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Optical Rotation of Soluble Feather Keratin

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A new structure has recently been proposed for feather keratin by Ramachandran & Dweltz (1961), which is based on a collagen-like triple helix and which is in good agreement with its detailed X-ray diffraction pattern. In support of this, they showed that feather keratin and collagen exhibit remarkable resemblances in their infrared-absorption spectra and birefringence, in addition to the nature of oriented crystallization of inorganic salts on the two fibrous proteins and in their behaviour on treatment with chemical reagents. In the present paper details are given of the measurement of the optical rotation of soluble feather keratin, which again is found to be close to that of collagen both in sign and magnitude.

MATERIALS AND METHODS

White goose-feather keratin was used in these experiments. The samples were first thoroughly degreased by washing in a number of changes of benzene, then cleaned by washing in a number of changes of distilled water and subsequently air-dried.

The solution of soluble SH-keratin of feather was prepared according to the method of Woodin (1954, 1956). A weighed amount (about 1 g.) of dry feathers was taken

and the extraction made in a 50 ml. mixture of 10M-urea, 0.1M-NaHSO₃ and 0.05M-sodium phosphate buffer at a pH of about 8.5 for 48 hr. at 60°. The extract was cooled to room temperature and filtered, and the clear protein solution collected. This was then made up to 50 ml. with the urea mixture and its optical rotation measured accurately at 25° with a Hilger standard polarimeter.

The protein concentration of feather keratin in solution was determined from the non-diffusible N content. For this purpose 4 ml. portions of the urea-bisulphite-protein solution were pipetted into cellophan sacs and dialysed against running tap water for various periods ranging from 1 to 7 days in steps of 1 day each. After the fourth day the estimated N was constant, but decreasing very slowly, probably because a fraction of the protein content was also diffusing out of the cellophan sac. A correction for this was made from the rate of change of concentration observed from the fourth to the seventh day.

The estimation of the N content in each case was carried out as follows: The contents of each dialysis sac was quantitatively transferred after dialysis to a 50 ml. flask and digested with conc. H₂SO₄ (A.R.) for 3 hr., over a microburner. Reaction was completed by adding 1-2 ml. of 30% H₂O₂ so as to remove any faint-yellow colour produced during digestion. The digest was then cooled in an ice bath and neutralized with NaOH (A.R.) added in drops, to a pH of approx. 7, and then made up to 100 ml. The N in this neutralized digest was estimated by nesslerization. The colorimetric measurements were made in a Klett-Summerson photoelectric colorimeter by using a no. 54

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green filter with maximum transmittance in the range 540–600 m μ . The amount of N in each sample was obtained by comparison with a standard. The corresponding protein concentration was calculated, taking the N content obtained to be 16.5% of the dry solid feather keratin originally dissolved (Woodin, 1954).

RESULTS AND DISCUSSION

The specific rotation, $[\alpha]_D^{25}$, of soluble feather keratin was -430° at 25° . The estimated probable error is $\pm 45^\circ$. This was for a solution of protein concentration 3 mg./ml. The result of these experiments therefore clearly shows that the solution of soluble feather keratin has a large negative specific rotation that is of the same order of magnitude as that of collagen. The specific rotation of collagen (ichthyocol) in citrate buffer at 10° , $[\alpha]_D^{10}$, has been found by Cohen (1955) to be $-350^\circ \pm 30^\circ$. In general both collagen and the triple helical polypeptides poly-L-proline and poly-L-hydroxyproline exhibit a large negative specific rotation, $[\alpha]_D$, being in the range of -300° to -400° (Sasisekharan, 1961). On the other hand polypeptides of the α -helical form generally have a specific rotation, $[\alpha]_D$, in the range 0 – 50° , whereas for wool (α -keratin) $[\alpha]_D$ is of the order of -50° to 0° . In general, α -type proteins have a small negative rotation, whereas in the random-coiled or denatured form they have a specific rotation of the

order of -100° . The fact that feather keratin exhibits a large negative rotation of -430° provides further evidence that it has a structure similar to that of collagen. The optical-activity data also support the triple helical structure of feather keratin.

SUMMARY

1. The optical rotation of soluble feather keratin has been studied, and the specific rotation of the protein in solution at 25° , $[\alpha]_D^{25}$, has been found to be -430° . This value is very close to that observed for soluble collagen and the triple helical polypeptides, and very different from that observed for α -proteins. It thus supports the triple helical structure put forward for feather keratin from X-ray data by Ramachandran & Dweltz (1961).

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Insulin and Incorporation of Amino Acids into Protein of Muscle

CELLULAR AMINO ACID LEVELS AND AMINOISOBUTYRIC ACID UPTAKE

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Kipnis & Noall (1958) found that insulin *in vitro* enhances the accumulation of a non-utilizable amino acid, α -aminoisobutyric acid, by isolated rat diaphragm. This observation has led to the belief that insulin may bring about the stimulation of incorporation of amino acids into protein of diaphragm (Sinex, MacMullen & Hastings, 1952; Krahle, 1953; Manchester & Young, 1958*a*; Wool & Krahle, 1959*a*) by a promotion of entry of amino acids into the tissue. However, in opposition to this view, no significant effect of insulin on the accumulation of naturally occurring amino acids has been observed and there is substantial evidence

that insulin can stimulate incorporation of amino acids into protein under conditions in which it is not affecting their transfer into the cell (Wool & Krahle, 1959*b*; Manchester & Krahle, 1959; Manchester & Young, 1960). The possibility has been put forward (Manchester, 1960, 1961; Tepperman & Tepperman, 1960) that the enhanced uptake of α -aminoisobutyric acid in the presence of insulin arises as a consequence of an increased rate of protein synthesis and that the α -aminoisobutyric acid enters the cell to take the place of the extra amino acids incorporated into protein. This possibility rests on the tacit assumption that an in-