

Crosslinking in Keratins. II. Theory for Sulfur Disproportionation after Chain Scission and Crosslink Cleavage

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Synopsis

Formulas are derived for the sulfur concentration in the insoluble remainder and extract of a keratinlike material following either chain scission, crosslink cleavage, or both. If only chain breakage occurs, the sulfur in the insoluble fraction can approach double its original concentration. On crosslink cleavage, however, it is likely to decrease somewhat by loss as unbound sulfur, formation of lanthionine, or disulfide-sulfhydryl exchange.

INTRODUCTION

In studying the structure of keratin it is usual to put it partially into solution by treatments which cleave crosslinks, break main chains, or both. The difference in composition between the soluble fraction (sol) and the remainder (gel) often has been taken to mean one region is more reactive or accessible than another, and that these regions also differ greatly in sulfur content. This paper will point out that in interpreting these results it is important to consider the composition differences to be expected from crosslinking theory.

We proceed by calculating first the sulfur composition of the sol and gel fractions after only main-chain bonds have been broken (as would occur, for example, in pure hydrolysis). Next, similar compositions are calculated for an example in which only crosslinks are broken. Finally, we discuss the general case, extended to two components, and including complications such as lanthionine-crosslink formation and the disulfide-sulfhydryl interchange.

THEORY

We will, throughout this paper, use the approximation that each amino acid residue in the keratin protein has an average weight m . In wool, for example, the overall average residue molecular weight is 109, and that for a half-cystine monomer is 102; the error of using the approximation is small and well repaid by the algebraic simplification it allows.

When a crosslinked material partially dissolves the crosslink concentration usually rises in the gel but diminishes in the sol. Following Charlesby,¹ the number of crosslinked residues in the sol fraction is

$$\sum_{u=1}^U \sum_{c=1}^u N_c(u) C S_0^c$$

where the notation is the same as used in I.² Referring to eqs. (30) and (32) of that paper, the above sum becomes $QA_1S_0^2$. Letting the sterile coefficient S_0 equal the soluble fraction S , and dividing by the total number of residues in the sol, A_1S , we find for the density of crosslinks in the sol

$$Q_s = QS \quad (1)$$

The total number of crosslinked residues is QA_1 . Hence the number in the gel is $QA_1 - QA_1S^2$, or $QA_1(1 - S^2)$. Dividing by the number of residues in the gel, $A_1(1 - S)$, we find for the density of crosslinks in the gel

$$Q_g = Q(1 + S) \quad (2)$$

Equations (1) and (2) describe the general disproportionation phenomenon which is the subject of this paper.

Main-Chain Scission

We first define the following terms: N_0 = number of residues in the entire sample; N_{10} = number of residues that contain no sulfur; N_{20} = number of residues that contain sulfur not engaged in intermolecular crosslinking; N_{30} = number of residues that contain sulfur bound as intermolecular disulfide crosslinks. N , N_1 , N_2 , and N_3 are the same respective quantities for the insoluble fraction (gel).

As already mentioned, the number of crosslinked residues in the gel fraction is

$$N_3 = N_{30}(1 - S^2) \quad (3)$$

The total number of monomers in the gel is

$$N = N_0(1 - S) \quad (4)$$

or

$$(N_1 + N_2 + N_3) = (N_{10} + N_{20} + N_{30})(1 - S) \quad (5)$$

The relative amounts of noncrosslinking residues will remain constant

$$N_2/N_1 = N_{20}/N_{10} \quad (6)$$

Combining eqs. (3), (5), and (6) and solving for N_2 , we have

$$N_2 = N_{20}(1 - S) \left(1 - \frac{N_{30}S}{N_{10} + N_{20}} \right) \quad (7)$$

