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Crosslinking in Keratins. IV. Thermal Cleavage of Crosslinks

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Synopsis

When wool is heated at 205°C. under silicone oil, it becomes progressively more soluble in 88% formic acid. This solubility is due almost exclusively to breakage of disulfide crosslinks. Assuming first-order crosslink cleavage, and using the methods of the first paper of this series, we establish the presence of two independent components. One component, about 20% of the wool, has a molecular weight of at least 70,000; the other has a molecular weight of at least 3300.

INTRODUCTION

The use of heat to break primary chemical bonds for structural study is customarily considered to be a drastic course, applied only when other, gentler treatments have failed. Thermal cleavage of the disulfide bonds in keratins is, however, a rather selective process, as this paper will attempt to show. In fact, by dissolving in formic acid the portions of the material rendered soluble by heat we can reapply the principles of papers I[1] and II[2] of this series to obtain additional information about the gross molecular make-up of keratins.

We will again assume the components are independent and monodisperse, although we will later discuss the effect of relaxing the requirement of independence. We further assume that the pyrolytic decrosslinking is first-order, and that the amount of each component is a single exponential decay function. We showed in I that the simultaneous occurrence of crosslink and main-chain breakage could be accommodated theoretically. There is evidence, to be discussed in this paper, that the disulfide crosslinks in keratin are thermally broken at a considerably lower temperature than is the peptide chain; hence, we will be able to use the theory for the solubility after pure crosslink cleavage and thereby avoid many complications of calculation.

EXPERIMENTAL

A Lincoln wool with a slight crimp and a crimpy Dubois wool were studied. The samples were scoured, then washed with water, vacuum-dried at

110°C., and finally washed with cyclohexane and vacuum-dried again. The dry weight of the samples, about 0.7 g., was recorded, and they were immersed in test tubes half filled with a lightweight silicone oil. Two heating baths were used; one, held at about 170°C., was for preheating the tubes, and the other, held at 205°C., was for the pyrolysis. The tops were loosely corked. The inert silicone oil not only excluded atmospheric oxygen, but also transferred heat well. After heating for a time, the samples were removed to sintered filters where they were washed with cyclo-

TABLE I
Solubility of Lincoln Wool in 88% Formic Acid after Heating at 205°C. under Silicone Oil

Time, min.	Fraction dissolved, S	$\frac{\ln 1/S}{1 - S}$
0	0.003	5.80
4	0.0125	4.42
7.5	0.0224	3.85
10	0.0441	3.25
15	0.0679	2.87
15	0.0849	2.69
20	0.100	2.55
25	0.143	2.27
28	0.191	2.01
32	0.245	1.86
38	0.334	1.64
45	0.482	1.40
48	0.602	1.27
54	0.767	1.135
60	0.783	1.124
64	0.784	1.123
75	0.887	1.060
75	0.901	1.052
100	0.945	1.028
200	0.983	1.008

TABLE II
Solubility of Dubois Wool in 88% Formic Acid after Heating at 205°C. under Silicone Oil

Time, min.	Fraction dissolved, S	$\frac{\ln 1/S}{1 - S}$
0	0.0027	5.90
0	0.0036	5.65
7.5	0.0353	3.44
15	0.105	2.52
17	0.110	2.46
22	0.174	2.10
30	0.378	1.56
40	0.578	1.296
50	0.788	1.121
75	0.930	1.035
211	0.990	1.005

hexane, dried, and then treated repeatedly with 88% formic acid. The residues were vacuum-dried and weighed; some were analyzed for total sulfur. Solubility results are given in Tables I and II. Results for the Dubois wool were not used in the calculations; however, they follow closely those of the Lincoln wool.

RESULTS AND DISCUSSION

Calculated Results

The sulfur content of the gel fraction is an index to the kind of degradation that predominates. As we saw in III,[3] main-chain breakage increases sulfur in the residue. Table III shows relative sulfur content for the Lincoln wool as measured by x-ray fluorescence, and Table IV shows total sulfur for the Dubois wool as measured by standard chemical methods. Since the uncertainty of these data is of the order of $\pm 0.2\%$ units in sulfur and ± 0.05 in relative sulfur content, the Lincoln wool shows no significant change and the Dubois only a slight decrease in sulfur. Amino acid analysis of the residue showed no lanthionine had formed, so that the apparent decrease in sulfur of the Dubois sample cannot be due to simultaneous lanthionine formation and chain scission, as eq. (27) of III would imply. We may conclude that the slight decrease is due to volatilization of unbound sulfur degradation products and that the degradation may be treated as essentially pure crosslink cleavage. Additional evidence is given elsewhere[4] that disulfide bonds break before those of the main chain.

