THE CONFORMATION OF A SOLUBLE WOOL KERATIN DERIVATIVE

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Summary

The conformation of a low-sulphur soluble wool keratin derivative (SCMKA) has been studied by optical rotatory dispersion. On the assumption that the overall conformation consists of a mixture of α-helical and random-coil regions, this protein has a helical content of about 50% in aqueous solution at pH 9.1; this helical content does not vary with the degree of severity of the preparative procedure. The protein may be reversibly converted to the random-coil form by heating to 70°C, or by treatment with urea at concentrations exceeding 6M. An increase in pH to 12.5 causes very little change in conformation, such change as does occur being reversible. The maximum helical content which has been induced by solvents into the protein is about 62% in 2-chloroethanol. This probably represents the upper limit of the helical content of this protein as it occurs in the fibre. The changes in the conformation of the protein which occur in several other solvents are briefly discussed.

I. INTRODUCTION

During the past decade intensive studies have been made on wool keratin, both as it exists in the fibre and also as soluble derivatives extracted after rupture of either disulphide or peptide bonds. Electron microscopy and X-ray diffraction data on the fibre have been interpreted in terms of a structure in which the cortex of the fibre consists mainly of highly organized “micro-fibrillar” regions embedded in a matrix which is largely amorphous (cf. Fraser 1961). On rupture of the disulphide bonds, about 70–80% of the wool can be extracted into solution and the soluble derivatives can be fractionated into two main groups (Woods 1961). One of these groups, which represents about one-third of the extractable protein, has a sulphur content (derived almost entirely from cystine) much greater than that of the parent wool and the other group a sulphur content of less than half that of wool itself. The first of these fractions is believed to arise from the amorphous regions of the fibre and the latter from the more organized microfibrils. Although considerable progress has recently been made with the further fractionation and chemical and physico-chemical characterization of these soluble derivatives (Woods 1961; Gillespie 1962, 1963; O’Donnell and Thompson 1962; O’Donnell, Thompson, and Inglis 1962; Gillespie and Harrap 1963 and unpublished data), one would like to know more about their secondary structure† and conformation in solution so that eventually a more tangible link between the studies on the soluble derivatives and those on the parent fibre might be found.

† This term is used as defined by Linderstrom-Lang (1952).

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Recently, the technique of optical rotatory dispersion has yielded much data on the conformation of proteins in solution and of the optical rotatory properties of synthetic polyamino acids in both the random-coil and $\alpha$-helical conformations (Blout 1960; Urnes and Doty 1961). This has been applied in a semi-empirical manner to proteins by assuming that in the unaggregated state they are composed of both $\alpha$-helical and random-coil regions and that their optical rotatory properties are the result of the sum of the contributions by both regions. In order to relate the specific rotation $[\alpha]$ to the wavelength ($\lambda$) the equation of Moffitt and Yang (1956)

$$m' = \frac{3}{n^2+2} \cdot \frac{M}{100[\alpha]} = a_0 \left(\frac{\lambda_0^2}{\lambda^2-\lambda_0^2}\right) + b_0 \left(\frac{\lambda_0^3}{\lambda^2-\lambda_0^2}\right)^2$$

is used. In this equation $m'$ is the reduced residue rotation in which $[\alpha]$ is corrected for the refractive index $n$ of the solvent, and put on a molar (mole residues/l) basis by using the mean residue weight $M$; $\lambda_0$ is a dispersion constant with a value of 212 $m_\lambda$ for a wide range of proteins and polypeptides; $a_0$ is a parameter reflecting contributions from both the helix and the residue rotation to the dispersion characteristics; and $b_0$ is a parameter which represents only the contribution of the helix and so may be used as a measure of the fraction of the protein molecule in the helical conformation. For a number of synthetic polyamino acids in the right-handed $\alpha$-helical conformation and for a few proteins which appear to be almost entirely helical in the native state, $b_0$ has a value $\approx -640$. In the random-coil conformation $b_0$ is zero so that the ratio $-b_0/640$ gives a guide to the fraction of a protein in the $\alpha$-helical conformation (or, more precisely, the excess of right-handed over any left-handed helix). These semi-empirical correlations have been placed on a much firmer basis by the recent important work of Beychok and Blout (1961) and Urnes, Imahori, and Doty (1961), who have shown that the helical content of myoglobin in aqueous solution as measured by its optical rotatory dispersion properties is almost identical with its helical content in the crystalline state as deduced from X-ray diffraction patterns (Kendrew et al. 1961).

