The Reaction of Combined Cystine of Wool with Sodium Bisulfite

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THE DISULFIDE cross-linkages in wool, due to the combined cystine, are of fundamental importance concerning the physical and mechanical properties of the wool. The changes in the elastic properties of wool, brought about by different reagents, are mainly due to the breaking of these disulfide cross-linkages.

If wool is treated with sodium bisulfite, according to Clarke [5] and Speakman [17], the combined cystine undergoes the following reaction:

$$R{-}S{-}S{-}R + NaHSO_3 \longrightarrow R{-}SH + R{-}S{-}SO_3Na.$$

In 1946, Carter, Middlebrook, and Phillips [4] summarized the results of previous investigations [7, 8, 12]. They came to the conclusion that not all of the combined cystine reacts in the same way with sodium bisulfite, but that cystine can be divided into different fractions. According to these authors, about 25% of the cystine does not react with sodium bisulfite, another 25% gives combined α-amino-acrylic acid, and one-half of the cystine is converted into cysteine and cysteine sulfonate side-chains. From the latter fraction part of the cystine can be restored by rinsing with water.

Moreover, Speakman [18] found that when human hair is reduced with sodium bisulfite the cystine cross-linkages can be restored by rinsing with a solution containing no oxidizing agent.

In connection with the extensive application of bisulfite for various treatments of wool, it is very important to know which reaction takes place in the wool and how this reaction can be influenced.

Therefore, we investigated the final effect of the reaction between wool and sodium bisulfite, and the influence of rinsing.

Experimental Procedure and Results

Samples of 400 mg. of woolen yarn, degreased by extraction with a mixture of trichloroethylene and ethanol, were first soaked for 30 min. at room temperature in 125 times their weight of 5% sodium bisulfite at pH 5.2,* and then reduced in an identical solution which had been preheated in a boiling water bath to 92°–95°C and kept at that temperature for 30 min. Heating the solution in a water bath prevented it from reaching the boiling point. Boiling had to be prevented because dispersion of tiny wool particles by the movement of the liquid might cause errors in the analytical data.

The bisulfite-treated wool was then subjected to the following aftertreatments: (*a*) no rinsing, immediate hydrolysis; (*b*) before hydrolysis, rinsing for 20 hrs. in 0.5% sodium acetate adjusted to pH 5.2 by the addition of acetic acid; (*c*) before hydrolysis, rinsing for 20 hrs. in 95% ethanol.

Two additional wool samples were reduced for 60 min. and two more for 90 min. In each case, one sample was hydrolyzed without rinsing and one was hydrolyzed after 20 hrs. of rinsing with the sodium acetate buffer solution.

The hydrolyses essential for determining cysteine and cystine were carried out in open test tubes with 10 ml. of 6*N* sulfuric acid. Hydrolyzing under CO_2 atmosphere did not make any difference. Although after 5 hrs. of boiling the keratin had not been completely hydrolyzed to amino acids, hydrolyzing for more than 5 hrs. caused no change in the values found for cysteine and cystine.

The amounts of cysteine and cystine were determined according to a method indicated by Shinohara [16], which enables cysteine and cystine to be determined simultaneously.

We started with three equal parts of the hydrolyzate, and adjusted them to pH 5.2 with sodium acetate and acetic acid. To the first part an aqueous solution of $HgCl_2$ and phosphotungstic acid reagent (Folin and Marenzi [10]) was added. The color of

* This solution was maintained throughout the investigation because of its optimum reducing action.