TABLE III
Relative Sulfur Content of Lincoln Wool Following Heating at 205°C. and Leaching

Time, min.	Relative sulfur content
0	1.00
20	0.97
35	1.00
50	0.96

TABLE IV
Total Sulfur Content of Dubois Wool Following Heating at 205°C. and Leaching

Time, min.	Total sulfur, %
0	3.81
7.5	3.62
15	3.58
22	4.41

For the ith component, with no chain scission, eq. (28) of I may be written

$$S_i = (1 - Q_i + Q_i S_i)^{U_i - 1} \tag{1}$$

We may use the *a posteriori* findings from this paper that for the keratins U_t is large enough to allow this exact expression to be replaced by the approximation from eq. (13) of I:

$$S_t = \exp\{-\delta_t(1 - S_t)\} \tag{2}$$

where $\delta_t = Q_t U_t$. For two independent components the overall solubility is

$$S = S_1X_1 + S_2X_2 \tag{3}$$

where $X_1 = (1 - X_2)$ is the weight fraction of component 1. By the first-order degradation assumption we have

$$\delta_1 = \delta_{10} \exp\{-k_1t\} \tag{4}$$

and

$$\delta_2 = \delta_{20} \exp\{-k_2t\} \tag{5}$$

We have, then, the problem of fitting to the experimental data of Tables I and II the following expression with five adjustable parameters:

$$S = X_1 \exp\{-(1 - S_1)\delta_{10} \exp(-k_1t)\} + (1 - X_1) \exp\{-(1 - S_2)\delta_{20} \exp(-k_2t)\} \tag{6}$$

The task is simplified somewhat by postulating that after some time, t_m, of the pyrolysis, one component, say 1, will become completely soluble, leaving eq. (3) in the form

$$S(t > t_m) = X_1 + S_2X_2 \tag{7}$$

We may now choose a value for X_1, and solve for S_2. From eqs. (2) and (5) we may write

$$\ln\left[\frac{\ln 1/S_2}{1 - S_2}\right] = \ln \delta_{20} - k_2t \tag{8}$$

From a plot of eq. (8) we can extrapolate S_2 back to $t < t_m$, calculate S_1 by the relation $S_1 = (S - S_2X_2)/X_2$, and see if S_1 fits an equation of the form of eq. (8). If not, we adjust X_1 and begin again.

Proceeding in this way, we find the parameters that give the best fit of S to be those listed in Table V. For comparison we plot the experimental solubility data as $\ln (1/S)/(1 - S)$ against t in Figure 1. The solid line is the solubility calculated from the parameters of Table V. The ability of eq. (6) to fit most of this curve gives credence to the postulation of two independent components. The rise in the initial portion of the curve of Figure 1 indicates solubility less than that expected from extrapolation of the longer-time data to zero. Removal of a small soluble fraction during the initial cleaning process accounts for the discrepancy. The observed solubility values, S_{obs} can be adjusted for this effect by the relation

$$S = S_{obs}(1 - S_0) + S_0 \tag{9}$$

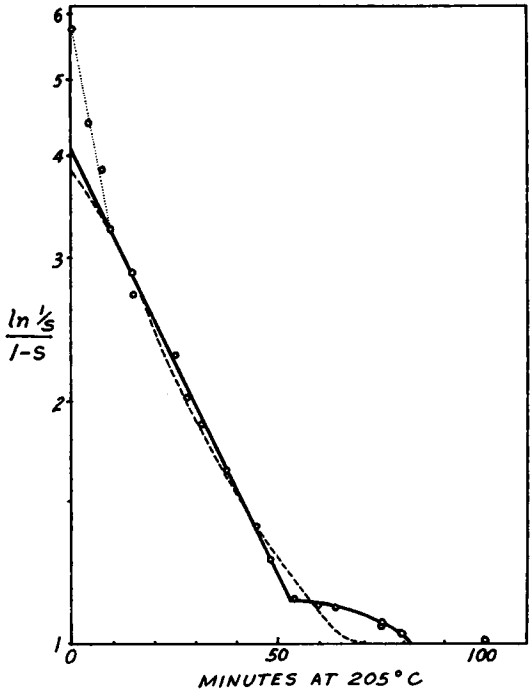

Fig. 1. Apparent δ as a function of heating time: (O) experimental points; (——) calculated assuming two independent components; (- - -) calculated assuming two intercrosslinked components; (· · ·) protein assumed to be leached out in the cleaning process.

where S_0 is the fraction initially leached out. The use of the value $S_0 = 0.018$ for Lincoln wool will provide a new set of S values which forms a more linear plot, but which is represented by essentially the same parameters as given in Table V. We assume this initial solubility of 1.8% is the sterile fraction that results when the monodisperse keratin protein is randomly crosslinked *in vivo* to the required extent.

The molecular weights also listed in Table V were calculated from δ_1 and δ_2 by eq. (19) of II:

$$M = 32\delta/f\phi \tag{10}$$

TABLE V
Parameters Obtained from Fitting Equation (6)

$X_1 = 0.78$
$\delta_{10} = 3.86$
$k_1 = 0.025$ min.$^{-1}$
($M_1 \geqslant 3300$, $U_1 \geqslant 26$)
$X_2 = 0.22$
$\delta_{20} = 82.$
$k_2 = 0.056$ min.$^{-1}$
($M_2 \geqslant 71,000$, $U_2 \geqslant 560$)

For the sulfur content ϕ, both components were taken to have the same weight fractional amount, 0.037. Furthermore assigning a value of unity to the fraction of intermolecular crosslinking f means that the molecular weights calculated are lower limits.

