Crosslinking Structure of Keratin. II. Intermolecular and Intramolecular Crosslinks in Potassium-Cyanide-Treated Wool Fibers

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Synopsis

The crosslinking structure of keratin fibers has been investigated. The reaction of wool with aqueous KCN was studied by means of chemical and physical methods. Quantitative conversion of disulfide (SS) groups of the cystine (Cys) residues into stable thioether (S) linkages was confirmed. In terms of mechanically effective and noneffective crosslinkages, the amounts of intermolecular and intramolecular crosslinks in the KCN-treated wools were determined from the analysis of the corresponding amino acids and mechanical experiment in which the shear modulus of swollen, rubberlike samples is determined from the relation between equilibrium stress and strain for simple extension of the fiber in a diluent. A modified elastic equation of state was used for the calculation of the number of intermolecular crosslinks. The front factor in the equation used was determined by combining the results obtained from purely chemical kinetics and the values of shear modulus of the swollen wools. The ratio of the number of intermolecular and intramolecular SS crosslinkages in the Lincoln wool was found to be 64 : 36. The reactivity of the former is much higher than that of the latter, and these two types of SS crosslinks form substantially the same type of S crosslinks. The fraction of the Cys residues accessible to cyanide ions depends mainly on the reaction temperature.

INTRODUCTION

The reaction of keratins with aqueous potassium cyanide has been extensively studied.[1-4] It has been postulated that the reaction proceeds via substitution mechanism of the type of reaction S_N2, in which cystine residues convert to lanthionine residues crosslinked by thioether linkage, and this causes negligible production of lysinoalanine residues.[1-3,5] It was decided, therefore, to investigate the crosslinking structure of keratins with particular emphasis on the use of potassium cyanide which reacts selectively with disulfide bonds in keratins and forms thioether crosslinkages.

It has been found that the rubbery conditions can be successfully achieved for swollen wools in a diluent system composed of equal volumes of 8*M* lithium bromide and diethylene glycol mono-*n*-butyl ether.[6-8] From extension modulus measured in the diluent, crosslink density could be determined by using rubber elasticity theory for swollen network.

Journal of Applied Polymer Science, Vol. 38, 29–44 (1989)

CCC 0021-8995/89/010029-16$04.00

Kajiyama et al.[4] investigated crosslinking structure of wools treated with potassium cyanide, and the number of crosslinks was evaluated by measuring the degree of swelling in formic acid according to the method of Caldwell and Milligan.[9] They reported that a part of intermolecular disulfide crosslinks converts to intramolecular monosulfide crosslinks.

Owing to the complexity of keratin structure and the nonuniformity throughout fiber structure, it is difficult to define the terms "intermolecular" and "intramolecular" crosslinkages in wool.[5] We can now differentiate only between "mechanically effective" and "noneffective" crosslinkages.

This article deals with the application of the elastic equation of state derived from rubber elasticity theory to the swollen wool fiber. The theory, however, can be used only in simple form when the polymer chain in network is in random-coil conformation. We have, therefore, studied by using a modified equation with a constant parameter characteristic in the keratin network.

of intermolecular and intramolecular disulfide crosslinkages. This is obviously related to the complexity found in wool. In this context, reaction of cystine residues with potassium cyanide has been treated kinetically and the reactivity for the two types of crosslinkage has been discussed.

EXPERIMENTAL

Materials

Lincoln wool fibers used were purified by the method described in a previous article.[6] Potassium cyanide, and tri-*n*-butyl phosphine (TBP) used as reducing agent for wool were special reagent grade. Acrylonitrile (AN) as blocking agent of free thiol groups was obtained by distillation of commercial product under reduced pressure after dehydration with anhydrous sodium sulfate.

Determination of Disulfide and Sulfhydryl Contents

Disulfide (SS) and sulfhydryl (SH) contents in some wool samples were analyzed by a polarographic method using methyl mercuric iodide.[10]

Preparation of Potassium-Cyanide-Treated Wool (KCNW) Fibers

The wools (0.5 g) were treated with 0.08*M* KCN solution (40 mL) at 50, 70, and 90°C for 1/6, 1/2, 1, 2, 3, 4, 5, 6, 7, 8, and 10 h, and thoroughly washed with a distilled water by rinsing several times with the fresh water and then air-dried. The KCNW fibers thus obtained were subjected to amino acid analysis and mechanical tests.

Preparation of Reduced and S-β-Cyanoethylated KCNW (RKCNW) Fibers

The KCNW fibers (0.1 g) were reduced with a 1% TBP solution containing 1-propanol (5 mL) and borate–phosphate buffer adjusted at pH 8.0 (5 mL) for 24 h at 25°C, washed three times with the same buffer containing 1-propanol

(10 mL), and then treated with a 1% AN solution composed of 1-propanol (5 mL) and the same buffer (5 mL) for 24 h at 25°C. Completely reduced and block wools were obtained by repeating two times the above procedure. The polarographic analysis showed that no SS and SH groups were involved in the RKCNW fibers thus obtained.

