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Reactions of β-Sulphatoethyl Sulphone Crosslinking Agent with Wool. Part IV: Topochemistry of Reactions by Fractionation and Extraction

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ABSTRACT

Topochemical studies have indicated that the crosslinking reaction of 2-chloro-4,6-di(-aminobenzene-4-β-sulphato-ethylsulphone)-1,3,5-s-triazine (XLC) occurs mainly in the low-sulphur microfibrillar proteins of the wool fibre. This can be explained by a high content of lysine and histidine residues, which have been shown to be the major sites of the reaction. Modification of these residues was shown to be pH dependent. Where crosslinking was greatest, it was shown that the lysine and histidine side chains were bridged by the compound. The non-keratinous proteins derived from the cell membrane complex were shown to react with the XLC crosslinking reagent at pH 6.

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1 INTRODUCTION

Studies have been carried out on the application of 2-chloro-4,6-di(-aminobenzene-4-β-sulphato-ethylsulphone)-1,3,5-*s*-triazine (XLC) to wool. HPLC analysis showed the compound to have good substantivity for the wool fibre when applied from boiling aqueous dyebaths in the pH range 3–6.[1] Solubility and swelling tests have shown that XLC introduces additional crosslinks into the fibre.[2] This was confirmed by amino acid analysis, and it was shown that lysine and histidine residues were the main sites of reaction to the compound.[3] In this work further evidence will be sought, as well as the exact morphological regions in the wool fibre where the crosslinking reaction occurs.

2 EXPERIMENTAL

Extraction and fractionation of wool was used to study the topochemistry of the XLC reaction with wool fibre.

2.1 Keratose fractionation

Keratose fractionation was carried out by the method of Asquith and Parkinson[4] after oxidation with performic acid. Wool samples (approx. 100 mg) were placed in weighed freeze-drier tubes, 3 ml of performic acid (cf Section 2.2)[2] added, and oxidation carried out for 16 h at 0°C. The samples were then freeze-dried to give known weights of oxidised wool. Oxidised samples were then transferred to 25 ml of 1·5 M ammonium hydroxide and allowed to stand at room temperature for 30 h. The insoluble β-keratose fraction was then filtered off on tared glass crucibles (porosity no. 3), washed with water, then acetone, and dried to constant weight at 105°C. The filtrates were diluted to 100 ml and their pH adjusted to 4 with glacial acetic acid. Coagulation of α-keratose was allowed to proceed for 24 h before collecting on tared glass crucibles, washing and drying as for β-keratose.

The results for α- and β-keratoses were expressed as a percentage of the weight of oxidised samples. The percentage of γ-keratose was obtained by difference from 100%.

2.2 Wool gelatin extraction

Wool gelatins were extracted using a procedure based on that of Baumann.[5] Wool samples (approx. 100 mg) were placed in stoppered test tubes and 0·05 M citric acid made to pH 2 by HCl (10 ml) was added. The tempera-

ture was raised to the boil in 45 min and held for times from 1 to 6 h. The samples were then filtered on glass crucibles (porosity no. 1) and washed with 3 × 10 ml of the above citrate buffer. The filtrates were then transferred quantitatively to 18/32 Visking tubing and dialysed overnight against distilled water. The volume of dialysed sample was then reduced by rotary evaporation at 40°C and finally lyophilised to dryness. Samples were then hydrolysed and the yield and composition of wool gelatin determined by amino acid analysis.

3 RESULTS

The topochemistry of the reaction of XLC with wool identifies the morphological regions of the fibre that are modified as a result of reaction. Amino acid analysis has already identified lysine and histidine residues as the main sites of reaction. These two compounds are present in the microfibrillar region, but it is uncertain if the reaction is involved with this region primarily. Fractionation of the wool should cast light on the situation.

3.1 Keratose fractionation of XLC-treated wool

Keratose fractionation is similar to the performic acid/ammonia solubility tests already carried out[2] in that the wool is completely oxidised with performic acid, and in a separate step the soluble α- and γ-keratose fractions are extracted from the insoluble β-keratose. The two procedures differ in that the latter only measures the insoluble β-keratose fraction, whereas the fractionation procedure goes one step further, and by precipitating the α-keratose all three keratose fractions are determined. The α-, β- and γ-keratoses have been shown to derive from the microfibrillar, the intermacrofibrillar and the matrix components of the wool fibre, respectively.[6] Thus, information on the topochemistry of the XLC reaction with the wool fibre may be obtained from changes in the relative yields of the three keratose fractions. Since the β-keratose fraction, corresponding to the intermacrofibrillar material, is insoluble in the first step of this procedure, no indication of the extent of the reaction of XLC with this component is given by this test. It should also be pointed out that the keratoses derived from chemically modified wool do not necessarily represent the particular morphological components with which the keratoses extracted from untreated wool could be associated. The 'keratose fractions' derived from modified wool only serve to indicate the changes that have taken place from the original distribution of keratoses.

