EXTRACTION OF REDUCED WOOL PROTEINS BY SOLUTIONS OF SALTS*

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Introduction

It has been shown earlier that the disulphide bonds in wool fibres are reduced specifically and almost quantitatively in aqueous solution using toluene-ω-thiol (Maclaren 1962) or tributyl phosphine (Sweetman and Maclaren 1966; Maclaren, Kilpatrick, and Kirkpatrick 1968). The next step was to find a suitable solvent system to extract the proteins from the reduced fibre.

These reductions can be carried out at room temperature and neutral pH and this is an advantage because these conditions minimize the side-reactions such as hydrolysis of amide and peptide bonds, and lanthionine formation. Hence it was desirable to use similar conditions for the extraction step.

Materials and Methods

The wool used was solvent-scoured Merino top 64's quality (MW129).

(i) *Extraction Technique.*—In extracts containing iodide it was not possible to measure the protein concentration by ultraviolet light absorption at 276 mμ, or by Kjeldahl nitrogen determination because of interference by the high concentration of iodide. Although sodium iodide was readily removed by dialysis, and this in fact was done for the preparative scale experiments, it was more convenient to measure the extent of extraction indirectly by weighing the undissolved wool.

Various shaking methods, such as the wrist-action shaker, the reciprocating box shaker, and the gyrotory table shaker, were tried and all gave identical results in the extraction experiments. In some preliminary experiments it was established that the extent of protein extraction was identical whether the wool was first reduced and then immersed in the solvent, or alternatively treated with the reducing agent dispersed in the solvent. This latter procedure, which is more convenient and avoids any adventitious re-oxidation, was used in all further experiments.

(ii) *Solubility Determination.*—Wool (250 mg) was treated with the extracting solvent (25 ml). Tributyl phosphine (0·06 ml, 250 μmoles) *or* toluene-ω-thiol (0·29 ml, 2·5 m-moles) was added and the solution was shaken gently in a stoppered flask at 18–20°C for 24 hr. After filtration through a tared sintered-glass Buchner funnel (porosity No. 2) the residue was washed with water (4×100 ml) and acetone (2×25 ml) and dried to constant weight. With sodium iodide (5M) in aqueous propan-1-ol (25% v/v) as solvent, the weight loss for the wool (MW129) was $71 \pm 0{\cdot}3\%$.

(iii) *Preparative Extraction.*—This has been adapted from the method of Harrap and Gillespie (1963). After extraction as described in the previous section, the reaction mixture was filtered as before and the residue was washed with oxygen-free water (4×10 ml). The combined filtrate and washings were stirred under a nitrogen atmosphere, iodoacetic acid (0·47 g) and boric acid (0·31 g) were added, and the solution was kept at pH 8·0 by addition of sodium hydroxide (1N). After 15 min, sodium sulphite (1·25 g) was added and the solution was again readjusted to pH 8·0. The solution was dialysed (Visking cellulose tubing 18/32) overnight against running tap water and then dialysed at 4°C against pH 9 buffer (0·01M borate). At this

* Manuscript received November 18, 1968.

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stage an aliquot was removed, diluted with an equal volume of glacial acetic acid, and the total protein content was estimated by the absorption at 276 mμ assuming $A^{1\%}_{1cm}$ at 276 mμ in 50% acetic acid = 7·5. (This value is somewhat arbitrary as the ratio of high-sulphur and low-sulphur proteins in the extract is variable.)

The remaining extract was made 0·5M in potassium chloride and then, with gentle stirring, acetic acid (25% v/v) was added to pH 4·4. The solution was filtered (Whatman No. 54) and the high-sulphur protein content of the filtrate was estimated as above assuming the value $A^{1\%}_{1cm}$ at 276 mμ in 50% acetic acid = 5·5.

Results and Discussion

In preliminary experiments wool was treated with reductant (either toluene-ω-thiol or tributyl phosphine) in alkaline buffer solutions (0·2M sodium borate, pH 8; 0·2M carbonate, pH 10; 0·15M phosphate, pH 12; 0·1M hydroxide, pH 12·8). To achieve an effective extraction (greater than 50%) it was necessary to use strongly alkaline solutions (pH *c.* 11) and here the results were probably complicated by formation of lanthionine cross links. These extraction conditions are not compatible with the stated intention of carrying out the whole process at near neutral pH (see Introduction).

Other solvents known to be effective for proteins were then tried; thus reduction in 8M urea solutions using toluene-ω-thiol or tributyl phosphine gave 50–60% extraction, depending on slight variations in the experimental conditions. Formic acid gave a maximum extraction of 26%, whether it was used with the reducing agent or used subsequently to extract the reduced wool.

Some neutral salts have a marked effect on the conformation of proteins both in solution (see reviews by von Hippel and Wong 1964; Berendson and Migchelsen 1965) and in the wool fibre (see review by Crewther *et al.* 1965). Aqueous alcoholic salt systems have been used as solvents for keratin, especially feather keratin (Lundgren *et al.* 1948). The solvent properties of neutral salt solutions for reduced wool fibres have therefore been studied.

Preliminary experiments showed that reduction of wool in the presence of high concentrations of certain salts caused marked extraction of the proteins, but *only* in partly alcoholic solutions (either ethanol or propan-1-ol). The effective salts were lithium bromide, lithium iodide, or sodium iodide, whereas lithium chloride or sodium chloride were ineffective. This solvent property of these salt solutions approximately parallels their effectiveness in the supercontraction of wool keratin fibres (Crewther and Dowling 1956). However, we find that supercontraction alone is not sufficient to bring about marked dispersion of the reduced fibres.

