THE CROSS-β MODIFICATION IN WOOL KERATIN

N. PEACOCK

Textile Physics Laboratory, University of Leeds (Great Britain)

(Received May 20th, 1958)

SUMMARY

1. Methods for producing the cross-β modification in wool keratin have been investigated and the optimum found to be treatment in urea-reducer solution at pH 6.4–8 and 40°, followed by stretching. Some α form remains.

2. Treatment in urea-reducer at pH 6 and below, and immersion in boiling water of fibres treated at pH 6.4–8, produce, in addition to cross-β, some parallel-β. This apparently results from conversion of residual α form.

3. The location of the material in the cross-β form within the fibre has been considered in the light of recent work on the fine structure of the cortex.

4. The cross-β form in keratin has similar stability to the "set" parallel-β form and is maintained by bonds in addition to hydrogen bonds.

5. The axial repeat of cross-β is 4.71 Å (or a multiple of this), which is thought to be the spacing between polypeptide chains linked by hydrogen bonds. This spacing is nearer to that in fibroin than to that in parallel-β keratin.

6. The 4.71 Å reflection is sharp and indicates that the chains as a whole run in the direction of the fibre axis and are folded or coiled, possibly in a form similar to that in the α configuration. Some models like the latter have been considered, but none found satisfactory.

INTRODUCTION

The cross-β configuration was first reported in films formed from denatured globular proteins and stretched[1,2]; and in the more labile of the α-type proteins it was found in thermally-contracted samples on stretching[3,4]. Only recently has this configuration been found to occur naturally, in bacterial flagella[5] and in the "egg-stalks" of *Chrysopa*[6]. Keratin fibres treated in boiling sodium bisulphite solution, in which they undergo supercontraction, show some cross-β form if they are stretched before being washed[7], but the most prominent appearance of this form in keratin was that discovered by MERCER[8] during his attempts to dissolve wool fibres in saturated urea solution containing a reducing agent. The treatment left a residue of about half the fibre—in merino wool, a distinct component, the paracortex[9]—and, on stretching this residue, it showed fairly good orientation of cross-β.

ASTBURY, DICKINSON AND BAILEY[1] suggested that the cross-β diffraction pattern resulted from orientation of straight-chain β-crystallites transverse to the direction of stretching in films of denatured proteins. The cross-β form was found generally to be converted to parallel-β on stretching[1-3], and RUDALL[4] also found that treatment in saturated urea solution converted cross-β to α in epidermin, from which he deduced that the cross-β configuration was a folded form of polypeptide chains, maintained by hydrogen bonding. This was supported by the observation of PARKER AND RUDALL[6] that the cross-β to parallel-β conversion, which occurred on stretching *Chrysopa* egg-stalks, was of the nature of an intramolecular transformation rather than a rotation of crystallites. MERCER[9], however, found no conversion of cross-β to parallel-β on stretching his wool keratin samples, and he thought that the cross-β configuration was a definite type of crystallisation of extended polypeptide chains. The X-ray photograph of *Chrysopa* egg-stalks[6] shows by far the most detailed cross-β pattern obtained so far, but there is no sign of any equatorial reflections corresponding to the repeat along the extended polypeptide chain, such as might be expected from MERCER's model.

Since it is found in both globular and fibrous proteins, the cross-β configuration has an important place in considerations of protein structure and particularly in the study of denaturation: it is therefore important to study this form in a protein as stable as wool keratin. Although the cross-β diffraction pattern from keratin is not sufficiently detailed for a choice to be made between the models discussed above, the relation between this and the α and parallel-β configurations has been further investigated in order to try to throw more light on the problem.

EXPERIMENTAL

Wool

All treatments were made on wool from the shoulder of a Lincoln fleece, which was soxhlet-extracted with benzene and ethyl alcohol. After being washed in distilled water, the wool was dried at room temperature and humidity.

