The Relation between the Number of Lanthionine Crosslinks and the Urea-Bisulfite Solubility of Alkali-Treated Wool

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It is possible to apply quantitatively the theory of the relation between the degree of crosslinking and solubility worked out for synthetic polymers irradiated by high-energy radiation[1,2] to the case of wool which has been crosslinked by an alkali pre-treatment and which is therefore less soluble than untreated wool in a standard urea-bisulfite solution at 65°C. in 1 hr. The experimental evidence is consistent with the theory that the cystine lost by the wool during the alkali treatment is the source of lanthionine thio-ether linkages.

We have carried out experiments which show that lanthionine is probably sufficiently stable under the conditions of the urea-bisulfite treatment to account for the increased insolubility of alkali-treated wool in the reagent.

Degree of Crosslinking Proportional to Cystine Lost

The results of Lees and Elsworth[3] show that 48% of untreated wool (2/32's worsted yarn) is soluble in a standard urea-bisulfite solution (50% urea, 3% $NaHSO_3$, pH adjusted to 7, liquor-wool ratio 100:1) in 1 hr. at 65°C., and that prolonging the time the wool is in the urea-bisulfite solution does not greatly increase the amount which dissolves. In further experiments described by Lees and Elsworth, wool samples were pre-treated in a 4.7 g./l. sodium carbonate solution for 30 min. at

TABLE I

Temperature of alkaline pre-treatment, °C.	Solubility, %	s	γ	Cystine lost, %
30	48	1	0.5	0.77
35	47	0.98	0.505	0.77
40	43	0.90	0.54	1.54
45	35	0.73	0.63	2.30
50	23	0.48	0.85	4.16
52	19.5	0.41	0.95	4.62
53	14	0.29	1.25	6.90
55	12	0.25	1.37	7.70
57	9	0.19	1.57	10.0
58	6	0.125	1.78	12.3
60	4	0.08	1.96	13.8

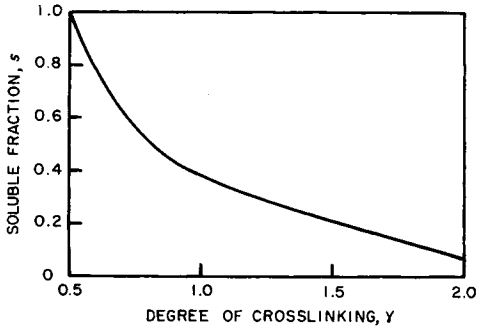

Fig. 1. The relation between the degree of crosslinking and the fraction of the polymer soluble.

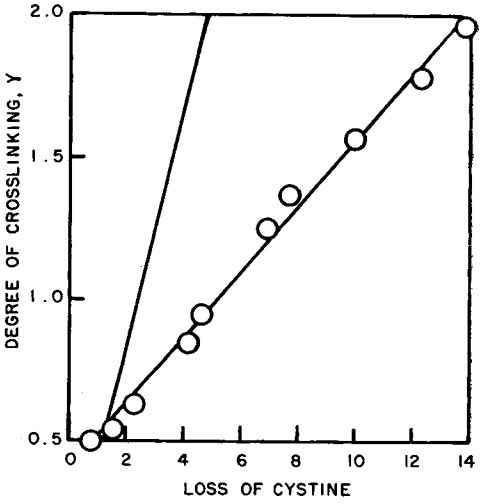

Fig. 2. The relation between the degree of crosslinking and the loss of cystine (in %) by wool samples during alkali pre-treatments.

different temperatures between 30 and 65°C. The solubilities of the alkali-treated wools in the urea-bisulfite reagent were measured, and the cystine contents of the wool samples after the alkali treatments were also determined and plotted as percentage cystine lost. The data in Table I are taken from the results of Lees and Elsworth (their Fig. 4).

In applying the theory of the relation between degree of crosslinking and polymer solubility, only the 48% of the wool which is soluble before any alkali treatment is considered, so that s, the fraction of the wool protein soluble, is taken as equal to unity for untreated wool (and, e.g., s would equal 0.5 if, after an alkali treatment, 24% of the wool were soluble). The degree of crosslinking γ, the average number of crosslinks per initial weight-average polymer molecule, is found from Charlesby's equation,[1]

$$s = \frac{(2 + \gamma) - (\gamma^2 + 4\gamma)^{1/2}}{2\gamma} \qquad (1)$$

by constructing the curve of s against γ for values of γ from 0.5 to 2.0 (Fig. 1). (It is inherent in the derivation of eq. (1) that if the degree of crosslinking ≤ 0.5, the polymer is completely soluble.)

From the solubilities of the wool sample after alkali treatments at different temperatures, given by Lees and Elsworth, the fractions, s, soluble after treatments at different temperatures can be calculated, and the values of γ read off from Figure 1. Figure 2 (lower curve) shows the values of γ plotted against the cystine lost during the alkali treatments. The linearity shows that, in fact, the degree of crosslinking is proportional to the cystine lost by the wool during the alkali pre-treatments.

