STUDIES ON REDUCED WOOL

I. THE EXTENT OF REDUCTION OF WOOL WITH INCREASING CONCENTRATIONS OF THIOL, AND THE EXTRACTION OF PROTEINS FROM REDUCED AND ALKYLATED WOOL

By E. O. P. THOMPSON* and I. J. O'DONNELL*

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Summary

The reduction of wool with mercaptoethanol and thioglycollate at pH 5 has been compared by measuring the disulphide contents of the reduced wool after alkylation of the thiol groups with iodoacetate at pH 8·5–9. Mercaptoethanol and thioglycollate are very similar in their extent of reduction of wool at low concentrations of thiol but the neutral thiol is more effective as a reducing agent at high concentrations. In contrast with the earlier findings of Patterson *et al.* (1941) the reduction was driven towards completion with increasing concentrations of thiol as would normally be expected for a reversible reaction. With 4M mercaptoethanol 4% of the disulphide bonds were intact in the reduced wool but the extracted proteins contained no disulphide groups. The limit of extractability of reduced wool prepared in this way, and subsequently alkylated with iodoacetate, was about 75%.

I. INTRODUCTION

In the early studies on the reduction of the disulphide bonds in wool (Goddard and Michaelis 1935; Patterson *et al.* 1941), thioglycollate solutions at various pH values were used. Although reduction occurs at all pH values, Patterson *et al.* showed that there was a marked increase in the level of reduction at pH values above 6. At pH 4·5 and at 35°C increase of the thiol concentration from 0·5 to 2M gave little or no increase in the extent of reduction. The use of thioglycollate solutions at pH values of 10·5–12·3 (Goddard and Michaelis 1935) for the extraction of wool proteins has been extensively studied in these Laboratories (Gillespie and Lennox 1955; Gillespie 1960). At pH values near 2, Savige (1960) has shown that thiols in the absence of salts can extract considerable amounts of protein and at the elevated temperatures used some lanthionine was formed. Since in general the rate of lanthionine formation increases markedly with both pH and temperature (Cuthbertson and Phillips 1945; Zahn, Kunitz, and Hildebrand 1960), the use of high pH values at elevated temperatures for the reduction and simultaneous extraction of proteins of wool is, from this point of view, less satisfactory than reduction at neutral pH values at room temperature followed by extraction of reduced proteins. At pH values of 11 (or higher) at room temperature we have found that there is an increase in ninhydrin colour after 24 hr with reduced and alkylated proteins (O'Donnell and Thompson 1962) which could be indicative of peptide bond hydrolysis.

* Division of Protein Chemistry, C.S.I.R.O. Wool Research Laboratories, Parkville, Vic.

An alternative method of reducing the disulphide bonds of wool, at a pH value where splitting of peptide bonds, lanthionine formation, and racemization are less likely, would be very useful. This paper describes experiments carried out with mercaptoethanol at pH 5 to test its effectiveness as a reducing agent. Almost quantitative reduction was achieved at high concentrations of mercaptoethanol. Some comparative experiments with sodium thioglycollate are also reported. Mercaptoethanol has previously been used for the reduction of the disulphide bonds in wool by Jones and Mecham (1943), Schöberl (1960), and Ward and co-workers (cf. Bartulovitch, Tomimatsu, and Ward 1960). An alternative method of achieving almost complete reduction of the disulphide bonds in wool has been developed by Maclaren (1962). In this method 0·1M benzyl mercaptan is used in aqueous alcohol solutions where the fibres are swollen by the solvents.

Having achieved complete, or almost complete, reduction of the cystine in wool at pH values near or below neutrality, the thiol groups formed can be stabilized by alkylation with reagents such as iodoacetate. In view of the side reactions between iodoacetate and proteins that have been reported (Gundlach, Stein, and Moore 1959) it is important that the time of alkylation should be minimized and the excess iodoacetate removed or destroyed. A study has therefore been made of the rate of alkylation and extraction of protein material from the reduced wool.

II. Materials

Merino 64's wool (MW127) in the form of solvent-degreased top was used. This wool was similar to that used previously (Thompson and O'Donnell 1959). Some experiments were carried out with a single fleece of Merino 64's wool (MW118). Disulphide content of MW127 estimated by the method of Leach (1960) was 486 μmoles/g while the –SH content was 30 μmoles/g.