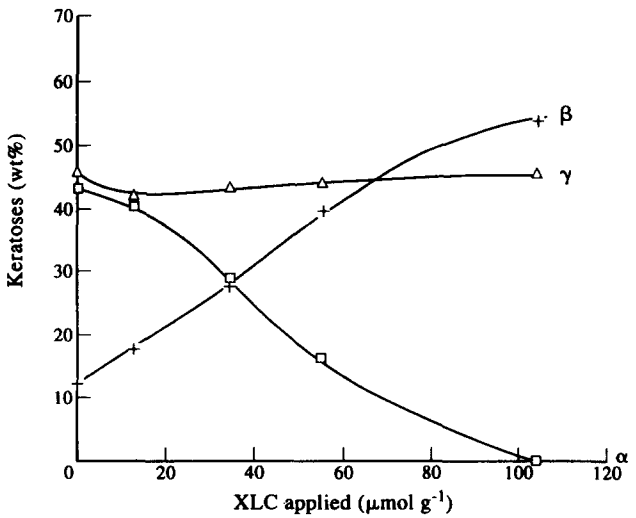

Fig. 1. The effect of XLC applied at pH 5 on the yields of the keratose fractions.

The changes in the yields of the three keratose fractions derived from wool treated with XLC at pH 5 and 6 are given in Figs 1 and 2. For XLC applied at pH 5 (Fig. 1) there was a fall in the α-keratose fraction, which was accompanied by a proportionate increase in the yield of β-keratose as more of the compound was applied. The α-keratose fraction loses its solubility on the introduction of crosslinks by 104 μmol XLC g^{-1}, the β-keratose yield at this concentration exactly matching the sum of the α- and β-keratose yields for blank treated wool. The yield of γ-keratose was unaffected by the application of XLC at this pH.

The same changes occurred at pH 6; however, a greater reaction of the compound was shown by the relatively lower concentrations of XLC required at this pH to insolubilise the same amounts of α-keratose. The maximum yield of insoluble keratose was greater at pH 6 due to some insolubilisation of γ-keratose. The γ-keratose appeared to be crosslinked by the compound at this higher pH value, but not at pH 5, and this different reactivity was clearly demonstrated by the final sets of points at the highest concentrations of XLC applied at pH 5 and pH 6 (see Figs 1 and 2).

It would thus appear from these fractionation data that crosslinking took place preferentially in the microfibrillar regions of the wool cortex, as was evident from the dramatic fall in the yields of α-keratose at pH 5 and 6. Some crosslinking of the matrix component has been demonstrated at pH 6 by a small reduction in the γ-keratose fraction.

Amino acid analysis of the keratoses[7] has shown the α-keratoses to be low in cysteic acid residues and to have five times more lysine than γ-

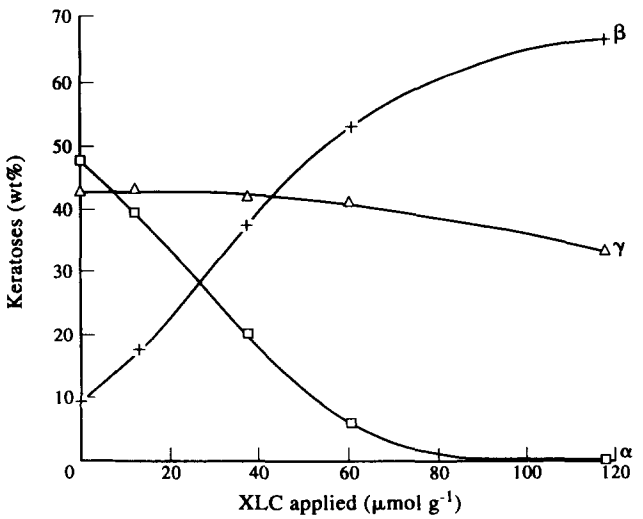

Fig. 2. The effect of XLC applied at pH 6 on the yields of the keratose fractions.

keratose. Since amino acid analysis of XLC-treated wool clearly exhibited a substantial reaction of lysine residues, the preferential crosslinking of α-keratose may be explained by this predisposition to extensive crosslinking by the compound.