TABLE I

	Cysteine S (%)	Cystine S (%)	Cysteine S + cystine S (%)
Untreated wool	—	3.04	3.04
Wool reduced in 5% $NaHSO_3$ for 30 min.			
(*a*) Unrinsed	1.43	1.68	3.11
(*b*) Rinsed in buffer, pH 5.2, for 20 hrs.	0.03	3.04	3.07
(*c*) Rinsed in 95% ethanol for 20 hrs.	1.64	1.35	2.99
Wool reduced in 5% $NaHSO_3$ for 60 min.			
(*a*) Unrinsed	1.48	1.64	3.12
(*b*) Rinsed in buffer, pH 5.2, for 20 hrs.	0.14	2.88	3.02
Wool reduced in 5% $NaHSO_3$ for 90 min.			
(*a*) Unrinsed	1.44	1.67	3.11
(*b*) Rinsed in buffer, pH 5.2, for 20 hrs.	0.21	2.61	2.82

this solution served as a blank. To the second part only phosphotungstic acid was added. With the aid of a standard calibration curve the cysteine content was calculated from the measured color intensity. To the third part sodium bisulfite and phosphotungstic acid were added; then, with the aid of the blank, the known cysteine content, and the standard calibration curve, the cystine content was calculated from the color intensity caused by this reaction.

The cysteine and cystine percentages were determined for the wool samples treated with bisulfite in the above manner. The results are given in Table I.

Table I shows that after reduction for 30 min. about one-half of the cystine was converted. If, however, after the reduction the sample is rinsed with pH 5.2 buffer, it appears that cysteine is almost completely reconverted into cystine. Rinsing with ethanol does not cause reconversion of cysteine into cystine; on the contrary, the reduction of cystine appears to continue.

After more intensive reduction—namely, for 60 or 90 min.—rinsing again caused a considerable reconversion into cystine. However, a small amount of cysteine appears to be irreversible. This amount increases with a longer reduction time, being greater after 90 min. treatment than after 60 min.

TABLE II

	Cysteine S (%)	Cystine S (%)	Cysteine S + cystine S (%)
Untreated wool	—	3.07	3.07
Wool reduced in 5% $NaHSO_3$ for 45 min.			
(*a*) Unrinsed	1.91	1.15	3.06
(*b*) Rinsed in buffer, pH 5.2, for 20 hrs.	0.39	2.58	2.97
(*c*) Rinsed in 95% ethanol for 20 hrs.	1.35	1.74	3.09
(*d*) Unrinsed, but treated with monoiodoacetic acid, pH 8.3, for 15 min. before hydrolyzing	1.04*	1.10	3.18

* This value must be multiplied by 2 in order to obtain the actual cysteine S content (see text).

Additional samples of wool yarn were reduced for 45 min. Samples that were not rinsed after the reduction were immediately hydrolyzed. It appears from Table II that about two-thirds of the cystine was reduced, the cysteine S content being 1.91%. By rinsing after reduction in the pH 5.2 buffer the cystine percentage increased, but 0.39% cysteine S could not be reconverted into cystine. Also, it appears that rinsing with ethanol hampers the reconversion into cystine.

A few reduced samples were treated with monoiodoacetic acid of pH 8.3 for 15 min. at 95°C. According to Sanford and Humoller [15] and Mirsky and Anson [13] the free thiol groups are blocked by this treatment.

The color intensity measured is due to cysteine formed during hydrolysis from the cysteine sulfonate groups. As there is an equal amount of cysteine blocked by acetic acid groups, the value found has to be increased twofold. The result of the measurement was 1.04%; according to this method, therefore, 2.08% cysteine S must have been present.

It is also possible to block the thiol groups with the aid of ethyl iodide [13, 15]. To enable a comparison with the previous treatment, the reduced wool was shaken for 18 hrs. in a suspension of ethyl iodide in water at room temperature. The values found for the cysteine S and cystine S contents showed a large divergence. It appeared, however, that much more cysteine was reconverted into cystine than with the monoiodoacetic acid treatment.

TABLE III

	Cysteine S (%)	Cystine S (%)	Cysteine S + cystine S (%)
Wool treated with 0.1*M* KCN	nil	1.50	1.50
After reducing for 45 min. in 5% $NaHSO_3$			
(*a*) Unrinsed	0.52	0.98	1.50
(*b*) Rinsed in pH 5.2 buffer for 72 hrs.	trace	1.51	1.51

Cuthbertson and Phillips [6] observed that in wool treated with a potassium cyanide solution the combined cystine is converted into combined lanthionine:

$$R—S—S—R + KCN \longrightarrow R—S—K + R—S—CN \longrightarrow R—S—R + KCNS.$$

In order to examine the effect of this reaction we treated wool with potassium cyanide, as described by Farnworth, Neish, and Speakman [9].

