CONTRIBUTION TO THE KINETICS OF KERATIN DISULFIDE BONDS BREAKING IN ALKALINE MEDIUM*

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The kinetics of disulfide bonds breaking in intact wool keratin induced in aqueous solutions of NaOH, $Ca(OH)_2$ and Na_2S was measured by stress relaxation technique in oxygen free medium. The rates of disulfide scission were derived from chemical relaxation times and parameters of activation and reaction order were estimated. The reaction with Na_2S is clearly of S_N2 type. In hydroxide solutions, the prevailing mechanism changes from elimination in milder alkalinity and temperature, to S_N2 type, if alkalinity stronger than 0·5M and temperature higher than transition temperature of keratin are used.

There have been little exact data concerning the kinetics of alkaline breaking of disulfide bonds in intact keratin of wool. The main experimental obstacle, due to the lack of a quick method for determination of disulfide bonds in insoluble keratin could be overcome with stress relaxation technique used by Katz[1] and others[2-4], provided that the rate of stress relaxation in moderately deformed wool fibre is equal to the rate of disulfide bonds breakdown. When alkaline reagent is used, also the possibility of peptide bonds hydrolysis must be taken in account. As peptide hydrolysis results in similar stress relaxation as disulfide breakdown, it cannot be distinguished by analysis of stress relaxation curves and must be estimated in independent experiments. In this paper kinetics of disulfide bonds breaking in keratin in alkaline medium is studied using stress relaxation technique.

EXPERIMENTAL

Goat wool taken from raw skin used in fur processing was applied. The samples were defatted by acetone and air dried. In each test fibres 0·05 to 0·12 mm thick and 8 to 12 mm long were used. Relaxation was measured at about 10% elongation in fibres dipped into stirred, oxygen free solution of agent. Inert atmosphere was secured by passing nitrogen stream beneath the surface of solution contained in temperature-controlled cell covered with rubber plate with small outlets. During the whole experiment the solutions were stirred. The apparatus used for stress measurements was a registering balancing tensiometer[5] having relative error in force measure-

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ment smaller than 1%. Specimens of wool were analysed for cystine and average content 408 μmol/g was found. The disulfide breaking agents tested were 0·1M, 0·5M and 1·0M-NaOH, saturated calcium hydroxide and saturated calcium hydroxide containing different amounts of sodium sulfide. The contents of sodium sulfide in these solutions were estimated by ferricyanide titration.

The effect of peptide bond hydrolysis was estimated by measurements of stress relaxation rates in elastin fibres about 0·2—0·3 mm thick, in treatment at the most strict conditions of alkalinity and temperature, used with keratin. Elastin samples were prepared according to Partridge[6] from fresh bovine ligamentum nuchae. As mature elastin is crosslinked through alkali stable covalent desmosine bonds, any stress relaxation in it must be accounted for peptide bonds hydrolysis.

RESULTS AND DISCUSSION

During relaxation two stages can be clearly distinguished which is visible from tensiometric record shown in Fig. 1. As it is supposed[7,8], the first stage involves breakdown of secondary bonds, such as van der Waals interactions, and diffusion of agents[9]. As starting point ($t = 0$) of disulfide scission is taken the intersection point of linear parts of the relaxation curve. The reaction rate can be characterised by chemical relaxation time, τ_{ch} which is the time interval when the remaining stress in sample (f_t) divided by the stress at beginning of reaction (f_0) has the value of $1/e$. Reliability of determination of chemical relaxation time was checked at conditions when short relaxation time (*i.e.* lowest precision) was obtained. As shown in Table I, even at such conditions the standard deviation for six repeated measurements is good and precision about $\pm 3\%$ can be expected.

Relative rates for keratin and elastin fibres are given in Table II. As stress relaxation in keratin is about 300 times quicker than in elastin, the effect of peptide bonds

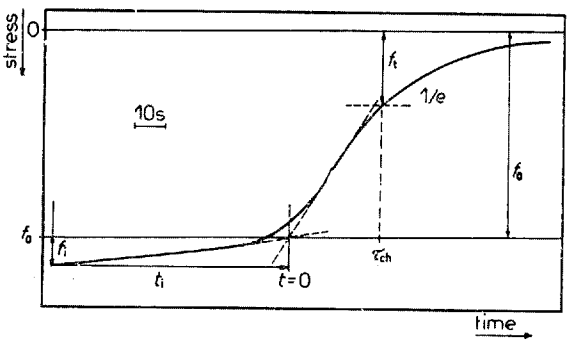

FIG. 1

Tensiometric Record of Wool Relaxation in 1M-NaOH at 52°C

Stress in fibre (f) at the beginning of reaction is corrected by subtraction of f_i, which is value of stress relaxation during interval t_i, when reaction rate is limited by diffusion of reagent.