Amino Acid Analysis

The chemically modified and unmodified wools were hydrolyzed with 6*M* HCl for 24 h at 110°C in deaerated conditions. According to the method of Gehrke et al.,[11] amino acids in the hydrolysates were derivatized into *N*-trifluoroacetyl-*n*-butyl esters, and total amino acid analysis was carried out by using a gas chromatograph Model 063 (Hitachi). Columns used were EGA and OV-17.

Relative molar response values (RMR) for 18 amino acids to ornithine used as internal standard were calculated from the experimental peak area of corresponding chromatograph of these amino acids. The RMR value for lanthionine (Lan) has been reported by Sakamoto et al.[12] and the value was used for present investigation. For both cystine (Cys) and Lan, OV-17 column was used and the analyses were performed three times for each sample; the average value was taken for the content of Cys and Lan.

Preparation of Swollen Fibers, Mechanical Tests, and Measurements of Fiber Density

The mechanical tests for swollen wool fibers were carried out by the method reported previously.[6,7] The densities of dry fibers, ρ, for Lincoln wool, KCNW, and RKCNW were measured by a density gradient column method using carbon tetrachloride and methanol at 25°C, and the corresponding values obtained were 1.28, 1.26, and 1.27 g/cm^3. These values were used for calculations in the text.

RESULTS

Amino Acid Compositions

Table I shows amino acid compositions of KCNW fibers prepared by varying the time of treatment with 0.08*M* KCN at 90°C. With increasing the time of treatment, Lan content is increased while Cys content is correspondingly decreased. For other amino acids, no significant changes in compositions are observed, and no Lan is present in the untreated Lincoln wool. Similar analytical results were also obtained for KCNW samples prepared at 50 and 70°C.

Figure 1 shows the variation of Cys and Lan contents with time of treatment at different temperatures. The rate of formation of Lan markedly increases with increase of treatment temperature. It should be noted that the amounts of Cys + Lan are approximately constant at different times. This suggests that all of the Lan originates from Cys residues. Throughout the amino acid analysis, the analytical data of Cys tended to scatter more than those of Lan. According to a good reproducibility for analysis of Lan content, formation reactions of Lan crosslinks were treated kinetically.

TABLE I
Amino Acid Compositions of Wool Fibers Treated with a 0.08*M* Potassium Cyanide Solution at 90°C

Amino acids	Untreated	Residues per 1000 amino acid residues, time of treatment (h)						
		1	2	3	4	5	7	10
Ala	59	67	72	68	68	66	70	71
Val	82	74	72	72	74	71	74	69
Gly	50	59	57	60	57	58	57	57
Thr	82	74	69	96	88	83	79	74
Ileu	48	50	48	47	47	48	44	39
Leu	101	106	100	107	99	101	100	105
Ser	102	90	91	84	93	91	92	94
Pro	70	68	75	64	63	68	64	63
Asp	59	77	80	68	65	76	78	74
Phe	21	25	26	37	33	29	22	25
Tyr	38	49	44	41	42	40	43	43
Glu	137	126	122	123	121	127	128	133
Lys	28	32	33	32	31	33	32	34
Arg	59	48	54	40	55	47	55	61
Lan	0	35	37	41	44	44	46	43
Cys	64	20	20	20	20	18	16	15

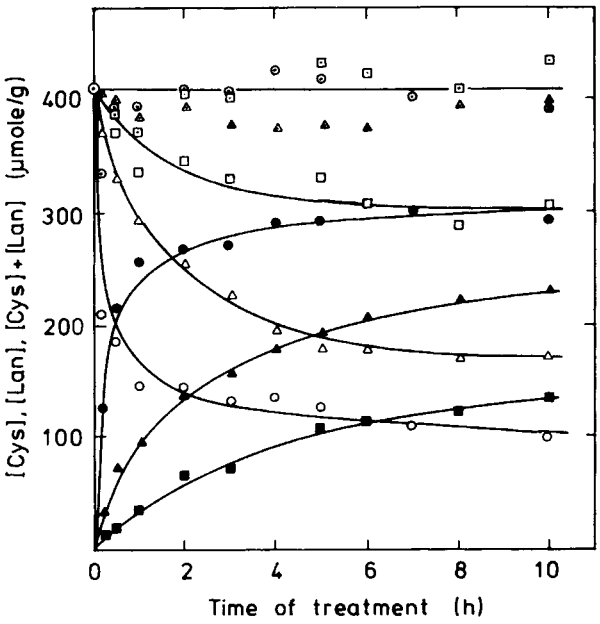
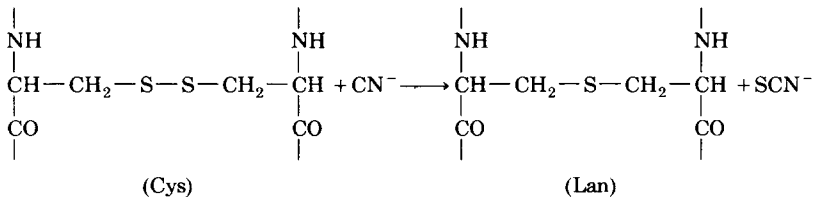


Fig. 1. The changes of cystine (Cys) and lanthionine (Lan) contents with time of treatment with aqueous KCN at different temperatures (°C). [Cys]: (□) 50; (△) 70; (○) 90. [Lan]: (■) 50; (▲) 70; (●) 90. The changes for Cys plus Lan contents are also shown, [Cys] + [Lan]: (⊡) 50; (◬) 70; (⊙) 90.