Nonindependence of Components

So far we have considered the two components to have no crosslinking between them. Since this is only a presupposition, it is of interest to see the effect of relaxing this condition—that is, to allow random crosslinking to occur not only within each fraction but to all available sites at once.

We again choose a two-component system, with weight fractions X_1 and X_2, molecular weights M_1 and M_2, crosslink densities Q_1 and Q_2, and containing U_1 and U_2 monomers per molecule. The numbers of molecules in each fraction are n_1 and n_2. We assume $Q_2 > Q_1$. Therefore, in order to apply random crosslinking statistics to both fractions simultaneously, we hypothetically lengthen the molecules of fraction 2 by the ratio Q_2/Q_1:

$$U'_2 = U_2(Q_2/Q_1) \qquad U'_1 = U_1 \tag{11}$$

The weight fractions of the two components must change also to accommodate the longer molecules of fraction 2. Designating the new weight fractions as Y_1 and Y_2, we have

$$Y_1 = n_1U'_1/(n_1U'_1 + n_2U'_2) = 1/[1 + (X_2Q_2/X_1Q_1)]$$

and

$$Y_2 = 1 - Y_1 \tag{12}$$

Without repeating a derivation similar to that already given in I, we find the sol fraction for this nonindependent case to be

$$S = Y_1[1 - Q_1(1 - S)]^{U_1} + Y_2[1 - Q_2(1 - S)]^{U_2} \tag{13}$$

Assuming again that Q_1 and Q_2 are small, U_1 and U_2 large, and that the degradation kinetics of eqs. (4) and (5) apply, this expression is converted to the form

$$S = Y_1 \exp\{-(1 - S)\delta_{10} \exp(-k_1t)\} + (1 - Y_1) \exp\{-(1 - S)\delta_{20} \times \exp(-k_2t)\} \tag{14}$$

The major difference between eqs. (14) and (6) is in the substitution of S for both S_1 and S_2.

A computer program was written to fit the experimental data of Table I. The parameters had the following values: $Y_1 = 0.88$, $\delta_{10} = 3.7$, $\delta_{20} = 8.7$, $k_1 = 0.018$ min.$^{-1}$, and $k_2 = 0.078$ min.$^{-1}$. A comparison of recalculated S values with the experimental data is seen as the dashed line of Figure 1. The agreement is in no way as good as that obtained by the use of eq. (6). Hence, we conclude that the two fractions found in this paper are not connected by crosslinks.

GENERAL CONCLUSIONS

We have herein adduced evidence that wool has two independent components, a minor one with large molecular weight and a major one with small molecular weight. The values of the parameters given in Table V should not be considered exact, or perhaps even more than qualitative, since they represent the results of only one kind of experiment out of many that could be done along similar lines.

In III we also concluded major and minor components were present, although no molecular detail could be given there except that the crosslinking in the minor component seemed to be predominately intramolecular. The temptation is strong, but resistible at this time, to associate the component split found in III with that of the present paper.

It has, we hope, been apparent throughout these papers that we have not tried to introduce the known gross structure of wool into the construction of a model. That is, such distinct entities as the cuticle, the cortex, cortical cells, ortho and para regions, microfibrils, and the matrix have been purposely omitted. Instead, the wool fiber has been treated as a "black box" polymer, with the notion that it is more logical to fit gross structure to overall chemical and physical behavior than to assume *a priori* detailed models with their necessary complexity. As an example, we tentatively associate the minor fraction of our present results with the cuticle (scale) region of wool, which comprises about 10% of the fiber. However, until the experiments discussed in these papers are repeated with descaled wool, this conclusion cannot be tested.

References

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Résumé

Lorsqu'on chauffe de la laine à 205°C dans de l'huile de silicone elle devient progressivement plus soluble dans l'acide formique à 88%. Cette solubilité est presque exclusivement due à la rupture des ponts disulfures. En admettant une rupture de ponts du premier ordre, et en employant les méthodes décrites dans le premier article de cette série, nous établissons la présence de deux composés indépendants. Un des composés, environ 20% de la laine, possède un poids moléculaire d'au moins 70,000; l'autre possède un poids moléculaire d'au moins 3,300.

Zusammenfassung

Bei der Erhitzung unter Silikonöl auf 205°C wird Wolle fortschreitend löslicher in 88%iger Ameisensäure. Diese Löslichkeit wird fast ausschliesslich durch Spaltung von Disulfidvernetzungen verursacht. Unter Annahme einer Vernetzungsspaltung nach erster Ordnung und mit dem in der ersten Mitteilung dieser Reihe angegebenen Methoden wird die Anwesenheit von zwei unabhängigen Komponenten nachgewiesen. Eine Komponente, etwa 20% der Wolle, besitzt ein Molekulargewicht von mindestens 70,000; die andere ein solches von mindestens 3,300.

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