Chemicals

All chemicals were of laboratory reagent standard. The term "urea solution" means (unless otherwise stated) a solution of 120 g urea in 100 g water, which is a saturated solution at 20–25°. Fresh sodium bisulphite (or metabisulphite), or fresh thioglycollic acid, was used as reducing agent, concentrations being expressed as percentage w/w of reagent added to a solution. The cuprammonium hydroxide solution contained 15 g copper and 200 ml of 0.88 ammonia solution per litre.

Treatment of fibres

Samples were mounted in frames of stainless steel, between a fixed and a movable jaw so that dimensional changes could be measured; the frame could be mounted in the X-ray camera without disturbing the fibres.

X-ray photography

Diffraction patterns were obtained with Cu Kα radiation. The specimen-to-(flat)-film distance of about 4 cm was calibrated with a silver specimen if necessary.

EXPERIMENTAL RESULTS

Supercontraction

Experiments on single fibres confirmed MERCER's[10] findings that a maximum of supercontraction was reached after 3 hours' treatment in urea +5% bisulphite at pH 7 and 40°, and also showed that the maximum supercontraction depended on the pH, being 42–47 % for pH 6 and below, and 26–30 % for pH 6.4–8.

Conditions for the production of cross-β

When the conditions of treatment in urea-reducer solution were varied considerably, it was found that the optimum treatment for the production of cross-β was that in urea +5% bisulphite at pH 6.4–8 and 40° for 24 h. At the end of the treatment the fibres were stretched to 1.5–1.8 times their original length, in the solution, and then washed and dried. They showed a fair orientation of cross-β, which was improved when they were stretched in cold water to 2.0–2.4 times their original length. Some α-form remained and there were also signs of the parallel-β form (Fig. 1).

Other results of these experiments were:

(1) reduction of the urea concentration by half reduced the amount of α to cross-β conversion considerably;

(2) temperature variation in the range 40°–65° affected the rate of reaction, but not the end-product;

(3) at pH below 6, *i.e.* in the range of greater maximum supercontraction, the production of cross-β was as great as at pH 6.4–8, but much parallel-β was also produced (Fig. 2);

(4) fibres treated at pH 6.4–8 and then immersed in boiling water showed the same diffraction pattern (and supercontraction) as those treated at pH below 6;

(5) ethylation of broken disulphide bonds did not affect the amount of supercontraction, but the diffraction pattern of the stretched fibres showed very little cross-β and much parallel-β;

(6) the parallel-β form in stretched fibres could not be converted to cross-β.

The treatment of fibres in boiling 5 % sodium bisulphite solution, with subsequent stretching[7], was found unsuitable because, even under the best conditions, there was a preponderance of the parallel-β form. Oxidation of fibres in peracetic acid, followed by treatment in boiling water[11] and stretching, gave only the parallel-β form.

Reversal of cross-β to α

Samples giving good cross-β diffraction patterns were treated both taut and slack in saturated urea solution at 40°, but little sign of reversal was noticed after 8–10 days' treatment. 100 % lithium bromide solution, used at room temperature, was also ineffective. However, treatment in cuprammonium hydroxide solution at room temperature, followed by removal of copper with dilute sulphuric acid (the method used by SIKORSKI[12] for the reversal of the permanently-set parallel-β form), was successful in reversing cross-β to α (Fig. 3).

Cross-β to parallel-β conversion

Extension of fibres which showed a good cross-β orientation to their breaking point in cold water caused an improvement in cross-β orientation and the appearance of some parallel-β, but there was no evidence of conversion of the former into the latter and it seemed most probable that the parallel-β resulted from the stretching of residual α form. Extensions beyond the cold-water breaking-point could be attained by stretching in boiling water, and there was then a considerable increase in the amount of parallel-β. This, however, appeared to be associated with the

