Reversible phenomena associated with a helix-coil transition in keratin

If wool-fibre keratin is treated at room temperature in aqueous LiBr of a sufficiently high concentration its X-ray α-pattern is destroyed, but returns if the salt is removed by washing the fibres in water[1]. Furthermore HALY AND GRIFFITH[2] demonstrated that supercontraction of wool fibres in LiBr at 100° takes place in two stages and that the first stage, which could be completed but not exceeded at room temperature, is marked by loss of birefringence. Both length and birefringence are precisely restored if the fibres are washed in water.

We have now found that first-stage supercontraction, birefringence, and the X-ray-diffraction pattern all show an abrupt change over the same small range of LiBr concentration.

All LiBr solutions were prepared by diluting aliquots of a standardised 8.0 *M* solution. Wool samples for X-ray-diffraction experiments were made up using 50 to 100 Corriedale fibres. A different sample was used for each LiBr solution, and was left in the solution at room temperature for at least 16 h. (No further changes have been observed in times up to many weeks.) After being straightened in a small jig, the fibre bundle and jig were placed in a perspex cell with mica windows (see inset to Fig. 1). The bundle was left wet with LiBr solution, and enough of the same solution was added to the cell to fill it to just below the level of the windows. The object was to maintain the correct relative humidity in the enclosure, so that the concentration of LiBr within the fibres did not alter. After being sealed the cell was placed in the camera of the X-ray-diffraction apparatus (Philips Type PW 1008). An exposure time of 20 min was required with a specimen to film distance of 3 cm and Kodirex film.

Supercontraction was measured using snippets of at least three fibres. These were mounted in the way previously described[2], and left for at least 16 h before measurement.

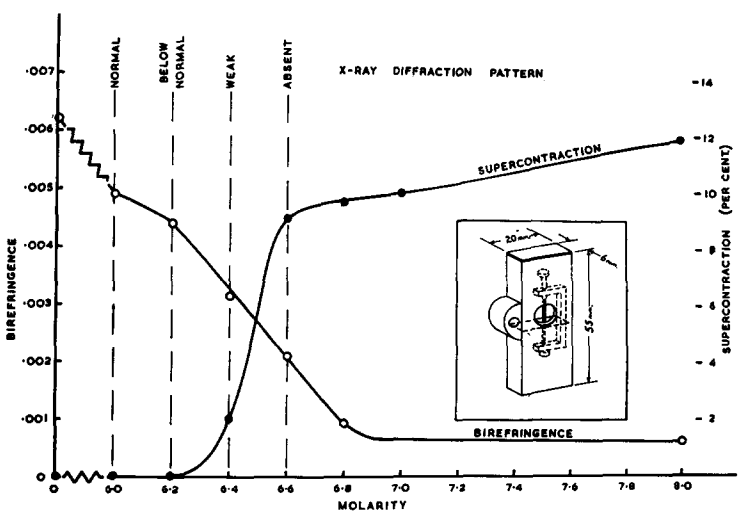

Fig. 1. Curves showing how degree of supercontraction (ordinates at right) and birefringence of wool fibres vary with concentration of aq. LiBr. Only first-stage supercontraction is involved. Qualitative statements about the intensity of the X-ray reflections (5.1 Å and 9.8 Å) are included. A drawing of the cell used for containing the sample during X-irradiation is in the inset.

Birefringence measurements by the method of GORANSON AND ADAMS[3] were made on these same snippets immediately after measurement of degrees of supercontraction. In calculating the birefringence the diameter of the water-saturated fibre was used, *i.e.* no correction was made for swelling due to absorption of LiBr or to longitudinal contraction. The correction is insignificant in the present context.

The results are shown in Fig. 1. Though we have been able to make only qualitative remarks about the loss of the α-pattern, it is clear that all three phenomena show a sharp change over the concentration range 6.2 to 6.6 *M*. There was no evidence from the X-ray photographs that disorientation of crystallites with respect to each other takes place, as arc lengths were the same in all photographs in which the pattern could be seen; this suggests that the structure was disorganized at the molecular level. In addition, extension of a contracted sample in LiBr to a length 50 % greater than the natural length gave no discrete X-ray reflections.