Thioglycollic acid and mercaptoethanol were purified by fractional distillation under vacuum. A nitrogen bleed was used. Schöberl (1948) and White (1960) have drawn attention to the formation of thioglycollides in thioglycollic acid. These were removed by the distillation and the purified thioglycollic acid was stored in a deep freeze.

III. Experimental

(*a*) *Reduction of Wool at pH* 5

Samples of air-dry wool were treated with a solution (liquor : wool ratio 100 : 1) of mercaptoethanol or thioglycollate (adjusted to pH 5 with potassium hydroxide) in deoxygenated water containing 0·02M acetate buffer at pH 5 and 0·001M "Versene" (disodium salt). The wool was wet by repeated evacuations at the water-pump and the vacuum released under nitrogen. The reduction mixtures were allowed to stand for various times before alkylation. An all-glass apparatus designed and constructed by Dr. J. P. E. Human for rapidly recycling (at the rate of 200 ml/min) the reducing medium (40 ml) through the wool (0·4 g), which was held on a sintered plate in an atmosphere of nitrogen, did not increase the rate of reduction.

(*b*) *Alkylation of Reduced Wool Samples*

(i) *Analytical Studies.*—In the early experiments the circulating apparatus described above was found useful for studying the rate of alkylation of the thiol groups. The reaction chamber could be coupled with a pH-stat and recorder to maintain the pH at any fixed value. With high levels of reduction, alkylation in the absence of additional salt was found to cause solution of the carboxymethylated wool protein, thus reducing the rate of flow through the sintered disk. The apparatus was most useful when studying levels of reduction of up to 75%.

In later analytical experiments the alkylation was carried out in a flask in the presence of salt to repress the solubility of the reduced and alkylated wool. After reduction of the wool (0·2 g) it was washed under nitrogen with 0·001N hydrochloric acid (made up in deoxygenated water) and transferred to 50 ml deoxygenated water containing 0·5 g iodoacetic acid and 1 g borax ($Na_2B_4O_7.10H_2O$) adjusted to pH 8·7 with potassium hydroxide (cf. Gillespie and Springell 1961). Alkylation was allowed to proceed overnight. The samples were then washed with 0·5M sodium acetate buffer at pH 5 to prevent solution of the wool (which can occur with water washing) and remove excess alkylating agent. Finally the samples were washed with water and air-dried before being analysed for residual thiol plus disulphide by the method of Leach (1960).

(ii) *Extraction of Soluble Proteins.*—In order to obtain maximum extraction of wool proteins during the alkylation of reduced wool with iodoacetate it is desirable to have a very low salt concentration (for a summary of the effect of salt on extraction of proteins from wool see Gillespie *et al.* 1960). Hence alkylation for this purpose was carried out under nitrogen at a range of pH values. A pH-stat and a modified Waring Blendor were used. The bottom stirring given by the Waring Blendor used was preferable to that given by an ordinary mechanical stirrer as the swollen wool fibres tended to wind up and around the stirring shaft. The speed of the Waring Blendor, which was fitted with a single horizontal blade, was controlled by a voltage regulator, and the lid of the Blendor was modified to support a combined glass electrode, a nitrogen supply lead, and an alkali lead. A hole was also made in the lid for the addition of samples.

Alternatively, aqueous ammonia was used to keep the pH at approximately 10·5. In all these alkylations 0·001M "Versene" (disodium salt) was present to inhibit the catalytic effects of metals on the reoxidation of thiols in alkaline solution. Usually 1–2·5 g iodoacetic acid was used per gram of dry wool and, after alkylation was complete (negative nitroprusside test on the fibres), 1 ml mercaptoethanol was added to destroy the excess iodoacetate, the pH being maintained at 9 or higher during this reaction.

