The oxidation of disulphide groups in proteins

The oxidation of proteins by per-acids has been widely used in structural studies to convert the disulphide groups of cystine residues into two sulphonic acid groups (cysteic acid residues) with subsequent separation of the component peptide chains. However, with performic or peracetic acids under a variety of conditions the oxidation of proteins including bovine serum albumin[1], insulin[2,3], papain[2,4], ribonuclease[5], chymotrypsinogen[6] and wool[7] is usually incomplete. Even with performic acid under optimal conditions[8], small amounts of partially-oxidized products have been isolated from insulin[9].

With incomplete oxidation a whole series of stages intermediate between disulphide and sulphonic acid is possible

$$-S\cdot S- \longrightarrow -SO\cdot S- \longrightarrow -SO_2\cdot S- \longrightarrow \cdots\cdots \longrightarrow 2\text{-}SO_3H$$

Such intermediate oxidation stages have been detected in wool after mild treatment with H_2O_2[10,11] or Cl_2[12]. These products are unstable under the conditions of acid hydrolysis normally used for analytical work on proteins and disproportionate to give cystine, cysteic acid and perhaps other products[10,11,13]. Hence in intact (*i.e.* unhydrolysed) oxidized protein, their existence could easily be overlooked; in addition, analytical results for hydrolysates from such incompletely oxidized proteins would not give the true disulphide and sulphonic acid contents of the unhydrolysed protein.

Recently, one of us has developed an amperometric method for estimating disulphide in intact proteins[14]. This has enabled us to estimate the disulphide contents of oxidized proteins using either the intact protein or its acid hydrolysate and hence for the first time to assess quantitatively the different oxidation states. In hydrolysates disulphide can be estimated by an amperometric method[15] and also by the Shinohara colorimetric method[16] and it will be seen that the results from these two methods are in very good agreement.

Since the action of peracetic acid on wool has been widely studied and conditions can be varied within wide limits we chose this system to test for the presence of partially oxidized disulphide residues. In Table I are listed the disulphide + thiol contents of the wool after various treatments; separate estimations have shown that the thiol content is almost unchanged by hydrolysis or by the ammonia treatment, and hence differences in these values represent changes in disulphide content. From Table I it can be seen that the disulphide contents are in all cases much lower in the intact oxidized wools than in the corresponding hydrolysates. Furthermore, the ammonia treatment causes a marked increase in the disulphide contents of the oxidized wool as measured on the intact protein, but little change in disulphide contents of the hydrolysates. We have also found that under these conditions cysteic acid does not give rise to any cystine.

The results in Table I can be explained by assuming that partially oxidized disulphide residues are present in oxidized wool. The increase in disulphide content after acid hydrolysis then arises from disproportionation of these partially oxidized residues, and evidently ammonia has a similar effect.

There are several currently-used oxidative treatments which are intended specifically to modify groupings other than disulphide, *e.g.* iodination of tyrosine residues, and recent work in this laboratory[17] using the analytical methods outlined above

TABLE I

DISULPHIDE + THIOL CONTENTS (μmoles/g DRY WOOL)*

Conditions of treatment**	Wool			Wool after treatment with ammonia***		
	Intact	Hydrolysed§		Intact	Hydrolysed§	
	A	B	C	A	B	C
Untreated	491	456	444	477	449	420
0.06 *M* peracetic acid for 1 h	105	318	314	236	312	305
0.13 *M* peracetic acid for 1 h	47	192	189	114	172	174
0.25 *M* peracetic acid for 2 h	22	82	77	56	83	76
0.25 *M* peracetic acid for 24 h	11	37	36	38	48	34

A: by the method of LEACH[14].
B: by the method of SHINOHARA[16].
C: by the method of STRICKS, KOLTHOFF AND TANAKA[15].

* Each figure the mean of at least two estimations. The wool used was Merino 64's.
** Oxidation was carried out at room temperature using the ratio of 50 ml solution to 1 g wool.
*** Wool was immersed in 3 *N* NH_4OH (in the ratio of 20 ml solution to 1 g) at room temperature for 16 h. The whole product was dried in vacuo, and in the case of B and C, then hydrolysed as in (§).
§ Hydrolysis was carried out with 1:1 mixture of formic acid, conc. HCl (in the ratio 20 ml mixture to 1 g wool) in a closed tube at 105° for 5 h.

has shown that the "specificity" of these treatments is illusory since partially-oxidized disulphide residues are formed which on hydrolysis are partly reconverted to cystine.

It should be borne in mind when interpreting cystine or cysteic acid analyses obtained from protein hydrolysates that partially oxidized disulphide residues can be formed in large amounts under various conditions of oxidation.

J. A. MACLAREN
S. J. LEACH
I. J. O'DONNELL

Division of Protein Chemistry (Formerly Biochemistry Unit), C.S.I.R.O. Wool Research Laboratories, Parkville N. 2, Melbourne, Victoria (Australia)

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