Wool with a cystine content of 3.07% was treated with 30 times its weight of a 0.1*M* potassium cyanide solution at 66°C for 2½ hrs. After this treatment the wool still contained 2.53% sulfur (determined by Blackburn's method [2]); a determination of the cystine content showed that 1.50% cystine S was still present. This wool was then reduced for 45 min. in 5% sodium bisulfite. The results are given in Table III.

From Table III it appears that one-third of the disulfide S was reduced to cysteine. After prolonged rinsing in the buffer solution practically all the cysteine had been reconverted into cystine.

Woolen yarn was treated in the usual method with potassium cyanide for various reaction times. As the duration of the treatment was prolonged, the amount of nonreduced cystine gradually approached a certain final value. After a period of 16½ hrs. the wool still contained 0.91% cystine S. A few samples of this wool were then, as in the previous experiment, reduced in 5% sodium bisulfite for 45 min. The results are given in Table IV.

Table IV shows that in neither case (*a*) nor (*b*) was cysteine S present, and no change in the cystine S content occurred.

TABLE IV

	Cysteine S (%)	Cystine S (%)
Wool treated with 0.1*M* KCN for 16½ hrs.	nil	0.91
After reducing for 45 min. in 5% $NaHSO_3$		
(*a*) Unrinsed	nil	0.95
(*b*) Rinsed in pH 5.2 buffer for 18 hrs.	nil	0.91

Discussion

If wool is treated with sodium bisulfite, the disulfide cross-linkages are affected. In the opinion of Clarke [5] and Speakman [17] the combined cystine is decomposed into cysteine and cysteine sulfonate:

$$R—S—S—R + NaHSO_3 \longrightarrow R—SH + R—S—SO_3Na. \quad (1)$$

Hydrolysis of this bisulfite-treated wool causes a decomposition of the cysteine sulfonate into cysteine and sodium bisulfate:

$$R—S—SO_3Na + H_2O \xrightarrow{H_2SO_4} R—SH + NaHSO_4. \quad (2)$$

Reactions (1) and (2) occur when bisulfite-treated wool is hydrolyzed without previous rinsing (see Table I (*a*)). About one-half of the cystine is converted into cysteine.

However, if before hydrolyzing the reduced wool is rinsed in a buffer solution, cysteine is reconverted into cystine (see Table I (*b*)). This phenomenon can be explained by assuming that reaction (1) is an equilibrium:

$$R—S—S—R + NaHSO_3 \rightleftharpoons R—SH + R—S—SO_3Na.$$

By rinsing in water, bisulfite is withdrawn from the reaction, thus shifting the equilibrium to the side of cystine.

Rinsing in ethanol does not shift the equilibrium in favor of cystine (see Table I (*c*)), the solubility of sodium bisulfite in ethanol being very low.

The assumption of the existence of an equilibrum is in close agreement with the result of Katz and Tobolsky [11]. These authors studied the relaxation of wool fibers in water, bisulfite, etc. For fibers treated with bisulfite, the rate of relaxation appears to be much greater than that for fibers immersed in water. If the bisulfite-treated fibers are rinsed before stretching, the rate of relaxation is equal to that of the untreated fibers. In the case of the increased rate of relaxation, a great part of the disulfide cross-link-

ages are broken. These linkages are recovered by rinsing, and the rate of relaxation then decreases to that of untreated wool.

Table II shows that a higher cysteine percentage was found after treatment with monoiodoacetic acid. This phenomenon can be explained by assuming that at the beginning of the reaction with monoiodoacetic acid the concentration of bisulfite in the fiber is still high. The rinsing effect during this short reaction time (15 min.), however, is small. This results in a continuing reduction, causing a somewhat higher cysteine content.