Some preliminary measurements of the optical rotatory dispersion of two subfractions isolated from the high-sulphur soluble wool proteins (Gillespie 1962; Gillespie and Harrap 1963) have shown that they exist in the random-coil form in aqueous solution. This might be expected both from the less-ordered nature of the region of the fibre from which they originate and also from their high content of prolyl residues (Gillespie 1962, 1963) which are known to interrupt the $\alpha$-helix in proteins (Cohen and Szent-Gyorgyi 1957). Attention has therefore been concentrated on a low-sulphur protein extract. Woods (1959) has already examined the optical rotation of low-sulphur soluble wool proteins in a variety of environments but because of instrumental limitations he was only able to make measurements at a single wavelength. In such circumstances changes in $[\alpha]$ may be due both to changes in conformation and the dependence of $[\alpha]$ on solvent (Urnes and Doty 1961); it was therefore difficult to interpret these results equivocally in some cases. Nevertheless Woods showed that three soluble derivatives, in which the cystine $-S-S-$ bonds were converted to $-\text{SCH}_2\text{COOH}$, $\text{S-SO}_3\text{H}$, and $-\text{SO}_3\text{H}$, were all equivalent in their behaviour with respect to change in environment.
The work described in this paper has been performed with a low-sulphur fraction from wool obtained by converting the cystine cross-links to the S-carboxymethyl (–SCH₂COOH) derivative. Attention has been focused on the effect of differences in the severity of the conditions used in several preparative procedures on the conformation of the final product, and also on the conditions under which the conformation of the protein may be altered.

II. Apparatus and Reagents

Optical rotatory dispersion data were obtained with a spectropolarimeter built from a photoelectric polarimeter (W. F. Stanley and Son), a quartz prism monochromator (Carl Leiss) and a 500-W high-pressure mercury arc (Philips SP 500). Measurements were made at 578, 546, 486, 436, 405, 365, and occasionally, 324 μm. Jacketed 1 dm cells were used through which water from a thermostat-controlled bath (Colora NB) was circulated. Solutions were filtered under pressure directly into the cells through a sintered-glass filter (porosity 4).

Protein concentrations were determined by semi-micro-Kjeldahl nitrogen analyses, taking the nitrogen content of the protein as 16.7% (Harrap and Woods 1958a).

Guanidine hydrochloride (B.D.H.) was recrystallized twice from methanol. 2-Chloroethanol (B.D.H.) was freed from water and HCl as described by Goldstein and Katchalski (1960) and stored over alumina. Dimethyl sulphoxide (Mathieson, Coleman, and Bell) was redistilled before use. All other chemicals were of A.R. quality and were used without further purification.

III. Results and Discussion

(a) Effect of the Severity of the Method of Preparation on the Conformation of the Protein

The original preparation of this low-sulphur S-carboxymethylkerateine (SCMKA) fraction (Gillespie 1956) involved treatment of the wool with 0.1M potassium thioglycollate at 50°C for 80 min at pH 10.5 and for 20 min at pH 11.5. Since then two methods have been developed by which the reduced protein can be extracted by less severe treatments (Gillespie and Harrap, unpublished data). In one, low-sulphur protein is extracted from the wool by 0.1M potassium thioglycollate at pH 8 at room temperature in the presence of 8M urea. The yield of protein obtained under these conditions is much less than with the original procedure. In the other, probably the mildest method, high-sulphur protein is first preferentially extracted with 0.5M potassium thioglycollate at pH 10.3 and 2°C for 24 hr. The low-sulphur protein is released by plasmoslysis from the residue by immersion in a large volume of iced water. In each case the extracted protein, in the –SH form, is alkylated with sodium iodoacetate at pH 9, and the low-sulphur protein purified by several precipitations at pH 4.4 and at an ionic strength of 0.5 (Gillespie, O'Donnell, and Thompson 1962) and subsequent re-solution in 0.05M sodium borate, pH 9.1.