Kinetic Analysis

The entire reaction represented by eq. (1) was assumed to occur under pseudo-first-order conditions:



(1)

Equation (2) is thus obtained:

$$[\mathrm{Cys}]_t = [\mathrm{Cys}]_{t=0} \exp(-kt) \tag{2}$$

where k is a pseudo-first-order rate constant, $[\mathrm{Cys}]_t$ is the concentration of Cys residues at time t, and $[\mathrm{Cys}]_{t=0}$ is the concentration of Cys at $t = 0$, i.e., initial concentration of the reactive Cys groups associated with the reaction itself in the untreated Lincoln wool.

From eq. (2), eq. (3) is obtained:

$$\ln\{[\mathrm{Cys}]_{t=0}/[\mathrm{Cys}]_t\} = kt \tag{3}$$

From the results shown in Figure 1, it can be assumed that the Cys residues are quantitatively converted into Lan residues. Then, eq. (3) is rewritten by

$$\ln\{[\mathrm{Lan}]_{t=\infty}/([\mathrm{Lan}]_{t=\infty} - [\mathrm{Lan}]_t)\} = kt \tag{4}$$

where $[\mathrm{Lan}]_{t=\infty}$ is the concentration of Lan residues which will be formed at $t = \infty$ and $[\mathrm{Lan}]_t$ is the concentration of Lan at time t.

The value of $[\mathrm{Lan}]_{t=\infty}$ was evaluated by using a method of equal time internals.[13,14] If Lan contents at time t_a, t_b, and t_c selected as being $t_b - t_a = t_c - t_b$ are known from the reaction rate curve, $[\mathrm{Lan}]_{t=\infty}$ can be calculated by

$$[\mathrm{Lan}]_{t=\infty} = \left([\mathrm{Lan}]_{t_b}^2 - [\mathrm{Lan}]_{t_a}[\mathrm{Lan}]_{t_c}\right)/ \left\{2[\mathrm{Lan}]_{t_b} - \left([\mathrm{Lan}]_{t_a} + [\mathrm{Lan}]_{t_c}\right)\right\} \tag{5}$$

Figure 2 shows the plots for the relation represented by eq. (4). The value of k can be obtained from the slope of the curve. All the curves consist of two different slopes. This means that there are at least two types of reactive Cys groups with different reactivities in wool. From the slope of the linear part toward the end of the reaction and extrapolation of the linear portion to the logarithmic axis, the pseudo-first-order rate constant for slower-reacting groups (k_s) and the values of $[\mathrm{Lan}]_{t=\infty}$ of both slower- and faster-reacting groups were determined. Again, from the estimated value of $[\mathrm{Lan}]_{t=\infty}$ of the faster-reacting groups, further semilogarithmic plots could be constructed as also shown in Figure 2 by dotted lines. The pseudo-first-order rate constant for the faster-reacting groups (k_f) was thus obtained.

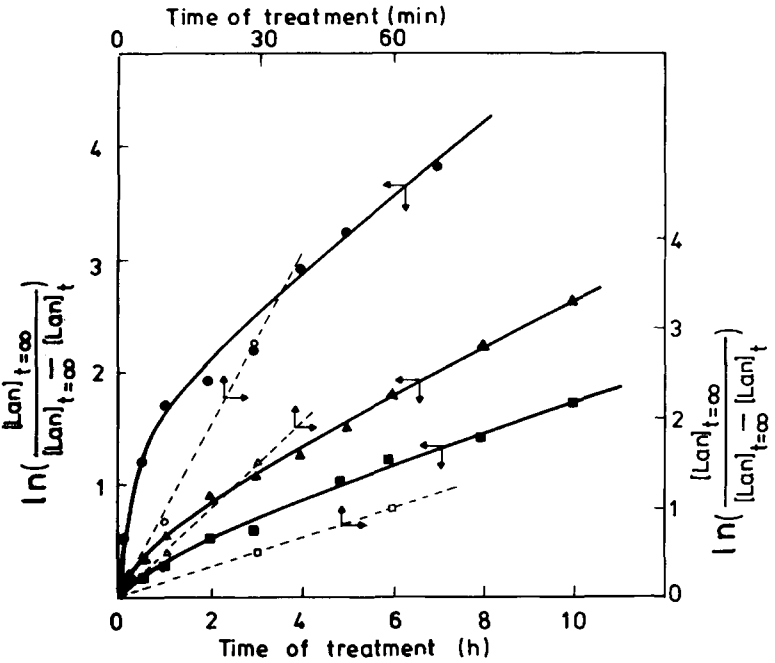


Fig. 2. Relationships of $\ln\{[Lan]_{t=\infty}/([Lan]_{t=\infty} - [Lan]_t\}$ vs. time of treatment with aqueous KCN at different temperatures (°C): (■) 50; (▲) 70; (●) 90. Relationships for the faster-reacting groups are shown by dotted lines: (□) 50; (△) 70; (○) 90.