(*c*) *Removal of Reagents from Reduced Wool before Alkylation to Extract Soluble Proteins*

Since the thiol remaining in the reduced wool is ionized when titrated to the pH of alkylation, several methods were used to reduce the reagent thiol to a low level before alkylation: (i) the mixture was filtered under nitrogen and washed

with 0·001M hydrochloric acid made up in deoxygenated water; (ii) the reaction mixture from 1 g wool was poured into 300 ml deoxygenated water containing 40 ml 4N trichloroacetic acid, centrifuged, and the precipitate washed once with 0·4N trichloroacetic acid; (iii) the reaction mixture from 1 g wool was poured into 100 ml of either 0·8N trichloroacetic acid or other acid (phosphoric acid, sulphuric acid, hydrochloric acid, or citric acid) contained in Visking 36/32 cellulose tubing and dialysed at 2°C on a rotating dialyser against several changes (at least three, each of 2 l.) of distilled water over a period of 48 hr; (iv) the reaction mixture from 1 g wool was poured into 100 ml deoxygenated water and dialysed at 2°C against 2 l. distilled water containing 20 ml 4N trichloroacetic acid or 4N sulphuric acid. After this first dialysis on the rotating dialyser against acid, the bag and contents were dialysed against several changes of distilled water to remove as much dialysable material as possible.

(*d*) *Recovery of Soluble Proteins from Reduced and Alkylated Wool*

Following alkylation of the reduced wools the excess reagents were removed by dialysis at 2°C on a rotating dialyser. The insoluble material was removed by centrifugation or filtration. If the suspension is first made 0·5M with respect to potassium chloride these processes are facilitated.

Separation into high-sulphur (SCMKB) and low-sulphur fractions (SCMKA) was carried out by repeated precipitation with 5N acetic acid at pH 4·4 in the presence of 0·5M KCl (Gillespie, O'Donnell, and Thompson 1962; O'Donnell, Thompson, and Inglis 1962).

(*e*) *Recommended Procedure for the Maximum Extraction of Protein from Reduced and Alkylated Wool*

Although higher concentrations give greater levels of reduction, the majority of our experiments have been carried out using 4M mercaptoethanol. The level of reduction of the wool cystine is approximately 96% of the whole wool. For the reduction of 1 g dry wool it is stood under nitrogen with 100 ml solution made up of 67 ml deoxygenated water, 28 ml freshly redistilled mercaptoethanol, 4 ml 0·5M sodium acetate–hydrochloric acid buffer at pH 5, and 1 ml 0·1M "Versene" (disodium salt). The wool is wetted by repeated evacuation at the water-pump followed by release of the vacuum under nitrogen. After 24 hr the excess reagents are removed in either of two ways: either (1) the mixture is diluted with 300 ml deoxygenated water and precipitated with 40 ml 4N trichloroacetic acid, centrifuged, and the precipitate washed once with 0·4N trichloroacetic acid (Mirsky and Anson 1936); or (2) the mixture is poured into 100 ml 0·8N sulphuric or trichloroacetic acids in 36/32 Visking cellulose dialysis tubing. It is then dialysed against successive changes of water at 2°C on a rotating dialyser for 24–48 hr.

For alkylation the reduced wool in 150–200 ml 0·001M "Versene" solution (deoxygenated water) is stirred under nitrogen by hand with a glass rod in the modified Waring Blendor. 10–50 ml of iodoacetate solution (2·5 g iodoacetic acid dissolved in oxygen-free water and adjusted to pH 9 with approximately

2·5 ml 4·5M potassium hydroxide) is added and the pH rapidly taken to 11 with concentrated potassium hydroxide. The hand-stirring is continued for 1 min before switching on the Blendor at low speed (one drop of sec-octyl alcohol is added to prevent frothing). This hand-stirring allows the reduced wool to swell and minimizes winding of the fibres round the paddle. Stirring is continued for 4 min more during which time the fibres disintegrate. Mercaptoethanol (1 ml) is then added, the pH being maintained at 9 until alkali consumption ceases (approximately 5 min). The solution is then dialysed free of reagents against 0·001M borax solution.