Optical rotatory dispersion data for SCMKA prepared by each of the above three procedures and dissolved in 0.025M borate, pH 9.1, at 25°C are plotted in terms
of the Moffitt–Yang (1956) equation in Figure 1. The slope of this plot gives the parameter $b_0$ and it is clear that within experimental error the three methods of preparation yield products with the same conformation. The proportion of a protein molecule in the $\alpha$-helical conformation is approximately equal to $-b_0/640$ (see Introduction); from the mean value of $b_0$ obtained ($= -320$, see Fig. 1), the proportion of the SCMKA molecule in the $\alpha$-helical conformation is thus 50%. This is a surprisingly high value when compared with data on proteins such as ribonuclease, bovine serum alumin, and insulin which have little or no helical content after treatments which rupture their cystine crosslinks (Yang and Doty 1957; Schellmann 1958).

![Moffitt–Yang plot for SCMKA prepared by three different procedures](image)

Fig. 1.—Moffitt–Yang plot for SCMKA prepared by three different procedures:
- Extraction with 0.1M potassium thioglycollate at 50°C (Gillespie 1956);
- Extraction with 0.1M potassium thioglycollate in presence of 8M urea;
- Cold-extraction method. Solvent for optical rotatory dispersion measurements was 0.025M sodium borate, pH 9.1.

Woods (1959) had previously found a change in $[\alpha]_D$ when the protein in the $-\text{SH}$ form was compared with that after alkylation; taken at its face value this indicated a decrease in helical content on alkylation. Dispersion measurements on the unsubstituted protein in the presence of 0.05M sodium thioglycollate gave a $b_0$ value of $-280$. This indicates an apparent slight increase in helical content on alkylation, the reverse of the conclusions drawn by Woods. However, the protein in its $-\text{SH}$ form is contaminated with about 10% of high-sulphur protein for which $b_0 = 0$; thus it is likely that no change occurs in the helical content of the low-sulphur protein on alkylation.

(b) Effect of Denaturing Conditions during Extraction Procedure on the Conformation of the Protein

It now became of interest to determine whether, during the various extraction procedures, the protein lost its helical conformation under conditions which are usually regarded as denaturing, viz. heat, urea, alkalinity, and then regained its helical conformation on removal of these conditions, or whether the protein was in fact particularly resistant to denaturing conditions.
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(i) Effect of Heat.—The optical rotatory dispersion of SCMKA prepared by the cold-extraction procedure was determined in 0·025m borate at pH 9·1 over the temperature range 25–80°C. The values of $b_0$ as a function of temperature are shown in Figure 2. At temperatures up to about 35°C no change in conformation apparently occurs; above this temperature there is a gradual decrease in $-b_0$, and hence helical content, up to about 70°C, where the protein is almost entirely in the random-coil conformation. If the solution at 80°C is slowly cooled to 25°C and its dispersion remeasured, a value of $-210$ for $b_0$ is found, suggesting that the molecule had regained only about two-thirds of its original helical content. A similar result was also found by Kauzmann and Saffer (personal communication) with $\alpha$-keratose.

![Plot](image)

Fig. 2.—Plot of $b_0$ v. temperature (◦) and urea concentration (△) for SCMKA. In each case the solution was 0·025m with respect to borate.

However, the turbidity of the SCMKA solution had increased considerably on cooling. The solution was therefore centrifuged at 40,000 r.p.m. for 30 min to remove this turbidity and the optical rotatory dispersion of the supernatant measured. A value of $-320$ was obtained for $b_0$, indicating a complete recovery of helical content. When the protein is converted to the random-coil form on heating and then cooled apparently two processes may occur: the protein may revert to its initial conformation or it may undergo intermolecular aggregation to give what is presumably a high-molecular weight $\beta$-type structure which may be removed by high-speed centrifugation. Determination of the concentration of protein in the supernatant showed that less than 5% of the protein was converted to this highly aggregated material.* However, unless it was removed an anomalously low value for $b_0$ was obtained. This indicates the errors which may be obtained if optical rotatory dispersion measurements are made on solutions of high turbidity.