The order of magnitude of k_f values obtained for wool treated at 50, 70, and 90°C were, respectively, 1.7×10^{-2}, 5.0×10^{-2}, and 8.8×10^{-2} min^{-1}. The k_s values were 1 order less than the corresponding k_f values, i.e., 2.1×10^{-3}, 3.6×10^{-3}, and 5.7×10^{-3} min^{-1}.

Kinetic results are summarized in Table II. The Lan contents at $t = \infty$ is equal to the amount of Cys reacted at $t = \infty$. Faster and slower reacting Cys, and nonreactive Cys contents are shown in Table II as $[Cys]_f$, $[Cys]_s$, and $[Cys]_{nonreactive}$, respectively. Comparing with the values obtained at 50°C, the

TABLE II
Faster and Slower Reacting Cys and Nonreactive Cys Contents in Lincoln Wool Treated with 0.08*M* KCN at Different Temperatures

Contents of Cys with different reactivities[a]	Treatment temperature (°C)					
	50		70		90	
	(μmol/g)	(%)	(μmol/g)	(%)	(μmol/g)	(%)
$[Cys]_{total}$	402	100	402	100	402	100
$[Cys]_{t=\infty}$	160	40	248	62	307	76
$[Cys]_f$	52	13	94	24	229	57
$[Cys]_s$	108	27	154	38	78	19
$[Cys]_{nonreactive}$	242	60	154	38	95	24

[a]Subscripts designated as total, $t = \infty$, f, s, and nonreactive denote total Cys, reactive Cys at time $t = \infty$, faster reacting Cys, slower reacting Cys, and nonreactive Cys, respectively.

reactive groups of Cys residues at 90°C increase by a factor of about 2 and faster-reactiong groups increase by ca. 4.4 times. However, 24% of the total Cys residues remains intact even at the highest temperature of treatments. Reactivity of Cys residues may probably be due to the difference in accessibility of CN^- ions and the ease of molecular motion of the chains near the Cys environment in fiber.

Crosslink Densities of KCNW and RKCNW Fibers

It has been shown that keratin fibers which have been treated with a 11*M* LiBr solution containing *N*-ethyl maleimide show typical rubber elasticity in a solution composed of equal volumes of 8*M* LiBr and diethylene glycol mono-*n*-butyl ether, and that crosslink density can be evaluated from the measurement of shear modulus of the swollen fiber.[6-8] According to the method reported in a previous paper,[8] swollen KCNW and RKCNW fibers were prepared and shear modulus G was measured under equilibrium conditions at 40°C.

Gaussian chain statistics of network system show that the relation between equilibrium forces τ and extension ratio α is represented by[15]

$$\tau = G(\alpha - 1/\alpha^2) \tag{6}$$

where τ is the stress referred to the swollen unstretched cross-sectional area of the sample. Here, G can be written

$$G = (\eta\rho RT/M_c)v_2^{1/3}(1 - 2M_c/M) \tag{7}$$

where η is the front factor introduced as a constant parameter characteristic in nature of the network, ρ the density of dry sample, R the gas constant, T the absolute temperature, v_2 the ratio of dry sample volume to swollen sample volume, M_c the number average molecular weight between crosslinks, and M the average molecular weight of polymer chain before crosslinking. The M value can be assumed to be 20,000 for the keratin system.[16] The crosslink density ρ/M_c represents the number of chains in unit volume of dry sample.

Relationships between $\eta\rho/M_c$ value obtained from eq. (7) and time of treatment with aqueous KCN are shown in Figure 3. The $\eta\rho/M_c$ values obtained for KCNW fibers prepared by varying temperature are approximately constant at definite times and have a sharp decreasing region followed by a leveling-off region continuing from ca. 1 to 10 h. The magnitude of the leveling-off density corresponds to about one-half the value obtained for the untreated wool. It is worth noting that the Cys or Lan contents are significantly varied at the region of a constant crosslink density (Fig. 1).

The $\eta\rho/M_c$ value of the Lincoln wool which has been completely reduced and blocked with AN is 1.5×10^{-4} mol/cm^3, and is shown in Figure 3 by the plot at $t = 0$. This means that nonreductive interchain crosslinks reside in wool. The $\eta\rho/M_c$ vs. time curves for RKCNW fibers are leveled off at ca. 1 h, and the levels observed are very different among the fibers prepared by varying the treatment temperature. It is noted that for the fibers obtained at 90°C, the $\eta\rho/M_c$ value at the leveling off region for the RKCNW fibers is