The sulfur content of the original material is

$$\phi = (32/m)(N_{30}/N_0) + \phi_0 \quad (8)$$

where $\phi_b = (32/m)(N_{20}/N_0)$ is the weight fraction sulfur in nonintermolecularly crosslinked form. The weight fraction sulfur in the gel is

$$\phi_g = (32/m)[(N_3/N) + (N_2/N)] \quad (9)$$

Using eqs. (3) and (7) to eliminate N_3 and N_2 , we obtain

$$\phi_g = \frac{32}{m} \left[\frac{N_{30}}{N_0}(1 + S) + \frac{N_{20}}{N_0} \left(1 - \frac{N_{30}S}{N_{10} + N_{20}} \right) \right] \quad (10)$$

On incorporating eq. (8), this becomes

$$\phi_g = (\phi - \phi_b)(1 + S) + \phi_b \left(1 - \frac{N_{30}S}{N_{10} + N_{20}} \right) \quad (11)$$

Defining $f = (\phi - \phi_b)/\phi$ as the fraction of the total sulfur engaged in intermolecular crosslinks, we can write eq. (11) as

$$\phi_g = \phi(1 + fS) - \phi_b \left[N_{30}S / (N_{10} + N_{20}) \right] \quad (12)$$

The last term is usually small and may safely be dropped, with the final result

$$\phi_g = \phi(1 + fS) \quad (13)$$

In a similar way the sulfur content of the sol is

$$\phi_s = \phi(1 - f + fS) \quad (14)$$

Equation (13) shows that if most of the sulfur is intermolecularly bound, then the sulfur content of the gel can approach twice that of the starting material after extensive chain scission. When both S and ϕ_g are measured, an expression of this kind can be used to estimate f . According to eq. (14) the sulfur content of the sol is always less than that of the starting material. In fact, the material first dissolved can contain very little sulfur when f is near unity.

Crosslink Cleavage

Most of the derivation of the previous section can be applied regardless of the nature of the degradation. When disulfide bonds are broken, however, we must allow for the additional possibility that some of the sulfur may end up unbound and be lost either as a volatile byproduct or to the soluble extract. This can be provided for by adding an unbound sulfur term, ϕ_c , to eq. (8)

$$\phi = (32/m)(N_{30}/N_0) + \phi_b + \phi_c \quad (15)$$

Then, eq. (11) for the sulfur content of the gel will become

$$\phi_g = (\phi - \phi_b - \phi_c)(1 + S) + \phi_b \left(1 - \frac{N_{30}S}{N_{10} + N_{20}} \right) \quad (16)$$

If we again define $f = (\phi - \phi_b - \phi_c)/\phi$ as the fraction of the total sulfur engaged in intermolecular crosslinks, then eq. (16) may be written

$$\phi_g = \phi(1 + fS) - \phi_c \quad (17)$$

where, as before, we have dropped the term containing $N_{30}S/(N_{10} + N_{20})$ as normally being negligibly small.

As crosslink cleavage proceeds, f now decreases, so we need a relation between f and S to complete the derivation. This comes from eq. (13) of I:

$$G = 1 - S = 1 - \exp \{-\delta G\} \quad (18)$$

We make the replacements

$$\delta = QU = (\phi - \phi_b - \phi_c)Um/32 = \phi fM/32 \quad (19)$$

where M is the molecular weight of the entire uncrosslinked protein molecule. Equation (18) now becomes

$$S = \exp \{(\phi fM/32)(1 - S)\} \quad (20)$$

Solving for f and combining with eq. (17) we have finally

$$\phi_g = \phi + \frac{32}{M} \left(\frac{S \ln 1/S}{1 - S} \right) - \phi_c \quad (21)$$

The quantity $(S \ln 1/S)/(1 - S)$ falls off rapidly from unity for $S < 1$. Thus, since $32/M$ is also small, one would expect little if any increase in the gel sulfur content following pure crosslink cleavage. Instead, loss as unbound sulfur, formation of lanthionine, or disulfide-sulfhydryl exchange will more likely decrease the sulfur content.

We may allow for simultaneous crosslink cleavage and chain scission by using eq. (13) of this paper along with eqs. (11) or (28) of I to relate S to P and δ (or f). Because of the resulting curve-fitting problems we prefer to avoid these mixed cases if possible.