In further experiments the effect of varying the concentration of components in the sodium iodide–water–propan-1-ol system on the solubility of reduced wool was studied (Figs. 1 and 2). Reduction with either tributyl phosphine or toluene-ω-thiol gives identical results in Figure 1, and essentially similar results in Figure 2. Clearly these solubility curves represent a property of the reduced keratin fibre and are independent of the nature of the reduction process. Further, within the limits of experimental error, identical results are obtained when sodium iodide (5M) is replaced by lithium bromide (5M), or when propan-1-ol (25% v/v) is replaced by ethanol (25% v/v). Hence this solvent property is a general one, associated with aqueous alcohol–salt systems. For routine extraction of reduced wools we have

adopted sodium iodide (5M) in aqueous propan-1-ol (25% v/v), which gives maximum extraction. This "standard" solution has pH 7·9 (measured with a glass electrode) and addition of alkali or acid to vary the pH in the range 5·0–10·2 caused no change in the extent of extraction.

Fig. 1

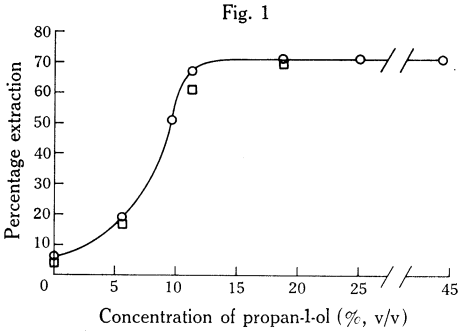

Fig. 2

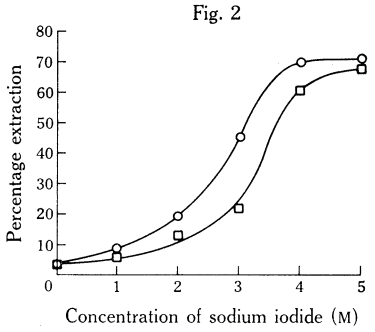

Fig. 1.—Effect of propan-1-ol concentration on the extraction of wool by sodium iodide (5M) using tributylphosphine (○) or toluene-ω-thiol (□) as reductants.

Fig. 2.—Effect of sodium iodide concentration on the extraction of wool by propan-1-ol (25% v/v) using tributylphosphine (○) or toluene-ω-thiol (□) as reductants.

This extraction technique has also been applied on a preparative scale, the extracted protein being then *S*-carboxymethylated with iodoacetate and separated into "low-sulphur" (SCMKA) and "high-sulphur" (SCMKB) fractions by the usual fractional precipitation technique (Gillespie, O'Donnell, and Thompson 1962). In order to explore the possibility of some selective extraction of particular components from the wool fibre, preparative runs have been made using different propan-1-ol concentrations in sodium iodide (5M) and different sodium iodide concentrations in aqueous propan-1-ol (25% v/v). The results in Table 1 show that at low levels of extraction the high-sulphur fraction is removed preferentially, and this is consistent with earlier observations (Gillespie 1962) on extractions by alkaline thioglycollate under conditions of high ionic strength. A more detailed study of the components in the SCMKA and SCMKB fractions isolated from wool by this tributyl phosphine–alcoholic sodium iodide method has recently been undertaken (Gillespie and Darskus, unpublished data).

It seems that this distinction between the approx. 70% soluble and 30% insoluble fractions must arise from morphological structures within the fibre. Under the microscope the insoluble residue appears as flattened ribbons of cuticle with an intact scale structure, and this material is now being examined further. Differences have been found in the extents of extraction of different wools in the "standard" solvent with tributyl phosphine, and to some extent this depends on the pretreatment of the wool, e.g. the conditions used for degreasing. This solvent is also effective with feather keratin and 84% extraction is obtained from chicken feathers using the reducing system above. Likewise in the absence of a reducing agent, this solvent has proved effective in extracting proteins from reduced and *S*-carboxymethylated fibres (Maclaren, Kilpatrick, and Kirkpatrick 1968).

The basis of the remarkable solvent properties of this system is not obvious. The solution contains strongly solvated sodium cations and weakly solvated iodide anions, and presumably water, alcohol, and polar groups in the protein are all involved in this solvation. Selective adsorption of anions by the fibre has been

TABLE 1

EXTRACTION OF COMPONENTS FROM THE WOOL FIBRE

The wool was reduced (tributylphosphine), extracted, and then *S*-carboxymethylated (see Materials and Methods section)

NaI Concn. (M)	Propan-1-ol Concn. (% v/v)	Amount of Material in Extract*		
		Total (%)	High-sulphur Fraction (SCMKB) (%)	Low-sulphur Fraction† (SCMKA) (%)
1	25	8	7	1
2	25	20	14	6
3	25	45	21	24
4	25	69	22	47
5	25	69	19	50
5	6·25	19	14	5
5	9·38	51	17	34
5	12·50	67	19	48
5	18·75	70	19	51

* All values are expressed as percentage by weight of the original fibre.

† Determined by difference. This fraction could also include some of the "high-glycine" fraction (Crewther *et al.* 1965).

proposed (Crewther and Dowling 1956) and this would confer an overall negative charge and tend to disrupt the secondary structure of the proteins. The marked influence which mixtures of aqueous and organic solvents exert on the physical properties (Atkinson and Speakman 1964; Zahn 1964) and on the chemical reactivity (Maclaren 1962; Gerthsen and Meichelbeck 1965) of wool, may also contribute to the dispersion of the keratin fibres.

Acknowledgments

The authors are indebted to Mr. I. J. O'Donnell and Dr. J. M. Gillespie for valuable advice.

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