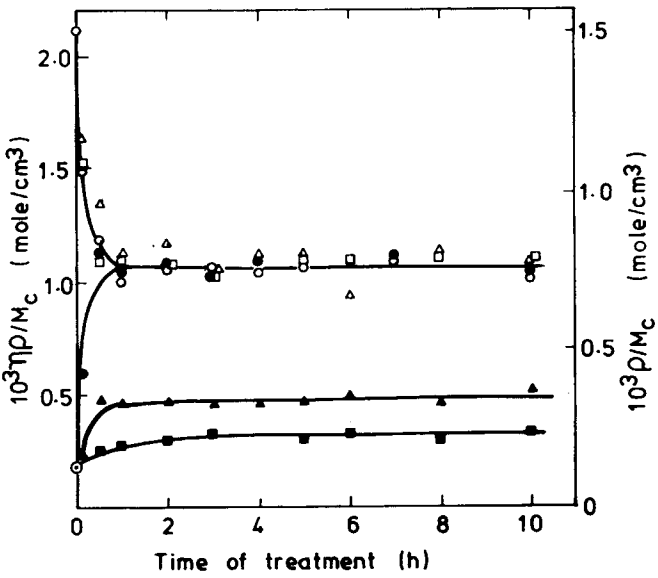


Fig. 3. Relationships of $\eta\rho/M_c$ vs. time of treatment with aqueous KCN at different temperatures (°C). KCNW fibers: (□) 50; (△) 70, (○) 90. RKCNW fibers: (■) 50; (▲) 70; (●) 90. The ρ/M_c scales in place of $\eta\rho/M_c$ are also shown. (⊙) ρ/M_c for completely reduced Lincoln wool.

approximately the same as that for the corresponding KCNW fiber which contains a considerable amount of intact Cys residues (Table I and Fig. 1). This is evidence for the presence of intramolecular disulfide crosslinks.

DISCUSSION

Determination of the Front Factor

If a randomly crosslinked network is formed by crosslinking with tetrafunctional crosslinkers such as Cys and Lan, the number of intermolecular crosslinks, n can be calculated from the ρ/M_c value by the equation: $n = (\rho/M_c)10^6/2\rho$ μmol/g. The number of intermolecular crosslinks, n_A for KCNW and n_B for RKCNW fibers, were calculated by assuming $\eta = 1$ and the results are shown in Table III. Here, the true number of intermolecular crosslinks, n_A/η and n_B/η are represented by eqs. (8) and (9), respectively:

$$n_A/\eta = [SS]_{inter} + [S]_{inter} + n_0/\eta \tag{8}$$

and

$$n_B/\eta = [S]_{inter} + n_0/\eta \tag{9}$$

where $[SS]_{inter}$ and $[S]_{inter}$ are the number of intermolecular crosslinks of Cys and Lan, respectively, and n_0 is the number of unreducible crosslinks in the untreated wool which could be estimated by assuming the crosslinks to be tetrafunctional. Therefore, the number of intermolecular and intramolecular

TABLE III
Number of Intermolecular Crosslinks for KCNW and RKCNW Fibers

Time of treatment (h)	Treatment temperature (°C)											
	n_A[a] (μmol/g)			n_B[b] (μmol/g)			$n_B - n_0$ (μmol/g)			[Lan]/($n_B - n_0$)		
	50	70	90	50	70	90	50	70	90	50	70	90
0	824	824	824	71[c]	71[c]	71[c]	0	0	0	—	—	—
1/6	586	647	583	—	83	232	—	12	161	—	2.83	0.77
1/2	432	524	468	95	185	437	24	114	366	0.75	0.64	0.59
1	437	444	389	102	177	402	31	106	331	1.03	0.92	0.76
2	417	464	405	114	185	421	43	114	350	1.44	1.22	0.75
3	405	413	421	122	185	402	51	114	331	1.35	1.29	0.82
4	—	437	409	—	181	429	—	110	358	—	1.58	0.81
5	437	444	413	118	185	429	47	114	358	2.21	1.66	0.82
6	433	361	—	122	193	—	51	122	—	2.18	1.66	—
7	—	—	425	—	—	437	—	—	366	—	—	0.82
8	440	448	—	114	181	—	43	110	—	2.79	2.01	—
10	441	437	397	114	201	413	43	130	342	3.02	1.77	0.85

[a] The number of intermolecular crosslinks of KCNW fibers calculated by assuming $\eta = 1$.
[b] The number of intermolecular crosslinks of RKCNW fibers calculated by assuming $\eta = 1$.
[c] The n_0 value.

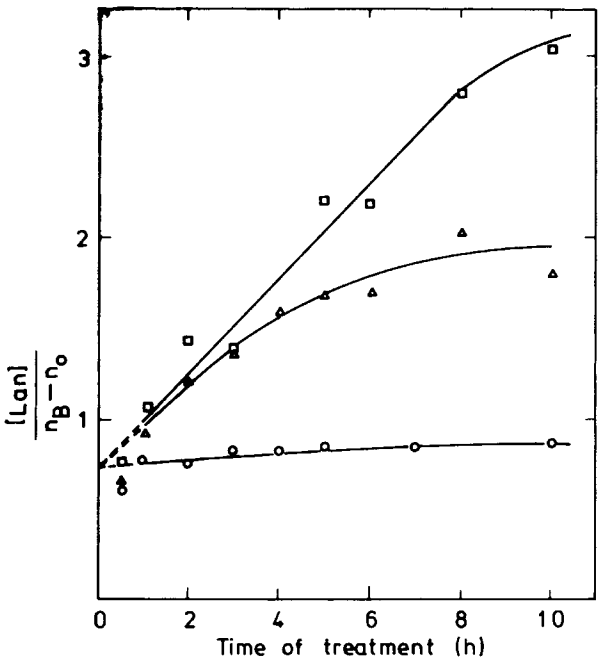


Fig. 4. Relationships of $[Lan]/(n_B - n_0)$ vs. time of treatment with aqueous KCN at different temperatures (°C): (□) 50; (△) 70; (○) 90.