Relation between Solubility and Number of Crosslinks

Under mild conditions—neutral pH and low temperatures—the urea-bisulfite reagent dissolves a polydisperse protein from wool which sedimentation and diffusion experiments show has a molecular weight of approximately 84,000.[4] Astbury[5] has estimated that there are approximately 100 g. moles of half-cystine residues in 10^5 g. wool, and therefore in the dissolved protein, MW 84,000, there are 42 cystine residues per molecule. For $\gamma = 1.0$, i.e., one crosslink per molecule, one cystine residue would be converted to a stable lanthionine residue. Therefore if, $\gamma = 1.0$, 2.4% of the cystine is converted to lanthionine; $\gamma = 1.5$, 3.6% of the cystine is converted to lanthionine; $\gamma = 2.0$, 4.8% of the cystine is converted to lanthionine; and so on.

The upper curve in Figure 2 shows the amount of crosslinking necessary to account for the observed solubilities of the alkali-treated wools in the urea-bisulfite reagent plotted against the cystine loss which would account for the crosslinking if each cystine molecule lost gave rise to one lanthionine crosslink. It shows that the cystine lost is more than enough to account for the decrease in solubility of the alkali-treated wools.

Certain assumptions are implicit in the application of the crosslinking solubility theory to the dissolution of alkali-treated wool in urea-bisulfite solution. (*1*) Some peptide bond scission as well as crosslink formation may occur during the alkali pre-treatment or during the treatment with the urea-bisulfite solution. (*2*) Some of the lanthionine crosslinks may be broken in the urea-bisulfite reagent. (*3*) The conversion of cystine to lanthionine during the alkali treatments may not be quantitative; other products may be formed. (*4*) The crosslinking-solubility theory assumes a certain distribution of molecular weight in the polymer[1,2] to which the molecular weight distribution of the heterogeneous wool protein may not conform. (*5*) The molecular weight of the dissolving protein may be either more or less than 84,000—reunion or further breakdown of dissolved molecules may take place after solution. (*6*) The distribution of cystine (and thus lanthionine) residues may not be random as is assumed in the derivation of eq. (1). (*7*) The cystine content of the dissolving wool fraction may not be the same as that of whole wool. (*8*) The ratio of the rates of crosslinking and peptide bond scission (if any occurs) may not be the same at the different temperatures of the alkaline pre-treatments. (*9*) The urea-bisulfite reagent may itself cause some crosslinking.

Only the last of these assumptions would definitely decrease the necessary amount of cystine lost to account for the observed solubilities. Assumptions (*1*) to (*3*) would, if not exactly true, mean that more crosslinks would be required to cause the observed fall in solubility as the alkaline pre-treatment of the wool is carried out at increasingly high temperatures. The effect of assumptions (*4*) to (*8*) may either be to increase or decrease the number of crosslinks necessary to account for the observed solubilities.

The linearity of the lower curve in Figure 2 shows that it is probably justifiable to make these assumptions. The difference between the lower curve and the upper curve in Figure 2 show that, for example, if all the assumptions except (*3*) were justified, then if only approximately half of the reacting cystine residues were converted to lanthionine, this would be sufficient to account for the observed solubilities.

Stability of Lanthionine Crosslink in Wool in Urea-Bisulfite Solutions

The work of Zahn[6] has thrown some doubt on whether lanthionine is sufficiently stable in urea-bisulfite solutions to account for the increased insolubility of alkali-treated wool in urea-bisulfite solution.

Experimental

A sample of Lincoln wool conditioned at 22°C., 65% R.H., was immersed for 25 hr. in excess 0.1*N* NaOH solution at room temperature, which has been shown[7] to convert 50% of the cystine residues to lanthionine. The loss in weight of wool, after conditioning at the same temperature and relative humidity, was less than $^1/_2$ % during this treatment.

A two-dimensional chromatogram of 0.035 ml. of the hydrolyzate from the alkali-treated wool (0.025 g. of conditioned wool refluxed with 6*N* HCl for 24 hr., evaporated twice over a water bath, dried, and dissolved in 2.5 ml. of 10% isopropanol) was compared with two parallel chromatograms of hydrolyzates from untreated wool with amounts of 0.1*M* lanthionine solution added corresponding to 40 and 60% conversion of cystine to lanthionine. The lanthionine spot from the alkali-treated wool was consistently between, in intensity and area, the lanthionine spots on the comparison chromatograms. [Solvents: (*1*) Butanol–glacial acetic acid–water, 4:1:5; (*2*) dicyclohexylamine–water–acetone–butanol,[8] 2:5:10:10.]