In the absence of a pH-stat, ammonia (0·3–2N) can be used. To the suspension of reduced wool (200 ml) in a flask which had little free air space was added the neutralized iodoacetic acid followed by concentrated ammonia. After 5 min 1 ml of mercaptoethanol is added and the mixture dialysed as above at 2°C. When using ammonia there is no danger of overshoot in pH as may happen when the pH-stat is used and if the hand-stirring is inefficient. However, the pH is maintained at 10·5 for longer periods than is the case when using the pH-stat. This high pH may not be deleterious since we have found with oxidized wool proteins (O'Donnell and Thompson 1962) that pH 10·5 causes dissociation of high-sulphur proteins held by secondary forces. The percentage extraction of the wool is the same in both procedures.

Variations in these alkylating conditions have shown that the extractions are slightly higher (an extra 3%) if the reduced proteins are stood with ammonia (where the ionic strength is lower) for 30 min before the alkylating agent is added. However, as pointed out earlier, there is more risk of reoxidation of disulphide bonds under these conditions.

(*f*) *Amino Acid Analysis*

The amino acid analyses were performed with a Spinco amino acid analyser which was operated under the conditions described by Spackman, Stein, and Moore (1958). Hydrolysates were prepared by refluxing with 6N HCl for 24 hr as previously described (Thompson and O'Donnell 1962).

(*g*) *Chromatography of SCMKA on DEAE-cellulose*

Chromatography with buffers containing 8M urea was carried out under exactly the same conditions as described previously for α-keratose (O'Donnell and Thompson 1961). The SCMKA was prepared from reduced wool (single fleece MW118) freed of excess mercaptoethanol by precipitation with trichloroacetic acid (see Section III (*e*)) and fractionated into high- and low-sulphur fractions by repeated precipitation at pH 4·0 in the presence of 0·3M potassium chloride.

IV. Results

(*a*) *Comparison of Mercaptoethanol and Potassium Thioglycollate*

Table 1 summarizes experiments in which mercaptoethanol and potassium thioglycollate were used as reductants. In general the results show that mercapto-

ethanol is a more effective reducing agent than potassium thioglycollate. With potassium thioglycollate, although the experiments were not all carried out in parallel, it appears that equilibrium is reached more slowly than would be expected from the findings of Patterson *et al.* (1941). The differences between the two thiols were most marked at high concentrations where the ionized carboxyl group of potassium thioglycollate gives the solution a higher ionic strength than is the case with mercaptoethanol.

TABLE 1

PERCENTAGE REDUCTION OF WOOL AT pH 5 BY THIOGLYCOLLATE AND MERCAPTOETHANOL AT VARIOUS CONCENTRATIONS

All reductions carried out either at 20°C or at room temperature

Thioglycollate or Mercaptoethanol Concentration (M)	Time of Reaction (hr)	Residual –S–S– + –SH (μmoles/g)	Reduction* (%)
Thioglycollate			
0·5	4 (room temp.)	291	40
0·5	24 (room temp.)	212	56
0·5	40 (room temp.)	169	65
0·5	98	114	76
4	24 (room temp.)	74	85
Mercaptoethanol			
0·5	4 (room temp.)	291	40
0·5	24	123	75
1	24	93	81
2	24	60	88
3	24	40	92
4	4	50	90
4	8	35	93
4	24	27	94†
4	48	27	94
4	72	23	95

* –SH contents were not usually determined. The percentage reduction will be greater if allowance is made for the –SH content and loss of weight of fully reduced protein during reduction and alkylation. The wool had a disulphide content of 486 μmoles/g while the thiol content was 30 μmoles/g.

† –SH content 8 μmoles/g. When this value is subtracted the reduction is 96% and will be higher if allowance is made for the 15% loss in weight during reduction and alkylation.

(*b*) *Loss of Weight of Wool during Reduction*

On reduction with 4M mercaptoethanol some of the wool dissolves in the reducing medium and is lost if it is filtered and washed with 0·001M hydrochloric acid. In a 24-hr reduction with 4M mercaptoethanol, 6% of the wool is lost; after 72 hr the corresponding value was 11%. The solubility is probably higher with 5M or 6M mercaptoethanol.

(c) *Effect of Increasing Thiol Concentration*

In Table 1 it is seen that as the concentration of mercaptoethanol increases so also does the level of reduction of the disulphide bonds in wool. The extent of solution of the alkylated protein also increases. There is no discontinuity evident in the values of percentage reduction.

