but since the methods for separating hydrolyzed compound (I) from polymer were identical with those used for dextran and polyvinylalcohol, the modified nucleic acid must also have covalently bound mercaptosuccinyl groups.

Thus a very simple method is available for the introduction of thiol groups into all three classes of biological macromolecule, as well as into certain synthetic polymers. These -SH groups can be readily oxidized to -S-S- linkages. Since disulfide cross-links play such an important role in protein molecules, it should be very interesting to examine the effect of such linkages on the configuration and behavior of these other classes of macromolecule.

## BIBLIOGRAPHY

R. E. Benesch, H. A. Lardy and R. Benesch, J. Biol. Chem., 216, 663 (1955).

R. Benesch and R. E. Benesch, Proc. Natl. Acad. Sci., 44, 848 (1958).

M. G. Horowitz and I. M. Klotz, Arch. Biochem. Biophys., 63, 77 (1956).

I. M. Klotz, J. Ayers, J. Y. C. Ho, M. G. Horowitz and R. E. Heiney, J. Am. Chem. Soc., 80, 2132 (1958).

I. M. Klotz and R. E. Heiney, J. Am. Chem. Soc., 81, 3802 (1959).

A. Schöberl, Angew. Chem., 60, 7 (1948).