The alkali-treated wool was immersed in boiling urea-bisulfite reagent (50% urea, 3% $NaHSO_3$, liquor-wool ratio 100:1) at pH 5 for 1 hr. Less than 1% of the alkali-treated wool dissolved, and the chromatograms of the wool after the urea-bisulfite treatment again showed a lanthionine spot which, in intensity and area, was between the lanthionine spots on comparison chromatograms of a hydrolyzate from untreated wool with lanthionine added corresponding to 40 and 60% conversion of cystine to lanthionine.

Thus, even under more drastic conditions of temperature and pH than in the solubility experiments, the majority of the lanthionine formed from cystine in wool by alkali treatment is stable in the urea-bisulfite solution.

Activation Energy of Reaction of Cystine with Hydroxyl Ions

The extent of the reaction of cystine with hydroxyl ions in 1 hr. at different temperatures is proportional to the rate of the reaction at least at the lower temperatures where only a small proportion of the cystine residues react, since the concentration of hydroxyl ions is constant throughout the reaction. The data in Table I can be used to calculate the activation energy of the reaction of cystine with alkali, the first step of which is the ionization of a proton from the α, main protein chain, carbon atom of the cystine residue.[9]

Figure 3 shows $\log_{10}$ (cystine lost) plotted against $(1/T°A.) \times 10^{-2}$. The gradient of this line,

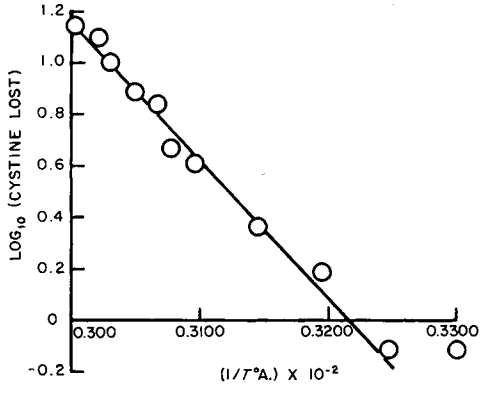

Fig. 3. The activation energy of the reaction of cystine residues with hydroxyl ions.

-0.535×10^4, multiplied by 2.303, multiplied by R, 1.98, is equal to $-A$. The activation energy, A, = 24.4 kcal./mole.

Discussion

Lees and Elsworth showed that a maximum of 48% of the protein from their untreated wool sample dissolved in urea-bisulfite solution under a variety of conditions. The changing proportion of this protein fraction which is soluble after alkali treatment of the wool is adequately explained by the application of Charlesby's equation connecting solubility and degree of crosslinking to the lanthionine crosslinks produced from cystine.

An explanation is still required of the stability of the insoluble protein fraction in untreated wool,

where some work on the stabilizing of the α-helical structure by cystine residues may be relevant.[10,11]

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Synopsis

Charlesby's equation connecting degree of crosslinking with polymer solubility will adequately explain the decreasing solubility of the urea–bisulfite-soluble protein fraction of wool after the wool has been pre-treated in alkaline solutions. It has been shown that lanthionine thio-ether linkages are probably sufficiently stable crosslinks in the urea–bisulfite solutions to account for the lower solubility of alkali-treated wool. They are formed from cystine residues during the alkali treatment. The activation energy of the reaction of cystine residues with hydroxyl ions is 24.4 kcal./mole.

Résumé

L'équation de Charlesby reliant le degré de pontage à la solubilité d'un polymère est capable de fournir une explication adéquate de la diminution de solubilité après traitement préalable en milieu alcalin de la fraction protéinique de la laine soluble dans le couple urée–bisulfite. Il a été démontré que dans ce solvant mixte on pouvait attribuer la décroissance de solubilité de la laine ayant subi préalablement un traitement aux alcalis à la formation de liens lanthionine thio-éther suffisemment stables. Ceux-ci sont formés durant ce traitement aux dépens des groupes cystiniques. L'énergie d'activation de la réaction de la cystine avec les ions hydroxyles s'élève à 24.4 Kcal/mole.

Zusammenfassung

Die Beziehung von Charlesby zwischen dem Vernetzungsgrad und der Löslichkeit eines Polymeren gibt eine befriedigende Erklärung für die abnehmende Löslichkeit der Harnstoff–Bisulfit-eiweissfraktion von Schafwolle nach Vorbehandlung der Wolle in alkalischer Lösung. Es wurde gezeigt, dass die Lanthionin–Thioätherbrücken in den Harnstoff-Bisulfitlösungen wahrscheinlich genügend beständige Vernetzungsstellen bilden, um die geringere Löslichkeit der alkalibehandelten Wolle erklären zu können. Diese Vernetzungsstellen werden aus Cystinresten während der Alkalibehandlung gebildet. Die Aktivierungsenergie der Reaktion von Cystinresten mit Hydroxylionen beträgt 24,4 kcal/Mol.

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