crosslinks of Cys and Lan can be calculated by eqs. (10)–(13), respectively:

$$[SS]_{inter} = (n_A - n_B)/\eta \tag{10}$$

$$[SS]_{intra} = [Cys] - (n_A - n_B)/\eta \tag{11}$$

$$[S]_{inter} = (n_B - n_0)/\eta \tag{12}$$

and

$$[S]_{intra} = [Lan] - (n_B - n_0)/\eta \tag{13}$$

Equation (14) can be obtained by rearrangement of eq. (13):

$$1/\eta = \{[Lan]/(n_B - n_0)\} - \{[S]_{intra}/(n_B - n_0)\} \tag{14}$$

The values of $n_B - n_0$ and $[Lan]/(n_B - n_0)$ are also shown in Table III. For the fibers treated with 0.08*M* KCN above 1 h, the value of $n_B - n_0$ is approximately constant at definite temperatures. This means that interchain links of Lan are substantially formed within 1 h. As compared with the curves in Figure 1, it is known that the intrachain bonds of Lan are formed in relatively slower rate than the interchain links. It can be assumed, therefore, that the second term of eq. (14) is negligible at the initial stage of the reaction. The first term of eq. (14), $[Lan]/(n_B - n_0)$, is plotted against time of treatment with aqueous KCN, and the results are shown in Figure 4. The $1/\eta$ values can be obtained from extrapolation of the curves to the vertical axis at time $t = 0$ and the values are approximately constant (0.72). The value of 1.39 is thus obtained as the front factor η for swollen keratin systems. It is of

TABLE IV
Number of Intermolecular and Intramolecular Disulfide Crosslinks of KCNW Fibers

Time of treatment (h)	Treatment temperature (°C), [Cys] (μmol/g)					
	$[SS]_{inter}$			$[SS]_{intra}$		
	50	70	90	50	70	90
0	542	542	542	−140	−140	−140
1/6	—	406	253	—	−33	−49
1/2	242	244	22	122	84	160
1	241	192	−9	93	101	152
2	218	201	−12	124	52	155
3	204	164	14	128	61	117
4	—	184	−14	—	8	152
5	230	186	−12	100	−10	134
6	224	121	—	86	53	—
7	—	—	−9	—	—	114
8	235	192	—	50	−24	—
10	235	170	−12	70	−3	110

TABLE V
Number of Intermolecular and Intramolecular Monosulfide Crosslinks of KCNW Fibers

Time of treatment (h)	Treatment temperature (°C)								
	[Lan] (μmol/g)						$100([SS]_{inter} + [S]_{inter})$		
	$[S]_{inter}$			$[S]_{intra}$			[Cys] + [Lan] (%)		
	50	70	90	50	70	90	50	70	90
0	0	0	0	0	0	0	(135)	(135)	(135)
1/6	—	9	116	—	25	8	—	(102)	(112)
1/2	17	82	264	1	−9	−50	(67.8)	(80.8)	(72.2)
1	22	76	238	10	22	15	68.9	68.5	57.8
2	31	82	252	31	58	9	61.6	72.0	59.4
3	37	82	238	32	65	33	60.1	66.1	62.7
4	—	79	258	—	95	32	—	71.9	57.0
5	34	82	258	70	108	37	60.4	73.2	59.0
6	37	88	—	74	114	—	61.8	55.6	—
7	—	—	264	—	—	36	—	—	63.0
8	31	79	—	89	142	—	65.7	69.7	—
10	31	94	246	99	136	44	61.2	66.0	60.3
						Avg	62.8	67.9	59.9

interest that the η value is constant and actually independent of the number of interchain crosslinks of the network. The number of inter and intramolecular crosslinks of Cys and Lan can now be calculated by eqs. (10)–(13). These results are shown in Tables IV and V. Thus, in Figure 3, a new scale for crosslink density ρ/M_c can be introduced as a measure of the true crosslink density.

Interpretation of the Number of Crosslinks in Untreated Wool

It is shown that the untreated Lincoln wool involves 542 μmol/g of interchain Cys links. This seems to be inconsistent with the fact that this

network system consists of the Cys links amounting to 402 μmol/g. It might be possible to explain the result obtained from this calculation if this system involves considerable chain entanglements which act as chemical covalent crosslinks. On the other hand, it is considered that this treatment for nonuniform crosslinking system gives rise to a deviation from ideal network, so that calculation by eq. (7) gives a relative value to the number of interchain crosslinks. Exact treatment or rubberlike elasticity originating from the nonuniform structure of keratin network needs further investigations.

Number of Intermolecular Crosslinks in Completely Reduced Wool

The crosslink density of the Lincoln wool fiber which has been completely reduced and blocked with AN is evaluated to be 1.3×10^{-4} mol/cm^3 which corresponds to 51 μmol/g as the value of n_0/η, namely, the number of the interchain crosslinks other than disulfide crosslinks in the native wool fiber.