* The supernatant was considerably less turbid than even the unheated cold-extracted protein. Moreover, the sediment packed to a hard pellet on centrifugation whereas with the unheated protein the sediment was in the form of a weak gel. It is as if any small aggregates in the original solution are "flocculated" on heating and cooling, the bulk of the protein remaining unchanged.
(ii) *Effect of Treatment with Urea.*—Values of $b_0$ for SCMKA in 0·025M borate, pH 9·1, as determined in the presence of different concentrations of urea are also shown in Figure 2. The conversion from the helical to the random-coil conformation takes place over the urea concentration range 2–6M. By superimposing the temperature and urea-concentration scales as shown in Figure 2, it is clear that in the transition region the effect of a 2M increase in urea concentration corresponds to that of a change in temperature of about 15 degC. In previous studies of conformational changes in the muscle protein paramyosin brought about by heating and by urea (Harrap and Doty, unpublished data) it was also found that a 2M increase in urea concentration was equivalent to a 15 degC increase in temperature. The change from the helical to the random-coil conformation in proteins probably involves the breakage of both backbone and side-chain hydrogen bonds as well as hydrophobic bonds between non-polar side-chains. The parallelism between urea concentration and temperature for two proteins of quite different amino-acid composition (and presumably different proportions of the various intra-chain bonds) suggests either that side-chain interactions are not involved in the helix–random-coil transition, which seems unlikely in view of the wide temperature range over which the transition occurs, or that urea does not rupture either the hydrogen or hydrophobic bonds involved in side-chain interactions any more specifically than does an increase in temperature.

If the solutions in 4M or 6M urea are diluted with 0·025M borate buffer to 2M with respect to urea there is a complete recovery of helical content without any production of turbidity. Woods (1959) had previously noted that changes in $[\alpha]_D$ in urea were completely reversible.

(iii) *Effect of Increased Alkalinity.*—Aqueous solutions of SCMKA were titrated to pH 11·6 and 12·5 and their dispersions measured. Values for $b_0$ of -270 and -260 respectively were obtained, indicating a small loss in helical content. On titrating back to pH 9·1 there was a complete recovery of the original helical content. Woods (1959) reported some evidence for a small degree of racemization of the protein on prolonged standing at high pH. We have not investigated this further; it would not be expected that the small degree of racemization reported would markedly influence the conformation of the protein since studies on synthetic polyamino acids (e.g. Wada 1961) have shown that a considerable proportion of D-residues can be accommodated in an L-polymer without disrupting the helix.

The above data confirm that, from the point of view of the conformation of the molecule, it is of little consequence by which procedure the SCMKA is prepared. Whether it is prepared in urea solutions, at high temperature, or at high pH, the final product eventually has the same conformation as that prepared by the less severe cold-extraction procedure. This does not of course imply that other changes in the non-helical regions of the molecule do not occur during any of the methods of preparation, since the conformation is only one of many characteristics of the molecule.

(c) *Conformation in Different Solvents*

The conformational changes which SCMKA undergoes in several other solvents has also been studied. Some of these have already been investigated at a single
wavelength by Woods (1959) but in view of the well-known solvent dependence of \([\alpha]\) (Urnes and Doty 1961), possible changes in conformation need to be confirmed by dispersion data.

Of particular interest was the behaviour of the protein in formic acid solutions since this reagent has been extensively studied in this Laboratory, both as a solvent for proteins (Harrap and Woods 1958b, 1958c, 1961) and as a medium for dyeing the wool fibre itself (Harrap 1959; Milligan 1961). The variation of \(b_0\) with formic acid concentration is shown in Figure 3. Up to \(\approx 65\%\) formic acid there is no loss (possibly even a small increase) in the helical content of the molecule, and even in 100% formic acid the molecule retains about 15% helical conformation (i.e. \(-100b_0/640\)). On dialysing the solution in 100% formic acid against 0.025M borate, pH 9.1, a small amount of turbid material was obtained similar to that obtained after heating. On removing this turbid material by centrifugation, complete recovery of the helical content was achieved.