TABLE I
Reliability of Chemical Relaxation Time Determination

Relaxation time, s	14·3—12·7—14·8—12·0—13·0—10·9
Average, s	12·95
Standard deviation	0·401
Relative error	±3%

TABLE II
Breaking Rates of Disulfide Bonds in Keratin and Peptide Bonds in Elastin during Treatment in 1·0M-NaOH at 70°C

Protein	Chemical relaxation time, s	Average
Keratin	9·66—8·99—7·83—7·20—8·99	8·53
Elastin	270—285—254	270

hydrolysis is probably within the limits of experimental error of stress measurements and therefore is considered as unimportant in this case.

Experimental results concerning the breakdown of disulfide bonds in wool in solutions of sodium sulfide and in solutions of hydroxides are given in Tables III and IV. From the slopes of chemical relaxation time *vs* $1/T$ the energy of activation and pZ factor of Arrhenius were estimated. The average value of activation energy for disulfide breaking reaction with sodium sulfide was found to be 13·7 ± 2·2 kcal . . mol^{-1} (57·4 ± 9 kJ mol^{-1}). This is in agreement with the values reported for other agents reacting with disulfides by bimolecular displacement of S_N2 type, *e.g.* 13 kcal . . mol^{-1} for sulphite[10-12] or 10—15 kcal mol^{-1} for thioglycolate and 18·2 kcal mol^{-1} for cystein[4]. Kinetics of the first order with respect to Na_2S at concentrations above 0·02M was found.

At temperatures higher than 70 or 60°C and at concentrations of NaOH above 0·5M resp. 1·0M, sharp drop in activation energy from 32—35 kcal mol^{-1} (134 to 147 kJ mol^{-1}) to the 10—13 kcal mol^{-1} (42—54 kJ mol^{-1}) was observed. Similar drop takes place in frequency factor pZ, the measure of activation entropy, which changed from 18—20 to 5—7. The alkaline scission of keratin disulfide bonds with low activation energy of 10—13 kcal mol^{-1} is very probably of S_N2 type, corresponding to the mechanism suggested by Schöberl[13]. This reaction is clearly bimolecular (first order with respect to NaOH) and its activation energy is comparable with

that of the sodium sulfide reaction. According to present results this termodynamically favored way of disulfide scission is inhibited at low temperature and at low activity of hydroxide. This can be attributed to steric hindrance, because of the sensitivity of bimolecular nucleophilic substitution to steric arrangement in proximity of displaced group. Prevailing reaction in milder conditions is probably of elimination type, which is free of steric hindrance effect.

The activation energy 32–35 kcal mol^{-1} found for alkaline breaking in milder conditions is of a rather high value. To this date an activation energy higher than 30 kcal mol^{-1} was reported only by Gawron and Odstrchel[14]. Other reported[15] values (in all cases for simple, soluble disulfides) lie in range of 15–20 kcal mol^{-1}. For the formation of olefins by bimolecular elimination (E_2) of thiols with hydroxides the activation energy value of 25·1 ± 1·1 kcal mol^{-1} was estimated while for the same reaction, but proceeding as monomolecular elimination of E_1 cb type (conjugated base intermediate) higher activation energy (32·3 ± 0·7 kcal mol^{-1}) is reported.