(d) *Rate of Alkylation of Reduced Wool*

Alkylations in the circulating apparatus (for wool reduced up to 50%) were studied both in the presence of buffer salts and in the presence of as low a concentration of salts as possible. It was found that with a concentrated buffer (1M Tris at pH 8·5 and containing 2 g iodoacetic acid per g wool) the nitroprusside test was negative in the circulating liquor after 40 min but that water-washing of the fibres gave a solution with a strongly positive test for thiol particularly on the second washing, when most of the iodoacetate had been removed. When the partially reduced wool was carboxymethylated in a closed flask at pH 9·0 in a solution of borax the alkylation was also slow to reach completion as judged by a nitroprusside test on the fibres, which was strongly positive after 3 hr and still faintly positive after 7·5 hr. This latter method of alkylation was suitable for analytical studies since the reduced and alkylated wool was less soluble than in the absence of added salt. However, with high levels of reduction considerable solution of the protein still took place. Thus by taking into account the 6% increase in weight expected for theoretical reduction and alkylation, the loss in weight for samples reduced in 4M, 5M, and 6M mercaptoethanol were 15, 24, and 25% respectively.

If a pH-stat was used to maintain the pH in the range 8·5–9 and 1·25 g iodoacetic acid was used per gram of wool the alkylation reaction was almost complete in 30 min (nitroprusside test on the fibres). However, the reaction was complete in 3 min when maintained at pH 11 with the pH-stat or with 0·3–1N ammonia.

(e) *Effect of Treatment with Acid on Extractability of Reduced Wool*

It was found that the amount of protein extracted from reduced wool alkylated at pH 10·5–11 was greater (70% *v.* 55% of the wool nitrogen) when the excess reducing agent was removed by treatment with trichloroacetic acid than when washing with 0·001N HCl was used. That this was a general effect of acid was shown by the close similarity in behaviour when other acids were substituted for trichloroacetic acid during dialysis for removal of excess reagent. Furthermore, the effect of acid was much the same whether the added acid was initially placed inside or outside the dialysis bag.

(f) *Amino Acid Analysis of SCMKA Fraction*

Table 2 shows the amino acid content of the low-sulphur protein fraction isolated from reduced and alkylated wool (MW118). The analysis is very similar to those of α-keratose and (α—X)-keratose (O'Donnell and Thompson 1962), with the exception of serine which is considerably lower. The reason for this is not known

but it may be due to destruction during hydrolysis since the overall recovery of nitrogen is also lower than usual (94%). Both the isolated wool protein and reduced and alkylated bovine plasma albumin (Thompson and O'Donnell 1961), when analysed by the Spackman, Moore, and Stein (1958) method, gave no evidence of unknown peaks which would indicate unwanted side reactions during the reduction with the high concentration of mercaptoethanol or the alkylation for a short time at pH 11.

The reduced bovine plasma albumin gave 33 moles thiol groups per mole when analysed by the method of Tsao and Bailey (1953) after washing free of reagent with seven washes of trichloroacetic acid (Mirsky and Anson 1936) and

TABLE 2

AMINO ACID COMPOSITION OF SCMKA*

Amino Acid	Amino Acid Nitrogen (as % total N)	Amino Acid	Amino Acid Nitrogen (as % total N)
Lysine	5·44	Alanine	4·32
Histidine	1·33	Valine	3·98
Ammonia	8·54	Methionine	0·37
Arginine	19·53	Isoleucine	2·46
Aspartic acid	5·46	Leucine	6·89
Threonine†	2·95	Tyrosine	2·88
Serine†	4·90	Phenylalanine	2·02
Glutamic acid	9·49	*S*-Carboxymethyl cysteine	4·55
Proline	2·85	Cystine	Nil
Glycine	5·91	Unknown‡	0·53
Total			94·4

* Prepared from a single fleece, MW118.

† Uncorrected for decomposition.

‡ This is the unidentified peak near cystine present in wool hydrolysates (O'Donnell and Thompson 1962).

dissolving in 8M urea. After alkylation, amino acid analysis showed the *S*-carboxymethyl content was 34 residues. These values are within experimental error of the known cystine plus cysteine content calculated from the sulphur content.