![Fig. 3.—Plot of \(b_0\) v. concentration of formic acid for SCMKA.](image)

Values of \(b_0\) and percentage helical content for SCMKA in several other solvents are given in Table 1. These solvents may be divided into two groups: (1) solvents which increase and (2) those which decrease the helical content of the proteins. It is clear from Table 1 that in none of the group (1) solvents is there a large increase in the helical content of the protein. Even in pure 2-chloroethanol the increase is small when compared to the effect of this solvent on many globular proteins (Doty 1958). This suggests that the SCMKA takes up almost its maximum helical content even in aqueous solution. The contrast between formic acid as a random-coil-favouring solvent and acetic acid as a helix-favouring solvent is interesting. In formic acid, which has a relatively high dielectric constant (58.5) intramolecular repulsions along the polypeptide chain would tend to disrupt the helix whereas this effect would be largely eliminated in acetic acid with a dielectric constant of only 9.7. The changes in \(b_0\) in the solvents in this group are proportionally much less than the changes in \([\alpha]\) previously observed by Woods (1959).
The data on lithium bromide solutions are of interest in view of the numerous investigations on the mechanical properties of wool fibres in this reagent (cf. Crewther and Dowling 1961; Mandelkern et al. 1962). The optical rotatory data indicate that as the concentration of lithium bromide is increased the SCMKA becomes more random in conformation. This is consistent with the supercontraction shown by the wool fibre in these solvents. Mandelkern and Roberts (1961) have shown that ribonuclease is also converted to a more random form in lithium bromide solutions. For these two proteins, at least, the optical rotatory dispersion data do not support Harrington and Schellman's (1957) conclusion that lithium bromide solutions favour the helical conformation.

**Table 1**

VALUES OF b₀ FOR SCMKA IN HELIX-FAVOURING AND IN RANDOM-COIL-FAVOURING SOLVENTS

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Concentration of Aqueous Solution</th>
<th>b₀</th>
<th>α-Helical Conformation* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td></td>
<td>-320</td>
<td>50</td>
</tr>
<tr>
<td>2-Chloroethanol</td>
<td>80%</td>
<td>-370</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>-400</td>
<td>63</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>67%</td>
<td>-350</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>90%</td>
<td>-380</td>
<td>59</td>
</tr>
<tr>
<td>Methanol</td>
<td>80%</td>
<td>-360</td>
<td>55</td>
</tr>
<tr>
<td>Dimethyl sulphoxide</td>
<td>50%</td>
<td>-300</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>75%</td>
<td>-70</td>
<td>11</td>
</tr>
<tr>
<td>Lithium bromide</td>
<td>5M</td>
<td>-50</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>8M</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Guanidine hydrochloride</td>
<td>5M</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* i.e. \(-100b_0/640\).

The SCMKA protein is entirely in the random-coil form in guanidine hydrochloride at a concentration of 5M. However, considerably higher concentrations of dimethyl sulphoxide (a reagent which has recently been used as a solvent for proteins, e.g. Vratsanos 1960) are necessary before there is any loss of helix. The random-coil-favouring effect of dimethyl sulphoxide for this protein contrasts with its helix-favouring effect on some synthetic polypeptides (Fasman 1962).

There are obvious difficulties in attempting to relate the data on the soluble keratin derivative with the conformation of the unreduced protein as it occurs in the fibre. However, one might generalize by setting as an upper limit of helical content that found in 2-chloroethanol solutions. This upper limit probably reflects both the content of proline, the imino acid which must necessarily interrupt the helical content of the protein, and the disposition of other residues such as threonine, serine,
valine, which may also inhibit helix formation if they are in unfavourable sequential arrangements. In reality the helix content of the intact protein may be considerably less than this upper limit since the presence of unbroken cystine residues themselves may prevent the molecule taking up its maximum helical content especially if intra-chain cross-links occur. Recent evidence on this point is provided by the work of Narita, Kakutani, and Imahori (1961) who showed that if only one of the three disulphide bonds in insulin is broken the helical content of the protein increases from 50 to 90%.

IV. Acknowledgments

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V. References

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