Formation of Monosulfide Crosslinks in Wool

After treatment with aqueous KCN for 1/2 h at 50°C, conversion of Cys to Lan is only 18 μmol/g which corresponds to 4.5% of the initial Cys content (Table V), and the ρ/M_c value dramatically decreases to one-half the value obtained for untreated wool (Fig. 3). Similar behavior has also been observed for wools and hairs treated with 0.05M sodium carbonate solution for 1 h at 40, 50, and 60°C.[17] It is appropriate, therefore, to consider the fact that the sharp decrease of the crosslink density at the initial stage of treatment with aqueous KCN does not correlate with the formation reaction of Lan crosslinks. Following scission and rearrangement reactions of crosslinkages are thus considered: (1) scission of alkali-labile bonds such as cystine oxides, (2) rearrangement of intermolecular SS links by SH/SS interchange reaction, and (3) disentanglements followed by the scission of some covalent links and the interchange reactions. The cystine oxides content being around only 25 μmol/g in keratin fibers has been reported.[18] It seems unlikely, therefore, that the abrupt change in crosslink density is caused by the hydrolytic breakage of these oxide bonds. It is probably considered that at the initial stages of the treatment with aqueous KCN, anisotropic swelling of fiber in alkaline media initiates the SH/SS interchange reactions, and this causes the decreases of the entanglement effect and the structural nonuniformity existing like domains of block copolymers.[8]

Here, it must be presumed that the modulus of elasticity of the swollen network obtained for the chemically modified wools includes more or less effects of the hydrolytic cleavage of peptide bonds. Although it is difficult to evaluate the amount of bond scission of the chains, it is likely to consider that the effects are negligible, because of the fact that, for KCNW fibers, the ρ/M_c values obtained at different temperatures are approximately constant at definite times of treatment (Fig. 3).

In Figure 5, the number of intermolecular or intramolecular crosslinks of Lan are plotted against the time of treatment with aqueous KCN. It should be noted that the rate of formation of the interchain links is faster than that of the intrachain links and tends to level off after ca. 1 h. The intrachain links

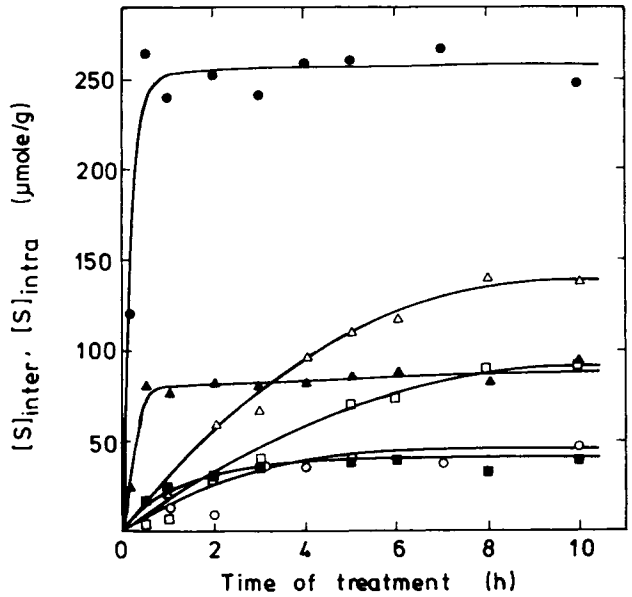


Fig. 5. Relationships of $[S]_{inter}$ and $[S]_{intra}$ vs. time of treatment with aqueous KCN at different temperatures (°C). $[S]_{inter}$: (■) 50; (▲) 70; (●) 90. $[S]_{intra}$: (□) 50; (△) 70; (○) 90.

are formed slowly and tend to saturate to a constant value at each temperature. It is suggested that the slower and faster rate processes are analogous to the kinetically observed two different reactions in which the slower and faster reacting groups of Cys are concerned (Fig. 2 and Table II). The value of $[Lan]_{inter,\, t=\infty}$, the number of interchain links of Lan at time $t = \infty$, was evaluated from the curve directly by assuming that the value is equal to the content at leveling off region. While the value of $[Lan]_{intra,\, t=\infty}$ was determined by the same way as described for the kinetic analysis of the formation reaction of Lan. These results obtained are shown in graphs B of Figures 6(a), (b), and (c). Here, the percentage ratios are referred to 402 μmol/g of the Cys content in the untreated wool. Graphs A show the percentage values from the data in Table II. The number of the intermolecular crosslinks of Lan is approximately the same as the number of faster-reacting groups of Cys residues (equivalent to Lan). The number of intramolecular crosslinks of Lan corresponds well to the number of slower-reacting groups of Cys. The rate constants obtained for the intrachain bond-forming processes were 2.6×10^{-3}, 3.5×10^{-3}, and 6.0×10^{-3} min^{-1} at 50, 70, and 90°C, respectively. For the interchain-bond formation, the corresponding rate constants were also obtained, i.e., 1.7×10^{-2}, 4.0×10^{-2}, and 6.7×10^{-2} min^{-1}. The order of these values is the same as the corresponding reaction-rate constant obtained from the kinetic analysis of the reaction.

