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**Cleavage of Disulfide Bonds in Proteins by the Method of Swan**

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### Cleavage of Disulfide Bonds in Proteins by the Method of Swan

Cleavage of disulfide bonds in proteins is of prime importance as a preliminary step in elucidating the structure of proteins. The performic acid oxidation has been proved to be very useful for this purpose in proteins not containing tryptophan (1, 2). Application of this method, even under the mildest conditions, to proteins such as  $\alpha$ -lactalbumin or  $\beta$ -lactoglobulin, however, is of limited value due to the total destruction of tryptophan and partial (over 50%) oxidation of tyrosine and phenylalanine. The present communication describes the successful application of the recently published method of Swan (3) for the cleavage of disulfide bonds to  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin. This procedure is based on the conversion of the cysteinyl and cystyl groups into S-sulfocysteinyl groups.

Three-times crystallized  $\alpha$ -lactalbumin and four-times crystallized  $\beta$ -lactoglobulin were used. To 1 g. of the crystalline protein dissolved in 50 ml. of water by adjusting the pH to 9.0 with 0.5 *N*  $\text{NH}_4\text{OH}$  was added 40 ml. of a cupric-ammonium solution (containing 0.249 g. of  $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$  dissolved in water and adjusted to pH 9.0 with conc.  $\text{NH}_4\text{OH}$ ) and 10 ml. of a  $\text{Na}_2\text{SO}_3$  solution (containing 0.83 g.). The reaction mixture was left at room temperature for 2 hr. and dialyzed against 2 l. of 0.1 *M* sodium citrate solution (adjusted to pH 7.0) at 4° over a period of 3 days (citrate solution changed each day) to remove the cupric ions. The pH of the dialyzed protein solution then was adjusted with 0.5 *N*  $\text{HCl}$  to 4.0 in the case of  $\alpha$ -lactalbumin and to 5.0 in the case of  $\beta$ -lactoglobulin. The resulting precipitate was washed into a cellophane bag and dialyzed against distilled water to remove the electrolytes. After centrifugation, the protein was freeze-dried. Since there is no satisfactory analytical procedure to determine the S-sulfocysteinyl content of the proteins, because of the lability of this compound during acid hydrolysis, the increase in total sulfur measured by the titrimetric Carius combustion method (4) has been used as a measure for the chemical conversion.

The total sulfur contents<sup>1</sup> of the native and modified proteins are shown in Table I.

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<sup>1</sup> We wish to thank Mrs. Ruth B. Kelly of this Laboratory for carrying out the total sulfur determinations.

TABLE I  
Per cent Total Sulfur Content of Native and S-Sulfoproteins

Protein	Native protein		S-Sulfoprotein	
	Found	Calculated <sup>a</sup>	Found	Calculated <sup>a</sup>
$\alpha$ -lactalbumin	1.86	1.88	3.36	3.37
$\beta$ -lactoglobulin	1.50	1.54	2.40	2.35

<sup>a</sup> Calculations were based on the molecular weight of  $\alpha$ -lactalbumin as 15,500 and in the case of  $\beta$ -lactoglobulin as 37,300 (5) and on the basis of the known amino acid composition of these proteins (6, 7). In the case of S-sulfo- $\alpha$ -lactalbumin, calculation was based on a molecular weight of 16,140 (due to the addition of 8 SO<sub>3</sub>-groups), while for S-sulfo- $\beta$ -lactoglobulin, it was based on a molecular weight of 38,100 (due to the addition of 10 SO<sub>3</sub>-groups).

As Table I shows, the method employed resulted in the complete conversion of all the cystyl groups into S-sulfo-cysteinyl groups, based on the increase of total sulfur. The ultraviolet absorption spectra of both S-sulfoproteins were identical with those of the parent protein, thus demonstrating that during the conversion, the tryptophan, tyrosine, and phenylalanine contents of these proteins were not affected. Both protein derivatives gave clear water solutions above pH 6.0.

A successful application of Swan's (3) method to insulin (8), trypsinogen, and chymotrypsinogen (9), and to kerateines (10) has been reported recently. The procedure of these workers differs in details from the method described above, particularly in the employment of 8 M urea solution which proved to be unnecessary for the proteins reported here.

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