It is important to remember that keratin fibres which are supercontracted, to the end of the first stage in LiBr behave as elastomers[4], *i.e.*, the stress in an extended fibre is entropic and not due to strained bonds. ELÖD AND ZAHN had previously found elastomeric behaviour of keratin under other conditions. These results imply the presence of permanent cross-links separated by many residues, and few or no intra-chain H-bonds.

We thus have evidence that LiBr solution causes disorganization at the molecular level, and that this is accompanied by supercontraction and chain freedom over many residues. A reasonable conclusion would be that inter- and intra-chain hydrogen bonds are ruptured, and that the chains then tend towards the random-coil form. Disorganization at room temperature is not so complete that return to the original configuration is forbidden once the salt is washed out. Such a transition is consistent with the data presented here.

To set against this we have the evidence of HARRINGTON AND SCHELLMAN[6] who worked with solutions of silk fibroin, serum albumin, clupein, and oxidized and native ribonuclease, and deduced that LiBr, while able to destroy the tertiary structure, actually increased the stability of intramolecular H-bonds. Their work was chiefly in the field of optical rotation. STRYER[7] remarked that the increased efficiency of energy transfer in chymotrypsinogen in LiBr, found by SHORE AND PARDEE[8], was consistent with the view that LiBr strengthens peptide H-bonds.

HARRINGTON AND SCHELLMAN concluded that LiBr was a "contra-denaturant", in the sense that it appeared to increase the amount of protein in the α-form. SIKORSKI[9] has, however, reported or quoted cases in which at least partially analogous increases in quantity of α-protein were produced by the action of urea on myosin, and by the action of urea, NaOH, LiBr, formic acid, or cuprammonium hydroxide on denatured keratin. Detection of the α-state was by X-ray diffraction. The denaturation was usually produced by the action of hot water on extended fibres, but in one case it was produced by high-pressure steam which caused supercontraction. (The properties of the native fibre were not restored by the treatment giving additional α-protein.) Of the above reagents at least urea and formic acid are regarded as denaturants.

Thus when results for solid proteins and proteins in solution are considered together some apparent contradictions are found. In an attempt to reconcile the data the following suggestions are made. (a) In solid keratin large numbers of new, small segments of helix might be produced by the LiBr and these would go un-

detected by the X-ray-diffraction technique whereas a similar phenomenon in solution is seen by optical-rotation methods. The disappearance of the original α-pattern might be explained in terms of small distortions not requiring wholesale breakdown of the secondary structure. It is not clear how supercontraction and elastomeric behaviour would be explained on this basis. (b) In solid protein the reaction due to LiBr may be very different from the reaction in solution. LiBr is absorbed in substantial quantity by the solid and its penetration alone causes a physical disturbance which must involve some forcing apart of molecular chains. We thus have physical and chemical effects to consider and both inter-chain and intra-chain bonds might be weakened or broken. In solution the question of penetration in the same sense does not arise and H-bonds may increase in stability.

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The application of the Sakaguchi reaction to the determination of DNP-arginine

During the study of an N-terminal amino acid residue of a protein by the dinitrophenylation procedure[1] it is necessary, when the end-group is arginine, to be able to determine accurately the amount of DNP-arginine present in the mixture of amino acids resulting from the acid hydrolysis of the dinitrophenylated protein. A method whereby this may be achieved has been outlined by WISSMANN AND NITSCHMANN[2]. In this, the DNP-arginine is first separated from the accompanying free amino acids by chromatography on talc columns, and subsequently subjected to a modification[3] of the Sakaguchi reaction, the colour produced being compared with that from similarly treated standards. The validity of the determination is therefore dependent on the assumption that DNP-arginine behaves similarly to arginine when treated with alkaline hypobromite.

When attempts were made to determine the DNP-arginine released by hydrolysis of dinitrophenylated α-casein using this method, very variable results were obtained. It was found that in order to obtain reproducible results with DNP-arginine, the rate

Abbreviation: DNP-, 2,4-dinitrophenyl-.