By assuming an equilibrium, it can also be seen why so much cysteine is reconverted into cystine after the ethyl iodide treatment, for in this case the wool has been exposed to a certain rinsing effect for 18 hrs.

After an intensive reduction and subsequent rinsing in pH 5.2 buffer a small part of the cysteine and cysteine sulfonate is not reconverted into cystine (see Table II (*b*)), but remains in the form of cysteine. This phenomenon led to the conclusion that the wool disintegrated to such an extent that some cysteine groups were not able to react with cysteine sulfonate groups in order to form combined cystine and sodium bisulfite. This may be explained by assuming displacement of the corresponding cysteine and cysteine sulfonate groups so that they are beyond the reach of each other.

This conception is supported by the results of the reduction of KCN-treated wool. Here, all disulfide cross-linkages are re-formed because the more stable lanthionine groups prevent the wool from disintegrating.

Brown and Harris [3] observed a similar phenomenon when wool was reduced with sodium hydrosulfite and new cross-linkages were subsequently formed on treatment with alkyl halides. If there was some lapse of time between these treatments, the wool appeared to be badly damaged, as the newly formed cross-linkages were insufficient in number. However, if the treatments were carried out almost simultaneously, the combined cysteine groups had no opportunity to shift, and damage was avoided.

From Table IV it is apparent that the cystine fraction remaining after prolonged treatment with potassium cyanide, being about 30% of the original cystine content, cannot be reduced by sodium bisulfite.

This means that the cystine groups which could not be changed by potassium cyanide were also unaffected by sodium bisulfite. This cannot be a matter of equilibrium but must be due to the structure of keratin.

Thus, we conclude that the reaction of the combined cystine with sodium bisulfite must be considered to be an equilibrium reaction; also, it is probable that not all cystine groups are equally reactive. This assumption holds for the reaction with sodium bisulfite as well as for that with potassium cyanide and probably also for the reaction with thioglycolic acid [14]. This difference in reactivity must be attributed to a difference in accessibility of the keratin. For various reagents the accessibility has not the same magnitude. Alexander, Hudson, and Fox [1] studied the reaction of oxidizing reagents with combined cystine of wool and observed that different amounts of cystine were oxidized by $KMnO_4$, peracetic acid, and chlorine. There is a great similarity between these results and the estimations of the amount of crystalline matter in cellulose. Using chemical methods, the degree of crystallinity observed depends upon the method. Hence, in the case of cellulose it is also better to speak about accessibility.

The accessibility of wool for sodium bisulfite and for potassium cyanide is about the same. With both reagents, about two-thirds of the combined cystine can be converted, whereas one-third is not affected.

It seems possible that, under certain conditions, the combined cystine forms combined α-aminoacrylic acid by splitting off hydrogen sulfide. However, we did not find any indication of this reaction taking place. In a few cases the sum of the cysteine S and cystine S contents was, after the treatments of reducing and hydrolyzing, somewhat lower than the original cystine S content of wool, but the difference never exceeded 10%. This difference need not be caused by the formation of α-aminoacrylic acid, but may be due to other causes—for instance, the formation of lanthionine.

Conclusions

1. The effect of sodium bisulfite on wool can be represented by the reaction

$$R{-}S{-}S{-}R + NaHSO_3 \rightleftharpoons R{-}SH + R{-}S{-}SO_3Na$$

2. At 95°C about two-thirds of the disulfide cross-linkages of the wool react according to this equation. By rinsing in water, all of these cross-linkages are re-formed. The remaining one-third does not react with sodium bisulfite at all.

3. After a prolonged reduction the rinsing does not reconvert all of the cysteine and cysteine sulfonate into combined cystine. This phenomenon is due to molecular shiftings in the fiber, which prevent the reformation of some of the disulfide cross-linkages.

4. The cystine groups which cannot react with potassium cyanide to form lanthionine do not react with sodium bisulfite either. This may be explained by assuming different accessibilities for different parts of the keratin fiber.

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