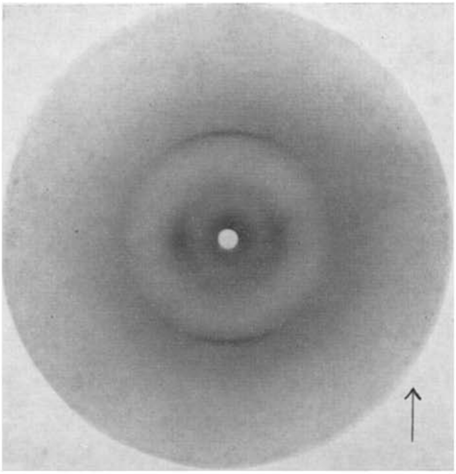

Fig. 1. X-ray diffraction pattern of Lincoln wool treated in urea-reducer solution at pH 7 and 40° for 24 h, stretched in solution to 1.5 times, and then in water to twice original length: cross-β and α forms. The fibre-axis direction is indicated in each Figure by an arrow.

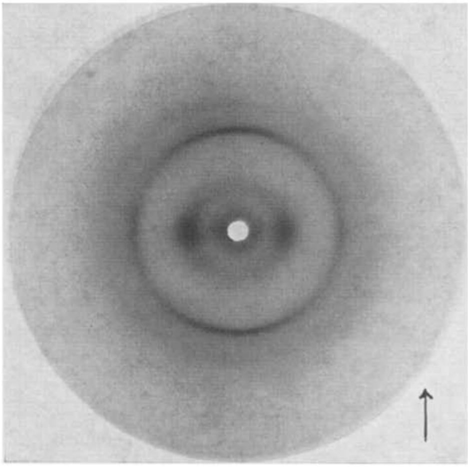

Fig. 2. X-ray diffraction pattern of Lincoln wool treated in urea-reducer at pH 5 and 40° for 24 h, stretched in solution to 1.5 times and then in water to twice original length: cross-β and parallel-β. A similar pattern is obtained when fibres treated as for Fig. 1 are immersed in boiling water.

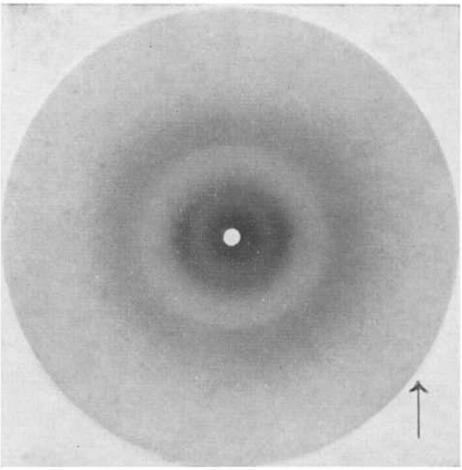

Fig. 3. Lincoln wool treated as for Fig. 1, then in cuprammonium hydroxide for 4 h and in sulphuric acid till all copper was removed, stretched 50%: cross-β reversed to α.

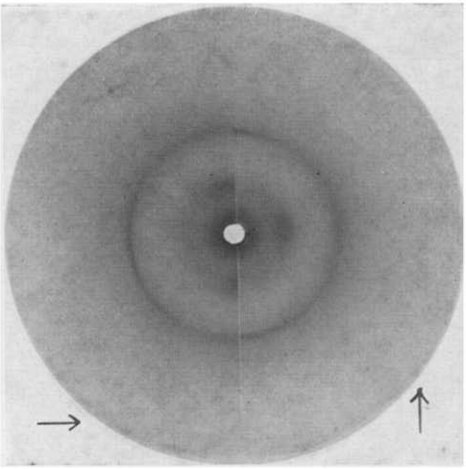

Fig. 4. As Fig. 2, sector photograph: L.H.S. fibres horizontal; R.H.S., fibres vertical. Showing difference in spacing between 200 of parallel-β and the axial spacing of cross-β.

disappearance of residual α-form, which always occurred on treatment in boiling water whether the fibres were stretched simultaneously or not.