Multiple Components

For a system containing i independent components the weight balance of sulfur in the gel is

$$\phi_g W_g = \sum_i \phi_{ig} W_{ig} \quad (22)$$

where W_g refers to the entire gel weight and W_{ig} that of the i th component. For each component we can write, from eq. (13)

$$\phi_{ig} = \phi_i(1 + f_i S_i) \quad (23)$$

We have also $G = W_g/W$, $G_i = W_{ig}/W_i$, and the total weight fraction of i , $X_i = W_i/W$. Combining these relations with eqs. (22) and (23) gives

$$\phi_g = \sum_i \phi_i X_i (1 + f_i S_i) G_i / G \quad (24)$$

Additional relations are, for the original sulfur content,

$$\phi = \sum_i \phi_i X_i \quad (25)$$

and, for the solubility S ,

$$S = \sum_i S_i X_i \quad (26)$$

In eq. (26) S_i values are determined for each component from eqs. (11) or (28) of I.

Miscellaneous Effects

If intramolecular disulfide bonds form from intermolecular ones as a result of sulfhydryl-disulfide interchange,³⁻⁵ the net effect is formally the same as simple crosslink cleavage, except no sulfur is lost. We may apply eq. (21) to this case by omitting ϕ_c .

A troubling factor enters many keratin degradation experiments when a lanthionine crosslink, or single sulfur linkage, forms from a double sulfur linkage, with loss of the extra sulfur atom in an unbound form. In the original material, after lanthionine forms (assuming there were originally only disulfide crosslinks), eq. (8) becomes

$$\phi = [16(1 + \alpha)/m](N_{30}/N_0) + \phi_b + [16(1 - \alpha)/m](N_x/N_0) \quad (27)$$

where α is the fraction of total crosslinks that are in the —S—S— form. The last term, which refers to unbound sulfur, drops out of the relation for the gel fraction after extraction. Equation (9) then becomes

$$\phi_g = [16(1 + \alpha)/m](N_3/N) + (32/m)(N_2/N) \quad (28)$$

Combining as before we obtain

$$\phi_g = [(1 + \alpha)/2](\phi - \phi_b)(1 + S) + \phi_b \quad (29)$$

or, defining f again as the fraction of total bound sulfur in the form of intermolecular crosslinks,

$$\phi_g = \phi \{ f[(1 + \alpha)/2](1 + S) + 1 - f \} \quad (30)$$

For the case in which only decrosslinking occurs we may use eq. (20) to replace f in eq. (30) and obtain

$$\phi_g = \phi + \frac{16}{M} \left(\frac{\ln 1/S}{1 - S} \right) [(1 + \alpha)(1 + S) - 2] \quad (31)$$

In both eqs. (30) and (31) diminishing α reduces the amount of sulfur remaining in the gel. For small α the effect on ϕ_g , according to eq. (30) can be appreciable, although the ϕ_g for pure decrosslinking would, from eq. (31) again show little difference from the original composition ϕ .

References

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Résumé

On déduit des formules pour la concentration en soufre du résidu insoluble et de l'extrait d'un matériau semblable à la kératine en suivant soit la rupture de chaîne, soit la cassure de la réticulation ou les deux. S'il y a seulement rupture de chaîne, le soufre de la fraction insoluble peut atteindre le double de sa concentration initiale. Par cassure de la réticulation, cependant la teneur en soufre peut diminuer quelque peu par perte de soufre non lié, par formation de lanthionine ou par échange disulfuresulfhydryle.

Zusammenfassung

Formeln für die Schwefelkonzentration im unlöslichen Rückstand und im Extrakt eines keratinartigen Materials nach Ketten- oder Vernetzungsstellenspaltung oder beiden werden abgeleitet. Bei Auftreten von Kettenspaltung allein kann der Schwefel in der unlöslichen Fraktion bis zum doppelten Wert seiner ursprünglichen Konzentration ansteigen. Bei Vernetzungsstellenspaltung nimmt er jedoch durch Verlust an ungebundenem Schwefel, Bildung von Lanthionin oder Disulfid-Sulfhydrylaustausch wahrscheinlich etwas ab.

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