NEW X-RAY EVIDENCE ON THE CONFIGURATION OF POLYPEPTIDE CHAINS

Polypeptide Chains in Poly-γ-benzyl-L-glutamate, Keratin and Hæmoglobin

POLYPEPTIDE chains in certain synthetic polymers, in fibrous proteins of the keratin–myosin–fibrinogen group, and also in hæmoglobin, appear to be coiled or folded to about half the length of a fully stretched chain. Many different chain configurations have been proposed to account for the X-ray diffraction data[1], the latest being those of Pauling, Corey and Branson[2]. Until now, however, the lack of any simple and decisive criterion in the X-ray diffraction pattern has made it difficult to test the validity of proposed models. This communication describes a new reflexion, not hitherto observed, which is given by the proteins mentioned above. The spacing at which this reflexion appears excludes all models except the 3·7 residue helix of Pauling, Corey and Branson, with which it is in perfect concord.

This model has two types of repeat: (*a*) the distance between successive turns of the spiral (5·55 A.), and (*b*) the spacing along the chain of successive amino-acid residues (1·5 A.). Thus there are in this model 3·7 residues per turn. Pauling and Corey[3] quote a calculation by V. Shoemaker showing that the 5·1-A. reflexion can be explained by the turn of the spiral. I have found a new reflexion from planes perpendicular to the fibre axis at a spacing of 1·50 A. which corresponds to the repeat of the amino-acid residues along the chain. This reflexion was discovered by oscillating the specimens about a direction normal to the fibre axis, so as to satisfy Bragg angles for planes perpendicular to that axis, and by taking photographs on cylindrical films of 3-cm. radius instead of the flat plates normally used.

Structure proposed by	Illustration	Repeat of pattern	Screw axis	Spacing of first 0*k*0 reflexion
Ambrose, Elliott and Temple (ref. 4)	Fig. 6, ref. 1	5·0–5·6	2	2·5–2·8
Bragg, Kendrew and Perutz (ref. 1)	Fig. 10, ref. 1	5·4	3	1·8
Huggins (ref. 5)	Fig. 11, ref. 1	5·2	3	1·7
Bragg, Kendrew and Perutz (ref. 1)	Fig. 12, ref. 1	5·6	4	1·4
Astbury and Bell (ref. 6)	Fig. 8, ref. 1	10·2	2	5·1; 1·7
Pauling, Corey and Branson	Fig. 2, ref. 2	*X* × 1·5	–	1·5

The appearance of a 1·50-A. reflexion from planes perpendicular to the fibre axis is incompatible with any other model so far proposed, for the following reason. All these models possess screw axes of symmetry. Thus if the chain axis *Y* is an *n*-fold screw, 0*k*0 should be absent for $k = nm$, where m is any integer. The first 0*k*0 reflexion should occur at a spacing of $d = b/n$, where b is the repeat of pattern along the chain. The table lists the structures so far proposed, together with their repeat distances, their types of screw axes and the spacing of the first 0*k*0 reflexion to be expected. It is seen that the only structure to give a 1·5-A. reflexion is that of Pauling, Corey and Branson; the one that comes closest to it (1·4 A.) is a topographically similar model with a fourfold screw axis proposed by Bragg, Kendrew and Perutz.

Bamford, Hanby and Happey[7] attempted to interpret their X-ray photograph of poly-γ-benzyl-L-glutamate in terms of a structure possessing a repeat of 5·0–5·6 A. and a screw dyad, which should give a strong reflexion at 2·5–2·8 A. Oscillation photographs of an oriented film of the polymer, kindly lent to me by Dr. A. Elliott, were taken about the normal to the fibre axis, but showed no such reflexion. On the other hand, an extremely powerful reflexion was discovered at a spacing of 1·50 A. (Fig. 1), in agreement with the structure postulated by Pauling and Corey[8]. The presence of this reflexion does not in itself prove the correctness of Pauling and Corey's structure, but taken in conjunction with the favourable agreement of observed and calculated intensities of other reflexions already obtained by these authors, it leaves little doubt about their structure being right.

