ON THE INTRODUCTION OF DISULFIDE CROSSLINKS
INTO FIBROUS PROTEINS AND BOVINE SERUM ALBUMIN

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ABSTRACT

Hydroxyl groups in serine side chains of collagen, silk fibroin, and bovine serum albumin (BSA) were converted to SH by tosylation. In collagen film, 50% of the serine OH groups could be thiolated at most. In fibroin, only 13% because of its compact β-pleated sheet structure and low susceptibility to swelling. The SH groups introduced are near enough together to form -S-S- bonds by oxidation. The residual SH content after oxidation was 0.1% in collagen and 0.03 to 0.25% in fibroin. Disulfide crosslinking increased the shrinkage temperature of collagen and fibroin and decreased the amount of shrinkage. BSA was crosslinked to dimers (MBSA) according to gel permeation chromatography and sedimentation analysis by the analytical centrifuge. Because these crosslinked proteins can be metabolized by the usual processes, in contrast to those crosslinked by artificial, nonphysiological bridges, they may be used for biological or medical purposes.

INTRODUCTION

Conversion of serine OH groups to SH was investigated by Zervas and Photaki (1), Koshland et al. (2), and Photaki and Bardakos (3). Polgar and Bender (4) transformed the active serine residue in the active center of subtilisin into cysteine, showing that the activity of the enzyme was decreased by this change.

Replacement of OH groups by SH is also interesting for introducing this latter group into proteins free - or almost free - from sulfur for several reasons. One is the possibility of forming covalent crosslinks in proteins like silk fibroin and collagen.
This chemical modification should markedly change the physico-chemical behavior of these proteins. Cellulose has been crosslinked in this way by Schwenker (5) and by Sakamoto, Yamada, and Tonami (6).

RESULTS WITH COLLAGEN

Collagen fibers were treated with toluenesulfonyl chloride to esterify the OH group in aqueous phosphate buffer at pH 8 or 9, in dioxane-containing buffer solutions, or in pyridine (7).

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\begin{align*}
\text{OH} & \quad \text{Tosylchloride} \quad \text{O-Tos} \\
\text{CH}_2 & \quad \text{CH}_2 \\
\text{-HN-CH-CO-} & \quad \text{-HN-CH-CO-} \\
\end{align*}
\]

The most tosylation was obtained in a buffer/dioxane medium (2:1); in pyridine no tosylation was observed. This is probably due to failure of collagen to swell in pyridine, so that the reagent cannot penetrate the protein. In purely aqueous medium, on the other hand, tosyl chloride is poorly soluble.

The subsequent transesterification was carried out in 0.5M thioacetate solution of pH 5.5. Afterward, cleavage of the resulting thioester was performed with 0.5N sodium methylate.

By this procedure 40 to 50% of serine OH groups in collagen could be transformed into cysteine, according to results of amino acid analysis. The limiting factor for the OH to SH exchange seems to be the tosylation step; no more than 55% of the alcoholic serine side chains could be tosylated at most.

For covalent crosslinking, the SH groups introduced were oxidized to -S-S- bonds by air or by iodide (5). In both cases, the residual cysteine content was only 0.1%. This means that almost all SH groups introduced were situated close together enough to be oxidized to disulfide (Table 1).

These covalent -S-S- crosslinks should increase the stability of the collagen structure. For this reason, the temperature of shrinkage, T_s, should be shifted to higher values. As one can see from Figure 1, this is, indeed, the case.

Untreated collagen film (made from bovine achilles tendon by Freudenberg GmbH., Weinheim, West Germany) contracts in water at 54°C; collagen film with 1.43% cystine contracts at 59°C. Further-