Spacings in the cross-β pattern

1. *Meridional.* The spacing of the chief meridional reflection in the cross-β pattern was found to be slightly greater than the 200 spacing (4.65 Å) of parallel-β in patterns showing both forms: to show the difference distinctly, a sector photograph was taken with the fibres lying in two directions at right angles (Fig. 4). Measurements made on a calibrated photograph showed the values to be 4.71 Å for cross-β and 4.65 Å for parallel-β. There was no change in the 4.71 Å spacing when the fibres were dried over phosphorus pentoxide for some weeks, or when they were kept wet while being photographed. In the disoriented-β pattern given by supercontracted, but unstretched, fibres, the ring about 4.7 Å was sufficiently broad to cover both 4.65 Å and 4.71 Å, so that it could not be ascertained whether latter spacing resulted from the extension of the former during the stretching and orienting process.

Whenever it appeared, the 4.71 Å reflection was very sharp (Fig. 1), although it spread in a circular arc, about 30° on either side of the meridian. This arc was not resolved into two components when the fibres were inclined at 10° (a little greater than the Bragg angle for this reflection) to the normal to the X-ray beam, but its intensity increased.

Intense photographs showed a meridional arc at about 2.4 Å, which was probably the second order of 4.71 Å. The 4.2 Å reflection, reported by HAPPEY[13] in the cross-β pattern from regenerated keratin, did not appear regularly in our photographs. Such a reflection is often found in residues and extracts from keratin and is normally associated with fatty impurities.

2. *Equatorial.* In patterns showing the best orientation of the 4.71 Å reflection there were slight signs of an equatorial reflection of about 3.3 Å, corresponding to the 020 reflection for parallel-β, but this was never very distinct and could not be measured accurately. The other two equatorial reflections, at 10 Å (corresponding to 001 in parallel-β) and at 30–35 Å, both showed the same orientation, normal to the plane of a film made by compressing fibres between glass plates in cold water.

3. *Other spacings.* The only other prominent reflection was an unresolved arc at 3.8 Å, which spread about 60° on either side of the meridian. Since this was twice the spread of the 4.71 Å arc, it seemed likely that it resulted from the superposition of two reflections, equidistant on either side of the meridian and about 30° from it, thus indicating that it might be the cross-β reflection corresponding to the 210 reflection of the parallel-β pattern.

DISCUSSION

The action of urea-reducer solutions on wool keratin causes changes in configuration of the type associated with denaturation in the globular proteins, *i.e.* the appearance of the cross-β form. However, this is only half the story, since a fair proportion (25–40 % of Lincoln wool, depending on the pH of treatment) is dissolved during treatment[9,14]. MERCER[9] has found that in crimped fibres a definite segment of the cortex, the orthocortex, dissolves, and therefore the cross-β form appears in the residual segment, the paracortex. In Lincoln wool no such macroscopic segmentation is found, but ANDREWS AND SIKORSKI[15] have shown that there are two different types of organisation of microfibrils in the cortical cells, which may be correlated with the ortho-like or para-like properties of the cells. Thus it may be at this microscopic level that we must seek the source of the cross-β form. Since considerable swelling takes place during the formation of cross-β, its appearance could be wholly morphological, caused, for example, by transverse orientation of microfibrils, but the results obtained by JEFFREY, SIKORSKI AND WOODS[16] do not support this idea.

Both the supercontraction experiments and the X-ray photography show the dependence of the action of urea-reducer solutions on pH. In the region pH 6.4–8 the action on the insoluble material causes a conversion of the α form to the cross-β form; the extent of this conversion depends on the concentration of the urea solution, but is not complete even when a saturated solution is used. The results, described above, of treatments in urea-reducer at lower pH's, and of treatments in reagents at 100°, indicate that the component of the insoluble material which remains in the α form is very labile and is easily denatured to give the parallel-β form. If, as seems very probable, this α component is a small residue of orthocortex, or ortho-like material, this denaturation represents another difference from the paracortical material which gives the cross-β form.