Amino acid analysis of both reduced and carboxymethylated proteins showed that no trace of cystine was present and there was no evidence to suggest that decomposition of *S*-carboxymethyl cysteine had occurred. This was in agreement with the findings of Harrup and Woods (unpublished data) with reduced and carboxymethylated bovine plasma albumin (cf. Cole, Stein, and Moore 1958).

(*g*) *Chromatography of Low-sulphur Wool Protein* (*SCMKA*)

Figure 1 shows that both the gradient-elution and the stepwise-elution behaviour of SCMKA on DEAE-cellulose in 8M urea–Tris buffer at pH 7·4 was similar to that previously reported for other low-sulphur wool proteins (O'Donnell and Thompson 1961). It is apparent that the degree of heterogeneity exhibited by the reduced and alkylated protein under these conditions is similar to those previously reported. More of the reduced and alkylated proteins are eluted at low salt concentrations.

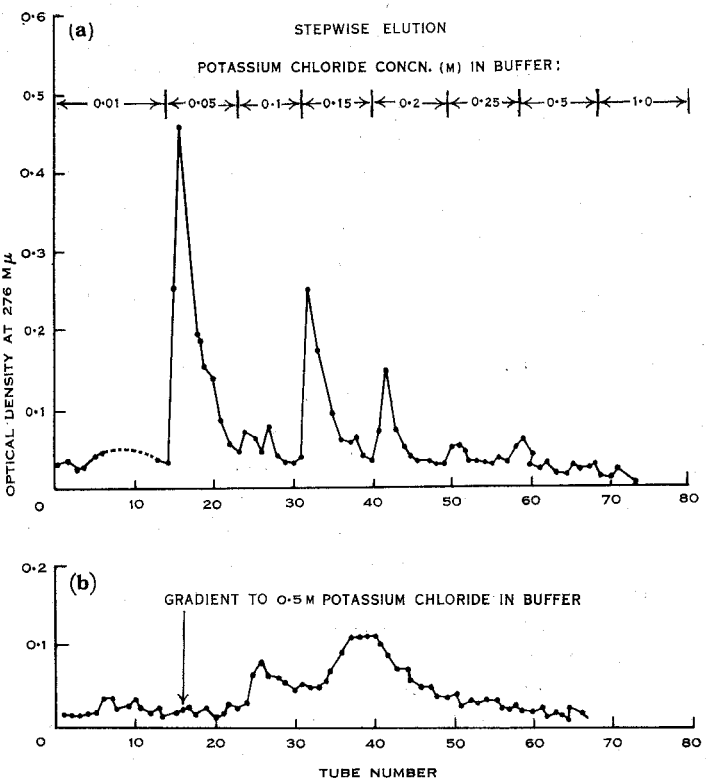

Fig. 1.—Chromatographic behaviour at 25°C of *S*-carboxymethyl kerateine A (SCMKA) in 8M urea–Tris buffer at pH 7·4. Fraction size was approximately 1·1 ml. (*a*) Stepwise elution with increasing concentrations of potassium chloride in the buffer; (*b*) gradient elution to 0·5M potassium chloride in buffer.

V. Discussion

Patterson *et al.* (1941) made an extensive study of the variables pH, temperature, concentration of reagent, and time of reaction on the reduction of the disulphide bonds of wool by sodium thioglycollate. Our results do not support their conclusion that concentrations of thioglycollate above 1M are ineffective in

producing greater reduction of the disulphide bonds of wool. With 4M thioglycollate at pH 5, 85% of the bonds are reduced, whereas with the same concentration of mercaptoethanol almost complete reduction (Table 1) can be achieved. The increased effectiveness of mercaptoethanol is probably due to the fact that at pH 5 it does not contribute to the ionic strength of the medium. The evidence from alkylation experiments suggests that salt impedes access of reagents to the thiol groups in reduced wool.