The amounts of reactive and nonreactive Cys groups and the rate constants obtained from physical measurements are in good agreement with the values obtained from purely chemical kinetics. This clearly suggests that: (1) as far as chemically modified wools are concerned, the ρ/M_c values from eq. (7) can be taken as reasonable values for actual number of interchain crosslinks with

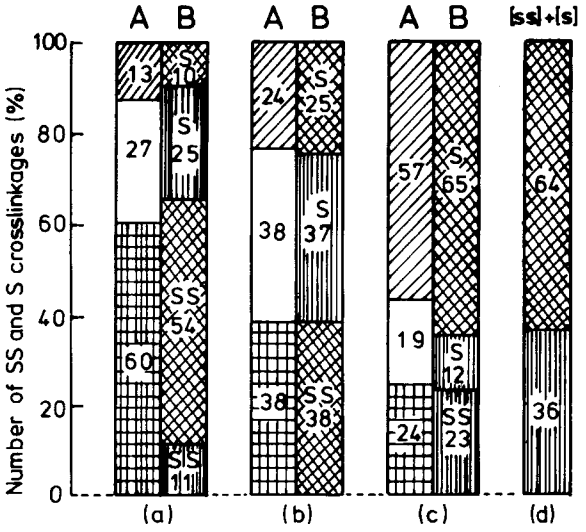


Fig. 6. Cys(SS) and Lan(S) contents in KCNW fibers obtained at time $t = \infty$ and at different temperatures (°C): (a) 50; (b) 70; (c) 90. Graphs A, percentage ratios of Cys residues with different reactivities (▨) faster; (□) slower reacting groups; (■) nonreactive groups. Graphs B, percentage ratios of Cys and Lan crosslinkages: (▨) intermolecular; (▩) intramolecular crosslinkages. The percentage ratio of the number of intermolecular and intramolecular crosslinks to total Cys plus Lan contents: (d) (▨) intermolecular crosslink (64%); (▩) intramolecular crosslink (36%).

covalent bonds, and (2) chain entanglement effects on network elasticity are less important.

At present, however, there has been no clearcut explanation for crosslinking mechanism at initial stage of the treatment below 1/2 h, and no analytical methods have been provided. This is due to the fact that the ρ/M_c value calculated from eq. (7) deviates from actual crosslink density, since the application of eq. (7) is only possible for lower crosslinking systems such as the fiber with the amount of interchain crosslinks less than about 300 μmol/g as the value of $[SS]_{inter} + [S]_{inter}$ (Tables IV and V).

As clarified in the preceding discussion, a sharp drop of crosslink density at the initial stage of KCN treatment is not correlated with the formation reaction of Lan crosslinks. This makes it possible to discuss the formation reaction of Lan within the limit of application of eq. (7) and to estimate the amounts of interchain crosslinks of Lan and Cys in the KCN-treated fibers.

Reactivity of Disulfide Crosslinks with Cyanide Ions

The percentage ratios of the total number of interchain crosslinks to the content of [Cys] + [Lan] are shown in the last column of Table V. It can be known that the percentage ratios are approximately constant for the fibers treated with aqueous KCN above 1 h at different temperatures. The average values of the ratios are 62.8, 67.9, and 59.9% at 50, 70, and 90°C, respectively. It seems unlikely that the values of the ratio are affected significantly by the treatment temperature. This leads to a conclusion that the amounts of both interchain and intrachain crosslinks are always constant during the treatment, introducing a constancy of [Cys] + [Lan]. This is evidence that the

interchain crosslinks of Lan residues are formed quantitatively from the interchain links of Cys residues. The situation of the formation of intrachain links is also the same. The number of interchain crosslinks can be taken to be 64% as average value of the above figures obtained at different temperatures and this is shown in Figure 6(d). Here, it is of interest to find the crosslinking structure of intact SS bonds remaining in the fiber. According to the ratio of 64 : 36, the nonreactive Cys residues shown in Table II can be divided into two parts, i.e., intermolecular and intramolecular crosslinks of Cys. The results obtained are shown in graphs B of Figures 6(a), (b), and (c).

Calculation shows that in the fibers treated with KCN at 50°C, SS bonds amounting to ca. 65% of the total number of crosslinks remain after the time at $t = \infty$ and the remaining SS bonds are divided into 54% interchain and 11% intrachain bonds. On the other hand, at 70°C, the SS bonds decrease to ca. 38% and all of them are attributed to interchain bonds. This means that with the increase of temperature from 50 to 70°C, approximately similar amounts of interchain (14%) and intrachain (12%) crosslinks of SS bonds are converted into reactive Cys groups [Figs. 6(a) and (b)]. Comparing graphs A and B of Figures 6(a) and (b), it can be known that, as already described above, the formation of interchain crosslinks always proceeds in a faster-rate process of the reaction. These clearly suggest that reactivity of the intermolecular SS bonds with CN^- ions is much higher than the intramolecular bonds, but the fraction of the Cys residues accessible to CN^- ions depend mainly on the reaction temperature.

At the maximum temperature in this experiment, the intact SS bonds decrease to 24% and only intrachain bonds remain. This implies that all of the interchain SS links are converted selectively to stable S links in faster rate. This may be due to the increase in the mobility of wool chains through the SH/SS interchange reactions. Stable Lan crosslinks can stabilize the structure at the remaining parts of intrachain links, and may lead to a decrease in accessibility.

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education (00555349).

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Received April 11, 1988
Accepted July 18, 1988