The formation of disulphides during hydrolysis of proteins containing oxidised thioether groups

ZAHN *et al.*[1] have shown that lanthionine SS-dioxide ("lanthionine sulphone") heated in 6 *N* HCl at 105° C for 16 h gives alanine, pyruvic acid, traces of cystine and "cysteine sulphinic acid", while lanthionine S-oxide, heated in 6 *N* or 12 *N* HCl at 105° C for 12 h gives high yields of cystine plus some cysteic acid, with a little of the sulphinic acid in the case of 12 *N* acid. We have had occasion to measure the cystine content of various wool proteins containing the $-CH_2SCH_2CO_2H$ and $-CH_2SCH_2CONH_2$ side chains, before and after oxidation of the protein with peracetic acid. These sulphide (thioether) groups, formed from cysteine residues by reaction with iodoacetic acid or iodoacetamide, can be oxidised to a sulphoxide and sulphone and are thus comparable to lanthionine which also contains the grouping $-CH_2SCH_2-$. Our results show that on oxidation and hydrolysis of such wools, and also of wools containing lanthionine, the apparent cystine content actually increases. This can only be due to decomposition of the sulphoxide or sulphone during hydrolysis, to yield new disulphide.

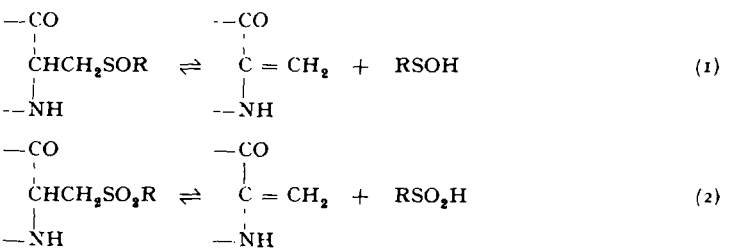
For example, after extraction of Merino 64's wool with alkaline thioglycollate solutions according to GILLESPIE AND LENNOX[2], the insoluble residue was treated with excess of iodoacetic acid at pH 9 for 2–4 h and was then washed thoroughly. After hydrolysis in a 1:1 mixture of 10 *N* HCl: 98% formic acid in a spring-loaded stoppered tube at 110° C for 5 h, the disulphide content calculated as cystine was shown to be 2.6% as determined by amperometric $HgCl_2$ titration[3,4]. After oxidation of a sample of the same residue for 5 h in 1.6% peracetic acid, the "cystine" content rose to 3.4%. It seems likely (see below) that in this case the new disulphide is not cystine itself, but bis(carboxymethyl) disulphide.

With S-carboxymethylkerateine-2, prepared from the wool protein which dissolves in the alkaline thioglycollate[2], the initial cystine content was zero, rose to about 4% in samples oxidized for 2–24 h, and fell again to zero in samples oxidized for 3–4 days. These values were determined after 16 h hydrolysis in HCl: formic acid, both by amperometric $HgCl_2$ titration and by the method of SHINOHARA[5]. The results could well indicate a different mode of breakdown for sulphoxide (short oxidation) and sulphone (long oxidation), as found also for lanthionine[1]. In a sample of wool which had been several times successively reduced with thioglycollic acid at pH 7 and then allowed to react with iodoacetic acid, the figures were: before oxidation, 1.4% "cystine"; after 5 h oxidation, 4.6%; after 24 h oxidation, 1.1%. A similar wool which had been substituted with iodoacetamide gave: before oxidation, 1.0% cystine; after 5 h oxidation, 5.2%.

Finally we treated Merino 64's wool having a cystine content of 12.7% with 0.5% KCN for 3 h at 50° C and obtained a product analysing for 5.2% cystine. In this reaction the cystine which disappears is converted to lanthionine[6]. After treatment with 1.6% peracetic acid, and hydrolysis for 16 h in 5 *N* HCl at 110° C, analysis[5] showed that 69% of this residual cystine was apparently destroyed after 3 h oxidation, and 78% after 24 h oxidation. After similar oxidation of the original wool, containing no lanthionine, the corresponding figures were 84% and 94%. These results suggest that new disulphide is formed during the hydrolysis of wool containing oxidized lanthionine residues.

Dr. H. LINDLEY of this laboratory has studied wools containing $-CH_2SCH_2CH_2SCH_2-$ groups prepared by reduction of the wool followed by cross-linking with ethylene dibromide. He reports[7] that after oxidation with peracetic acid none of the expected disulphone could be detected in hydrolysates. Oxidation and hydrolysis of SS-ethylene bis-cysteine gave rise to cysteic acid, alanine and three other unidentified amino acids.

In these various oxidised thioethers the sulphur atom is in the β relationship to a potentially ionisable hydrogen atom (the α-hydrogen of the amino acid residue). We therefore suggest that decomposition of these oxides occurs by a type of β-elimination (equations 1 and 2), although acid-catalysed elimination reactions must be regarded as exceptional. The analogous decomposition of alkyl sulphones in strong alkali to give olefin and alkyl sulphinic acid is known[8],



while the reverse reaction (addition of sulphinate to olefin to give sulphones) has also been reported[9].

The sulphenic and/or sulphinic acid formed could well undergo disproportionation and other reactions to give thiol, disulphide and sulphonic acid. In the case of lanthionine S-oxides, these three products would be cysteine, cystine and cysteic acid respectively, while S-carboxymethyl cysteine oxides should yield mainly bis(carboxymethyl) disulphide. Pyruvic acid and alanine are known to arise from aminoacrylic (dehydroalanine) residues[10,11]. The β-elimination mechanism explains the instability in the above cases and the contrasting stability of methionine SS-dioxide[12], where the sulphur atom is isolated from the α-hydrogen by an extra carbon atom.

EARLAND AND KNIGHT[13] have shown that after heating wool in cyanide solutions at 66° C or 100° C and then oxidising with peracetic acid, cysteic acid can be detected in hydrolysates. This result was interpreted as showing that unchanged disulphide remains after the cyanide treatment. It is apparent that some at least of this cysteic acid could arise by decomposition of lanthionine oxides, but EARLAND AND KNIGHT's conclusion is not questioned since it was shown already by CUTHBERTSON AND PHILLIPS[6] that some cystine is unaffected by the cyanide. Incidentally it seems to us that the latter workers claimed not that the cystine conversion is complete (as maintained by EARLAND AND KNIGHT) but only that the cystine which does react with cyanide is converted quantitatively to lanthionine. For the estimation of cystine as cysteic acid in the presence of sulphides it would seem best to oxidize after hydrolysis, as prescribed by THOMPSON[14].

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