The effectiveness of high concentrations of thiol in bringing about almost complete reduction of the disulphide bonds in wool is probably due to a combination of a mass action effect (cf. Leach and O'Donnell 1961) and the thiol acting as an organic solvent in promoting conformation changes in the wool proteins. As the concentration of mercaptoethanol is increased from 0·5M, where reduction of the disulphide bonds of wool is approximately 75%, to 4M, where reduction is approximately 96%, there is no marked discontinuity in the extent of reduction *v.* reagent concentration curve. Therefore it is concluded that there is no sharp point at which an onset of denaturation (or supercontraction) of the wool proteins occur. This is in agreement with the observations of Maclaren (1962) who has achieved high extents of reduction with low concentrations of thiols in aqueous alcohols. Mercaptoethanol was less effective in his system than benzylmercaptan.

The method we have described for almost quantitative reduction of the disulphide bonds of wool has also been shown to quantitatively reduce the disulphide bonds of insulin, lysozyme, and bovine plasma albumin (Thompson and O'Donnell 1961).

It has been shown recently (Zahn, Kunitz, and Hildebrand 1960) that lanthionine formation results from a direct nucleophilic attack of the ionized cysteinyl residue on a –C–SS– bond. Thus it might be expected that at pH 5, where the ionization of thiols is extremely low compared with almost complete ionization at pH values above 10·5, there should be much less likelihood of lanthionine formation during the reduction of the wool at the lower pH values. That it is still possible, however, is shown by the results of Savige (1960) and Zahn, Kunitz, and Hildebrand (1960). Paper chromatographic examination (Thompson 1956) of hydrolysates of the SCMKA fraction showed that some lanthionine was present but in much smaller amounts than was present in the original wool.

Although we succeeded in our aim of achieving almost complete reduction of the disulphide bonds of wool under conditions of pH and temperature which were less likely to result in lanthionine formation (cf. Maclaren 1962), it was not possible to extract the reduced wool proteins in large amount either before or after a short time of alkylation at pH values of 9 or less. At pH 8·5 the completion of alkylation is slow particularly in the presence of salt. The addition of urea did not reduce the time of alkylation to below 2 hr (cf. O'Donnell and Thompson 1962).

In the absence of reagents such as urea or detergent the wool proteins in the –SH form are only extractable at pH values where ionization of the thiol groups occurs (Goddard and Michaelis 1935; Gillespie and Lennox 1955). By

extraction at pH values of 10·5–11 of the wool reduced at pH 5 it is possible to extract the protein in the thiol form and subsequently this can be rapidly alkylated. Using these conditions, which are analogous to those used by Gillespie and Lennox, somewhat higher extractions are obtained (75%) than those reported by these workers (65%). However, they used liquor : wool ratios of only 30 : 1 at 50°C.

At pH values of 10·5–11, at which the ionization of tyrosine phenolic and thiol groups combine with the repression of ionization of amino groups to induce repulsive effects between negatively charged molecules within the fibre, and at which the rate of reaction between thiol groups and iodoacetate is extremely fast, the alkylation of the reduced wool is complete in less than 3 min. The alkylation is accompanied by disintegration of the fibres, particularly when beaten in a Waring Blendor, and the extent of extraction of the wool reaches 70–75% by weight, almost the same limit as when the –SH protein is extracted first.

The reason for the lower extractability of reduced wool at pH 10·5–11 compared with oxidized wool (O'Donnell and Thompson 1962) is not known. In the case of reduction the value is possibly limited by: (1) unavoidable presence of some salt (iodoacetate); (2) the small amount of residual disulphide (20 μmoles of disulphide per gram of protein (i.e. 96% reduction) is sufficient to cross-link two molecules of molecular weight 25,000); (3) any lanthionine formed. The possibility of some peptide-bond hydrolysis during performic acid oxidation cannot be excluded although this has not occurred with other proteins (for summary see Thompson and O'Donnell 1959).

It is of interest that high extractions of reduced wool were obtained after treatment with acid. This is interpreted as being due to a contribution (additional to that occurring when the fibre is initially at pH 5) of the bound acid and unionized carboxyl groups to the osmotic bursting of the fibres when the pH is taken suddenly from pH 2 to 11. Bond-splitting by the acids is a less likely explanation since the acid can be dialysed into the reduced protein at low temperature to give the same effect as precipitation with trichloroacetic acid or other acids.

VI. Acknowledgment

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VII. References

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