The reflexion at 1·50 A. was also discovered on oscillation photographs of horse hair and of porcupine quill tip. (It is actually listed as one of the meridional reflexions of porcupine quill tip by MacArthur[9], but its significance does not seem to have been realized.) Fig. 2 is a Geiger counter spectrometer record of horse hair kindly placed at my disposal by Mr. Andrew Lang, which shows the relative intensities of the 5·1-A. and the 1·5-A. reflexions. When hair is stretched to the β-form the 1·5-A. reflexion vanishes.

Pauling and Corey also propose a structure of feather rachis keratin[10]. So far as can be seen from their illustration, this consists to about three-quarters of its volume of 3·7 residue helices, the remaining quarter being made up of 'pleated sheets' of extended chains. If this structure were correct, feather rachis keratin should show the 1·5-A. reflexion. Prolonged exposure of seagull's feather rachis set at the appro-

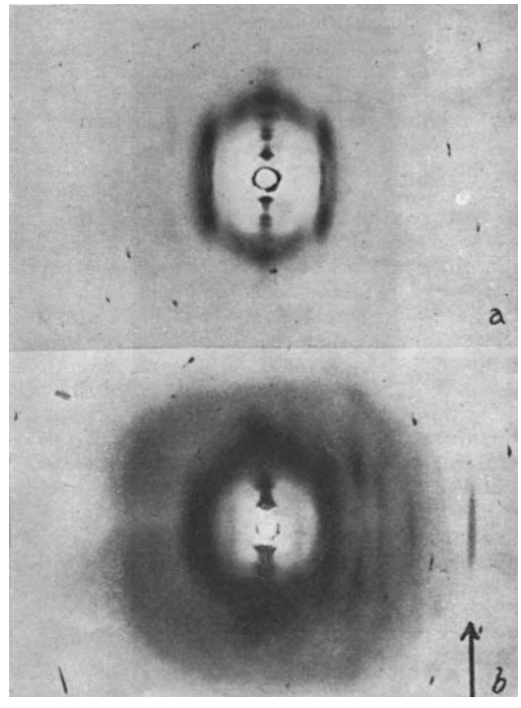

Fig. 1. X-ray photographs of poly-γ-benzyl-L-glutamate. (*a*) Conventional picture with fibre axis horizontal and normal to X-ray beam. (*b*) Fibre axis oscillated between angles of 55° and 85° from X-ray beam. Arrow indicates 1·50-A. reflexion. 3-cm. cylindrical camera, copper *Kα* radiation

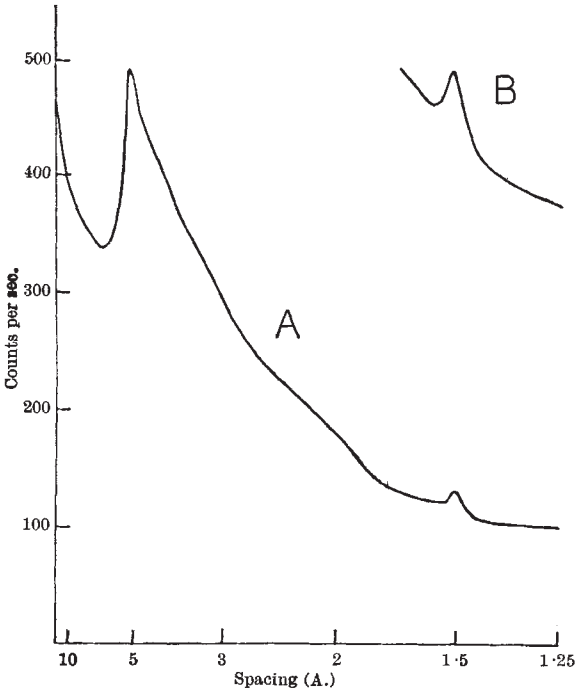

Fig. 2. Geiger counter spectrometer record of horse hair rotated about a normal to the fibre axis. (*A*) Bragg angle for 0*k*0 planes $2\theta = 7°–75°$, taken with reduced aperture of beam. (*B*) Repeat of $2\theta = 55°–75°$ with full aperture of beam

priate Bragg angle revealed no trace of such a reflexion, which shows that the structure proposed by Pauling and Corey must be wrong.