The crosslinking of the γ-keratose at pH 6 was consistent with the results obtained from the swelling of wool in formic acid,[2] which suggested that crosslinking of the matrix component may have occurred to some extent in wool treated with XLC at pH 6.

These results are in general agreement with the findings of Schepp and Baumann,[8] who carried out similar but more extensive work with an analogous bifunctional vinyl sulphone reagent BDVS.

3.2 The effect of XLC on the yield and composition of wool gelatins

The keratose fractionation procedure was not capable of indicating the reaction of XLC with the β-keratose fraction, since this fraction was insoluble in the first step of the procedure. Baumann[5] has demonstrated that the cell membrane complex can be specifically extracted from wool by a treatment in citrate buffer (pH 2) at 100°C for 1 h. Therefore, reaction of XLC with the cell membrane complex, and its possible stabilising effect, may be studied by determining the yields and composition of this component following the extraction procedure. Soluble wool proteins are generally termed 'wool gelatins' and, using the above extraction procedure, the wool gelatins obtained are specifically from the cell membrane complex.

TABLE 1
Yield and Amino Acid Composition of Wool Gelatins from Wool Treated with XLC at pH 5·0

Amino acid residue	*Blank wool (mol %)*	*Wool gelatin composition (mol %) XLC applied (μmol g^{-1})*					*Yield of amino acid (%) XLC applied (μmol g^{-1})*			
		0	*13*	*34*	*55*	*104*	*13*	*34*	*55*	*104*
Asp	6·32	9·39	9·60	9·31	9·64	8·45	76	41	25	26
Glu	11·45	12·39	15·05	25·32	20·87	24·89	91	85	41	58
Ser	9·57	7·32	5·73	6·04	5·95	6·92	59	34	20	27
Gly	8·48	12·99	13·18	11·87	12·55	12·07	76	38	28	27
His	0·81	1·68	1·42	0·91	1·54	0·62	63	23	22	11
Arg	7·00	7·00	6·29	6·31	7·11	5·56	67	37	24	23
Thr	5·36	4·10	3·22	2·72	3·15	2·96	59	28	18	21
Ala	5·31	5·71	6·09	5·25	6·22	4·75	80	38	26	24
Pro	6·88	7·73	8·15	5·33	7·24	5·15	79	29	23	19
Tyr	3·66	5·71	5·52	5·29	5·07	5·28	72	39	21	27
Val	5·64	5·52	6·20	5·37	5·15	5·78	84	40	22	30
Met	0·40	0·99	1·08	0·30	0·37	1·66	82	13	9	34
1/2 Cys	12·50	0·67	0·52	0·44	0·15	0·40	58	27	5	17
Ile	3·21	3·37	3·58	3·33	3·31	3·04	79	41	24	26
Leu	7·82	7·85	7·94	7·32	6·62	7·22	76	39	20	27
Phe	2·80	3·89	3·47	3·03	3·01	4·42	67	32	19	33
Lys	2·79	3·71	2·97	1·84	2·06	1·33	60	21	13	10
Yield of gelatin (%)		0·69	0·52	0·29	0·17	0·20				
Yield relative to yield of wool gelatin from blank (%)							75	42	24	29

Amino acid concentrations are given in mol %.
Yields of wool gelatin are given as a percentage of the dry weight of the wool.
Yields of amino acids in XLC-treated samples are given as a percentage of the yield of the amino acid in the wool gelatin extracted from blank-treated wool. Wool gelatins were extracted at pH 2 and 100°C for 1 h.

The term wool gelatin has often been used to describe soluble wool proteins extracted during severe chemical processes where large percentage weights of wool were extracted. As such, the composition of these wool gelatins was analogous to whole wool. In the present work the quantity of wool gelatine extracted was small (less than 1%) and, as it represented one specific morphological component (the cell membrane complex), its composition was very different from that of whole wool.