TABLE III

The Relaxation Times and Activating Parameters of Keratin Disulfide Bonds Breaking with Na_2S Solutions in Saturated $Ca(OH)_2$

τ_{ch} s	°C	Na_2S mol/l	τ_{ch} s	°C	Na_2S mol/l
9 956	19	0·01245	202	17	0·09964
4 016	29	0·01245	168	22	0·09964
2 558	38	0·01245	146	30	0·09964
			113	30	0·09964
E_a = 13 kcal mol^{-1}; pZ = 2·8			90	30	0·09964
416	38	0·02491			
360	38	0·02491	E_a = 12·9 kcal mol^{-1}; pZ = 5·5		
933	28	0·02491			
2 306	18	0·0249	383	2	0·1993
			316	6·5	0·1993
E_a = 15·9 kcal mol^{-1}; pZ = 5·2			158	12	0·1993
608	19	0·04982	E_a = 12·9 kcal mol^{-1}; pZ = 6·4		
356	26	0·04982			
326	31	0·04982	23 400	70	[a]
180	31	0·04982	9 000	82	[a]
			1 889	92	[a]
E_a = 13·7 kcal mol^{-1}; pZ = 5·1			1 675	92	[a]
270	13	0·09964			
247	17	0·09964	E_a = 33·2 kcal mol^{-1}; pZ = 16·5		

[a] Without Na_2S.

Thus the question could raise, if the activation energy of 32–35 kcal mol^{-1}, which is about 10 kcal mol^{-1} higher than in typical E_2 reaction, is needed for carrying parts of keratin molecule to condition favored for E_2 reaction, or if such mechanism is suppressed for account of E_1 cb type. This second possibility is suggestive and probably in accordance with previous view[16–18] concerning the importance of disulfide bond polarisation in alkaline scission. Unfortunately, unambiguous distinguishing between both elimination mechanisms is impossible on the basis of the present results, owing to variability in observed reaction order with concentration of hydroxide. According to our interpretation this variability could be the result of disulfide interchange, strongly affected by the presence of sulfhydryl group in keratin. Such groups are produced by scission and could catalyze disulfide interchange, leading to preferential breakdown of stress supporting crosslinks and finally resulting

TABLE IV

The Relaxation Times and Activating Parameters of Keratin Disulfide Bonds Breakdown in NaOH Solutions

τ_{ch} s	°C	NaOH mol/l
67 500	56	0·1
28 575	61·5	0·1
3 24	76	0·1
911	85	0·1
833	85	0·1
E_a = 34·9 kcal/mol; pZ = 18·3		
25 000	24	0·5
3 500	37	0·5
3 800	38	0·5
717	47·5	0·5
390	47·5	0·5
90	52	0·5
44	52	0·5
E_a = 33·9 kcal/mol; pZ = 20·4		
17	69	0·5
14	69	0·5
6	85	0·5
7	85	0·5
6	85	0·5
E_a = 13·5 kcal/mol; pZ = 7·1		
2 049	21·5	1·0
2 652	21·5	1·0
438	32	1·0
390	32	1·0
75	42	1·0
74	42	1·0
40	52	1·0
39	52	1·0
14	62	1·0
E_a = 32·0 kcal/mol; pZ = 19·8		
13	62	1·0
15	62	1·0
12	62	1·0
13	62	1·0
11	62	1·0
9·7	71	1·0
9	71	1·0
7·8	71	1·0
E_a = 10·2 kcal/mol; pZ = 5·6		

TABLE V

Comparison of Products of Relaxation Time and Reagent Concentration

Reagent mol/l Na_2S	°C	$\tau_{ch}\, n$	Reagent mol/l Na_2S	°C	$\tau_{ch}\, n$
0·01245	49·4	13·7	1·0	49·4	42·7
0·02491	49·4	4·04[a]	0·5	49·4	173
0·04982	49·4	4·45[a]	0·1	49·4	22 400
0·09964	49·4	2·75[a]	1·0	76·5	6·3[a]
0·19930	49·4	2·63[a]	0·5	76·5	5·0[a]

[a] Values stable with changing concentration, showing the validity of first order kinetics.

in stress relaxation without true change in disulfide content. Such effect is more pronounced in relatively slow elimination than in quick substitution.

When lower activating energy for alkaline breakdown is valid, the kinetics is of the first order with respect to hydroxide concentration (see constant value of product of $\tau_{ch}\, n$ for several concentrations of NaOH in Table V) while at higher activation energy the reaction order is variable with concentration. This confirms the existence of sudden change in prevailing reaction mechanism. The fact that reaction with higher activation energy is preferred at milder conditions is a striking one and probably cannot be explained without supposing the importance of some structural transformations in keratin, which facilitates mechanism with lower activation upon heating of wool. Such structural changes are possible, as observed temperature range 60–70°C is consistent with transition temperature of second order transition found in wool keratin[19].

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