The three-dimensional Patterson synthesis of horse methæmoglobin shows rod-like concentrations of high vector density parallel to the crystallographic *X*-axis[11]. My interpretation of that synthesis led me to the conclusion that the hæmoglobin molecule consists of a compact bundle of close-packed chains running parallel to the *X*-axis and having the α-keratin configuration. The X-ray diffraction pattern of hæmoglobin fades out at a spacing of about 2 A., and no diffraction pattern at smaller spacings had hitherto been observed. Taking the *X*-axis as a possible chain axis, a search was made for reflexions in the 1·5-A. region by taking a 5° oscillation photograph in the appropriate orientation of the crystal. A picture was obtained in which the bulk of the reflexions fade out at a spacing of 2 A. as usual, but protruding from this is a faint bulge with a distinct maximum of intensity at 1·50 A. A preliminary search has not revealed any such effect in other crystallographic directions.

The three-dimensional Patterson synthesis of hæmoglobin shows neighbouring chains to be 10·5 A. apart and arranged in cylindrical close-packing. Taking the density of the protein as 1·30 and the mean residue weight as 112·5 [11], the number of residues in a 'sub-cell', $10·5 \times 10·5 \times 1·5 \times \sin 60° = 143$ A.^{3}, can be calculated:

$$\frac{143 \times 1·3 \times N}{112·5} = 1·00 \text{ residue},$$

which is the number to be expected for the 3·7 residue helix. These results add to the evidence in favour of the hæmoglobin structure proposed by me[11] and indicate that the chains are coiled to form 3·7 residue helices in poly-γ-benzyl-L-glutamate, in α-keratin and in hæmoglobin.

The discovery of the 1·5-A. reflexion shows that even relatively disordered substances like hair may contain an atomic pattern of such high intrinsic regularity that it gives rise to X-ray diffraction effects at spacings where they had never before been suspected.

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Polypeptide Chains in Frog Sartorius Muscle

Oscillation photographs of dried frog sartorius muscle were taken about a direction normal to the fibre axis using a 3-cm. cylindrical camera and copper radiation. The specimens included muscle dried in the stretched, relaxed and contracted state (contraction by electrical stimulus). All photographs show the 1·5-A. reflexion from planes normal to the fibre axis, as described in the preceding communication. The reflexion is most intense on photographs of stretched muscle, slightly weaker on pictures of relaxed muscle, and very faint on photographs of contracted muscle. Thus both stretched and relaxed muscle seem to contain polypeptide chains coiled in the 3·7 residue helix and running parallel to the fibre axis. It is too early to say whether the weakening of the 1·5-A. reflexion on contraction is due to a change in chain configuration or to the disorientation of larger units. In any event, clear evidence on the mechanism of contraction cannot be expected unless X-ray photographs are taken during an actual twitch. In addition to the 1·5-A. reflexion, which can be recognized as an 0*k*0 reflexion by its small angular spread, the photographs also show a wide arc at 2·9–3·0 A. which is about as intense as the 5·1-A. reflexion. This arc is strongest in stretched and weakest in contracted muscle, and was also observed in oscillation photographs of hair.

Our results are incompatible with the mechanism of muscle contraction proposed by Pauling and Corey[1], who suggest that the chains in extended muscle are almost fully stretched, and that they coil up to form 3·7 residue helixes on contraction. On the other hand, our findings are in accord with those of Astbury and Dickinson[2], who showed both extended and relaxed muscle to have the α-keratin structure which becomes disorientated on contraction.

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