From the results of the "reversal" experiments, it appears that the cross-β form is similar in stability to the "set" parallel-β form and is maintained by covalent bonds as well as by hydrogen bonds. Since ethylation of the reduced disulphide bonds results in a lower yield of cross-β, it seems that broken disulphide bonds play a part in the formation of the new bonds which maintain this form. Further confirmation that the bonding maintaining the cross-β form in keratin is stronger than in other proteins is given by the absence of any conversion to parallel-β on stretching.

This last might be taken as evidence for the hypothesis of β crystallites oriented transversely to the fibre axis[1], but there are reasons for not accepting this theory. Firstly, cross-β cannot be obtained directly from parallel-β by the action of urea-reducer, nor does parallel-β appear as an intermediate in the formation of cross-β; and secondly, there is no clear sign of the expected equatorial reflection at 3.34 Å in the cross-β pattern. The difference between the meridional repeat in cross-β and the 200 spacing in parallel-β also indicates that the two "β forms" are different configurations rather than different orientations of polypeptide chains, although it does seem reasonable to suggest that the 4.71 Å spacing is due to hydrogen bonding.

The value of 4.71 Å is closer to the hydrogen-bonding spacing in silk fibroin[17] than it is to the 4.65 Å found in the parallel-β form in keratin. The difference between the spacings in fibroin and keratin is attributed to differences in their amino-acid composition, fibroin having shorter and less active side-chains than keratin. While swelling experiments[14] show that wool fibres treated in urea-reducer are more acidic than virgin fibres, it seems improbable that all the long and active side-chains have been damaged and the analogy with fibroin should not be taken too far, particularly since the cross-β does not appear to be a straight-chain configuration.

No quantitative evidence about the metrical relationship between cross-β and α may be deduced from the supercontraction results since there is disorientation as well as a configurational change, and there is no evidence as to the contributions of the crystalline and noncrystalline regions of the fibres to the supercontraction. However, a folded form for cross-β seems very probable[4] and, since the hydrogen-bonding will be more nearly intramolecular, as in the α form, than intermolecular, as in the parallel-β

form, it is worthwhile considering some α-like structures[18,19]. Such structures might be expected not to give a 3.34 Å transverse repeat, but might be expected to show a transformation to parallel-β on stretching and also to show different infra-red absorption spectra from the parallel-β form. This spectrum has not been obtained for the cross-β modification in keratin, but PARKER AND RUDALL[6] have found no such difference for *Chrysopa* egg-stalks. Of the forms considered by BRAGG, KENDREW AND PERUTZ[18], the most likely is $2_{11}\,{}^{1}/_{3}$, which has an axial repeat of 9.6 Å: since, however, only one-third of the possible hydrogen bonds are made intramolecularly along the fibre axis, it seems unlikely that it would show the necessary dichroism in its infra-red absorption[6]. PAULING AND COREY[20] consider that this, and any totally-bonded helical structure, is less stable than the α-helix[19], but just as modifications of the latter have had to be proposed to account for the diffraction pattern of α keratin[21,22], some other modification may account for the cross-β configuration. Such a modification, involving the relative positioning of adjacent chains, has been suggested by HAPPEY[23]. His fundamental postulate is that the 4.71 Å "reflection" is really an unresolved pair of off-meridian reflections[13], produced by the "staggering" of adjacent chains, which would give reflecting planes inclined to the fibre axis. However, our estimates of the spreading of the 4.71 Å arc at its best orientation show that the corresponding axial "repeat" for the α chain would have to be less than 5.10 Å, which does not agree with the observed value of 5.14 Å in α keratin or of 5.3–5.4 Å for the α-helix. An important point about this model is that the 1.5 Å reflection, characteristic of the α-helix, should appear[24]: so far we have no evidence for its appearance in the cross-β pattern for wool keratin, but in view of the circumferential spreading of reflections it may be difficult to detect.