The yields and amino acid composition[3] of the wool gelatins extracted

TABLE 2
Yield and Amino Acid Composition of Wool Gelatins from Wool Treated with XLC at pH 6·0

Amino acid residue	*Blank wool (mol %)*	*Wool gelatin composition (mol %) XLC applied (μmol g^{-1})*					*Yield of amino acid (%) XLC applied (μmol g^{-1})*			
		0	*12*	*38*	*61*	*118*	*12*	*38*	*61*	*118*
Asp	6·26	9·05	10·21	8·00	8·00	5·71	90	46	45	16
Glu	11·81	15·97	18·19	26·23	27·88	39·37	90	84	89	61
Ser	9·96	4·85	4·47	3·74	2·22	2·02	73	39	23	10
Gly	8·21	11·26	10·97	10·53	9·31	9·65	77	48	42	21
His	0·79	2·30	1·74	1·07	0·57	0·00	60	24	13	0
Arg	7·16	7·78	8·57	6·97	5·73	5·64	87	46	38	18
Thr	5·80	2·35	1·88	1·82	0·77	1·35	64	40	17	14
Ala	5·43	6·37	6·42	5·44	5·45	4·14	80	44	44	16
Pro	6·76	8·10	7·33	8·65	9·14	7·63	72	55	57	23
Tyr	3·71	5·65	5·21	5·52	7·30	5·71	73	50	66	25
Val	5·19	5·04	5·10	4·16	4·76	3·09	80	42	48	15
Met	0·42	3·81	3·80	3·37	2·71	2·56	79	45	36	17
1/2 Cys	12·30	0·29	0·25	0·27	0·23	1·53	68	48	40	131
Ile	2·86	3·27	3·07	3·46	4·03	3·40	75	54	63	26
Leu	7·65	7·61	7·20	6·36	7·75	4·99	75	43	52	16
Phe	2·86	3·44	3·16	3·04	3·13	2·47	73	45	46	18
Lys	2·81	2·88	2·42	1·39	1·03	0·74	67	25	18	6
Yield of gelatin (%)		0·70	0·56	0·36	0·36	0·18				
Yield relative to yield of wool gelatin from blank (%)							79	51	51	25

Amino acid concentrations are given in mol %.
Yields of wool gelatin are given as a percentage of the dry weight of the wool.
Yields of amino acids in XLC-treated samples are given as a percentage of the yield of the amino acid in the wool gelatin extracted from blank-treated wool. Wool gelatins were extracted at pH 2 and 100°C for 1 h.

from wool samples that had been treated with XLC at pH 5 and 6 are given in Tables 1 and 2 respectively. These data demonstrated a considerable fall in the yields of soluble wool gelatins on application of XLC. The yields levelled off at the higher concentrations of XLC, and minimum values of 24% at pH 5 and 25% at pH 6 were achieved, relative to the yields of wool gelatin from blank-treated controls. Overall, there was little difference in the yields of wool gelatins for wool samples treated with XLC at pH 5 and 6.

Comparison of the amino acid compositions of the blank-treated whole wools with those of the wool gelatins extracted from them, clearly demonstrated that they had vastly different compositions. The wool gelatins were low sulphur non-keratinous proteins, the latter having been defined by Baumann[9] as being proteins with a cystine content of <3 mol%. They may also be classified as high-glycerine/tyrosine proteins due to their high relative content of these amino acids. The wool gelatins had twice the amount of histidine and a higher content of lysine than whole wool. On this basis they were predisposed to a high degree of reaction with XLC, and this may explain the stabilisation of these proteins as evidenced by their much lower yield on treatment with XLC. The higher aspartic acid content of the wool gelatins probably reflected the mechanism of their formation. This was at least partly due to the hydrolysis of the particularly labile peptide bonds formed by aspartic acid residues[10] which would cause a preferential breakdown and dissolution of these proteins.

The effects of XLC on the amino acid compositions of the wool gelatins extracted are shown in Tables 1 and 2, where the ratios of the amino acids are given in mol % and the absolute yields are given relative to the yields in the wool gelatin derived from blank-treated controls. Examination of the relative amino acid concentrations (mol %) of the wool gelatins extracted showed that the relative concentration of glutamic acid rose dramatically with increasing amounts of XLC applied and a decreasing yield of wool gelatins. The rise in the mol % value of glutamate caused a fall in the mol % values of all the other amino acids. This effect was most noticeable at pH 6, where glutamate represented 39% of the protein extracted from wool treated with 118 μmol XLC g^{-1}. When the absolute concentrations of the amino acids were considered (percentage yield of amino acid), the glutamate concentrations were seen to fall to minimum values of 41% at pH 5 and 61% at pH 6. The reductions in percentage yield for glutamate were, however, not as great as those of the other amino acids, and a rise in the mol % value for glutamate was the result. The effects of XLC on the percentage yield of individual amino acid residues can be divided into four categories:

(i) amino acids that exhibited a consistent fall in percentage yield that reflected the fall in total percentage yield of wool gelatin;
(ii) amino acids that showed a fall in percentage yield moderately greater than the fall in total percentage yield of wool gelatin;
(iii) amino acids that showed a dramatic fall in percentage yield; and
(iv) amino acids whose percentage yield was consistently higher than the percentage yield of wool gelatin.

The majority of amino acids fell into category (i), and these included

Asp, Gly, Arg, Ala, Pro, Tyr, Val, Ile, Leu and Phe. Threonine and serine belonged to category (ii), and lysine and histidine belonged to (iii); glutamic acids alone belonged to (iv).

Two factors that could have contributed to a lower percentage yield of a particular amino acid in the wool gelatin from XLC-treated wool samples were: (a) stabilisation of the proteins from which the amino acid was derived, which would lead to a reduced extraction of these proteins; and (b) reaction of the amino acids residue with XLC, such that the modified amino acid was not detected by amino acid analysis. Stabilisation of the wool gelatins would generally explain the reduction in percentage yield of the amino acids in category (i). A combination of reaction with lysine and histidine residues, and the enhanced stabilisation of wool gelatins containing these residues, would account for category (iii), where the final percentage yields for lysine and histidine were very low: 10 and 6% for lysine at pH 5 and 6; 11 and 0% for histidine at pH 5 and 6, respectively.

The relatively high percentage yields of glutamic acid at pH 5, and particularly at pH 6, for high concentrations of XLC, indicated that wool gelatins rich in this residue or its amide form, glutamine (hydrolysed to glutamic acid on acid hydrolysis), were not stabilised to as great an extent as other proteins deficient in these residues. These high glutamic acid-containing proteins would have represented the major type of protein extracted from wool samples treated with high concentrations of XLC. This would have produced the observed increases in the mol % values of glutamic acid with increasing amounts of XLC applied and increased application pH. Examination of the amino acid composition of the wool gelatins extracted from wool which had been treated at pH 6 with 118 μmol XLC g^{-1} indicated that these proteins had very high contents of glutamic acid, as well as high contents of glycine, proline, aspartic acid, tyrosine and leucine. If aspartic acid and glutamic acid were present mainly as their acid forms, these proteins would have had a high negative charge. This would have discouraged the adsorption of negatively charged XLC onto the protein. This effect, along with their high content of aliphatic and hydrophobic amino acid residues, coupled with their low contents of lysine and histidine, would have resulted in a lower reaction of these proteins with XLC, and hence their extraction would not have been as retarded as the other wool gelatins.

The percentage yields of threonine and serine residues fell by a significantly greater extent than the fall in the percentage yield of wool gelatin. Since amino acid analysis of whole wool indicated that these residues were unreactive towards XLC, the greater fall in percentage yields of these amino acids would indicate that they were present in higher concentrations in the stabilised proteins than the extracted wool gelatins.

Baumann[11] stated in a review that '. . . after the addition of crosslinking agents only the yield of gelatins is changed significantly and there is little change in their amino acid composition'. The results presented in this work would at first appear to be in conflict with this statement; however, the statement is somewhat vague and may be subject to interpretation. It could be interpreted to mean that, as observed in the present work, the absolute concentrations of the majority of the amino acids were unchanged and the exceptions to this were few; lysine and histidine due to reaction with the crosslinker, and glutamic acid due to poor stabilisation of these proteins.

In summary, the results of wool gelatin analysis indicated that the non-keratinous proteins of the cell membrane complex were stabilised to a great extent by the application of XLC at pH 5 and 6.

4 CONCLUSION

By using keratose fractionation of XLC-treated wool it was shown that crosslinking took place preferentially in the microfibrillar region of the wool cortex. From previous amino acid analysis it was clear that lysine residues were responsible for this reaction with XLC. Some small reaction of XLC with the matrix component of wool was evident at higher pH values. Extraction of the cell membrane complex with citrate buffer, followed by amino acid analysis, indicated that the non-keratinous proteins of the cell membrane complex were stabilised to a great extent by XLC application at higher pH values.

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