Although none of the models suggested for the cross-β configuration is entirely satisfactory, the sharpness of the 4.71 Å reflection almost certainly shows that the polypeptide chains run as a whole along the fibre axis and therefore they must be folded in some way. For this reason a strict differentiation must be made between the two "β" forms, since the term "β" is usually applied to fully-extended chains, as in the parallel-β form, and not to folded chains.

APPENDIX

The indexing of the cross-β pattern

In indexing the diffraction pattern of *Chrysopa* egg-stalks, PARKER AND RUDALL[6] have used the same axes as for the parallel-β form, thus making the fibre-axis direction the *a* axis. For the α and the parallel-β forms it has been usual to call the fibre axis either the *b* axis[25] or the *c* axis[19]. There seems to be no reason to depart from the former of these two systems for the cross-β form and the 4.71 Å spacing should therefore be called 020 on the basis of PARKER AND RUDALL's results[6].

ACKNOWLEDGEMENTS

The author wishes to thank Mr. H. J. WOODS and Dr. J. SIKORSKI for many helpful discussions. He is indebted to Courtaulds' Scientific and Educational Trust Fund and to the International Wool Secretariat for providing the financial support for this work.

REFERENCES

[1] W. T. Astbury, S. Dickinson and K. Bailey, *Biochem. J.*, 29 (1935) 2351.
[2] F. R. Senti, C. R. Eddy and G. C. Nutting, *J. Am. Chem. Soc.*, 65 (1943) 2473.
[3] W. T. Astbury, *Proc. Roy. Soc., London*, B134 (1947) 303.
[4] K. M. Rudall, *Soc. Dyers and Colourists, Symposium on Fibrous Proteins*, (1946) 15.
[5] W. T. Astbury, E. Beighton and C. Weibull, *Symposia Soc. Exptl. Biol.*, 9 (1955) 282.
[6] K. D. Parker and K. M. Rudall, *Nature*, 179 (1957) 905.
[7] J. Sikorski, personal communication.
[8] E. H. Mercer, *Nature*, 163 (1949) 18.
[9] E. H. Mercer, *Textile Research J.*, 24 (1954) 835.
[10] E. H. Mercer, *Ph. D. Thesis*, Leeds, 1948.
[11] P. Alexander, *Kolloid-Z.*, 122 (1951) 8.
[12] J. Sikorski, *Nature*, 170 (1952) 275.
[13] F. Happey, *Proc. Intern. Wool Textile Research Conf. Australia*, 1955, B-153 and B-280.
[14] N. Peacock, *M. Sc. Thesis*, Leeds, 1952.
[15] M. W. Andrews and J. Sikorski, *Proc. Electron Microscopy Conf., Stockholm*, 1956, p. 300.
[16] G. M. Jeffrey, J. Sikorski and H. J. Woods, *Textile Research J.*, 25 (1955) 714.
[17] J. O. Warwicker, *Trans. Faraday Soc.*, 52 (1956) 554.
[18] W. L. Bragg, J. C. Kendrew and M. F. Perutz, *Proc. Roy. Soc., London*, A203 (1951) 321.
[19] L. Pauling, R. B. Corey and H. R. Branson, *Proc. Natl. Acad. Sci. U.S.*, 37 (1951) 205.
[20] L. Pauling and R. B. Corey, *Proc. Natl. Acad. Sci. U.S.*, 37 (1951) 729.
[21] L. Pauling and R. B. Corey, *Nature*, 171 (1953) 59.
[22] F. H. C. Crick, *Nature*, 170 (1952) 882.
[23] F. Happey, *Inst. Phys. X-ray Analysis Group Conf. on Biological Structures and Computational Methods, London*, Nov. 1956.
[24] F. Happey, *Nature*, 179 (1957) 194.
[25] W. T. Astbury and A. Street, *Trans. Roy. Soc. London, Ser. A* 230 (1931) 75.