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I. M. Squire [a] & A. Elliott [a]
[a] King's College Department of Biophysics, Medical Research Council Biophysics Research Unit, 26-29 Drury Lane, London, W.C., 2
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Liquid Crystalline Phases of Poly-γ-Benzyl-Glutamate in Solution

J. M. SQUIRE and A. ELLIOTT

King's College Department of Biophysics and
Medical Research Council Biophysics Research Unit
26–29 Drury Lane, London, W.C.2

Abstract—Recent work on oriented liquid crystalline solutions of the synthetic polypeptide poly-γ-benzyl-L-glutamate in the " complex " phase is reviewed and some new results reported. The similarity between some features of the X-ray diffraction pattern from this phase and the patterns from certain molluscan muscles suggests that the coiled-coil model for the protein chains in the paramyosin filament of these muscles may not be adequate. A highly ordered liquid crystalline phase of the racemic form of poly-γ-benzyl-glutamate is discussed and some features of its diffraction pattern are explained.

Introduction

Much of the past work on the molecular structure of polypeptides and proteins has been concerned with the possible configurations adopted by the backbones of the polypeptide chains. Quite detailed information about these configurations is now available.[1] The main forms known to occur are the α-helix, the ω-helix, the β-pleated sheet and the cross-β form. A further possible backbone configuration involves α-helices which are themselves wound into super-coil or coiled-coil structures. Each of the four structures named above and also the coiled-coil, has a characteristic X-ray diffraction pattern, although certain features of the diffraction pattern of the α-helix and coiled-coil are similar (since one is derived from the other).

One of the main features expected in the diffraction pattern from a coiled-coil is a strong near-equatorial streak such as that shown in Fig. 1.[2] This is the diffraction pattern from the posterior adductor muscle of *Mytilus edulis* (PAM) in a 50% solution of

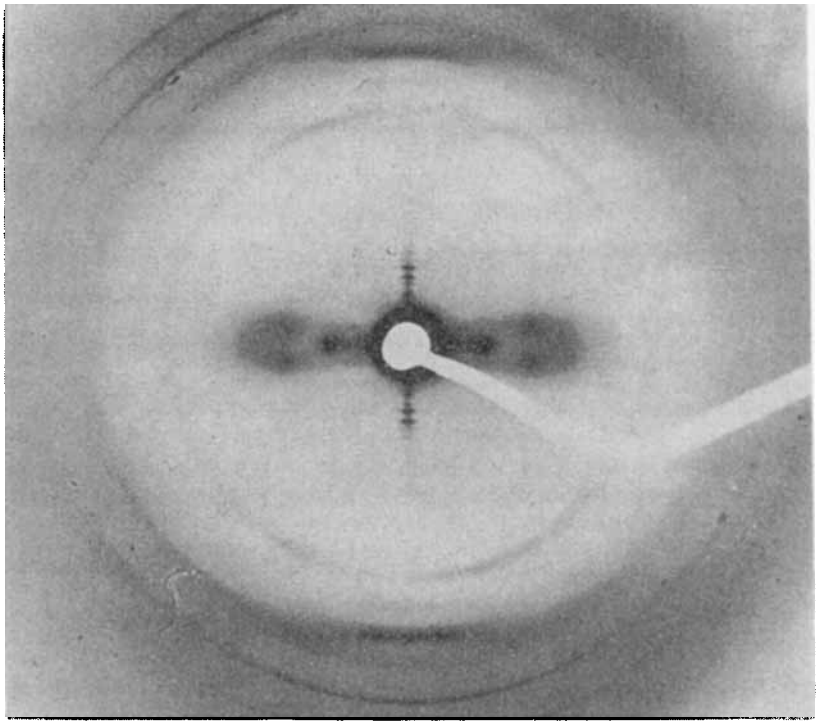

Figure 1. X-ray diffraction pattern from the posterior adductor muscle of *Mytilus edulis* showing the strong near equatorial streaks (muscle fibre normal to X-ray beam). The muscle was wetted with a 50% solution of acetone and water.

acetone in water. All the diffraction patterns shown in this paper have been photographed using a focusing camera with a toroidal mirror.[3]

It has long been supposed that the helical polypeptide chains in α-proteins (as for example in the paramyosin filament of PAM) are coiled-coils.[4, 5] Recently Parry and Elliott[6] have studied solutions of the synthetic polypeptide poly-γ-benzyl-L-glutamate (PBLG) in dimethylformamide (DMF) (see also Luzzati *et al.*[7]). By wetting oriented fibres of this polypeptide with DMF a " complex " phase was found which gave a diffraction pattern similar to that shown in Fig. 2. One feature of this pattern is the

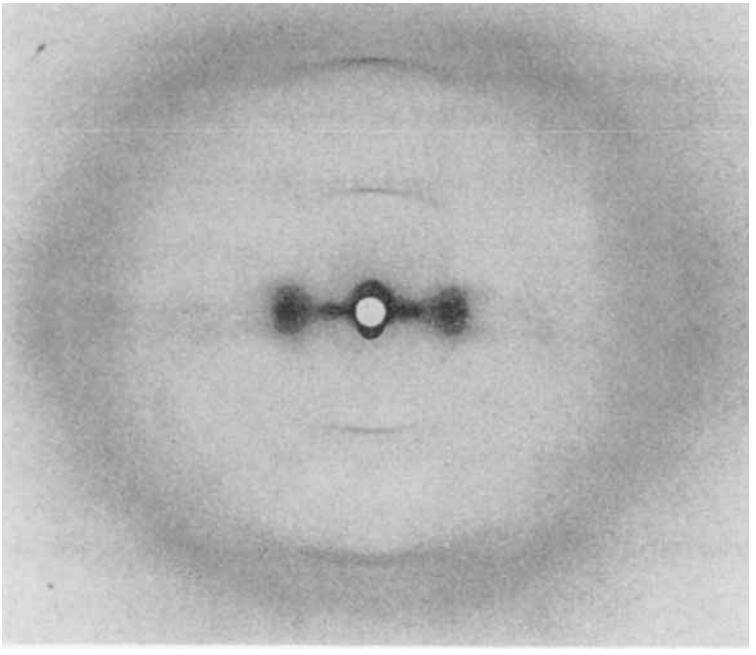

Figure 2. X-ray diffraction pattern from the " complex " phase of poly-γ-benzyl-L-glutamate wetted with benzene. (Flat film: fibre normal to X-ray beam.)

strong near-equatorial streak. It therefore seemed possible that the polypeptide backbone formed a coiled-coil. However a detailed study of other features of the diffraction pattern showed that a coiled-coil structure was not possible.

The " Complex " Phase of Poly-γ-benzyl-L-glutamate

PBLG is a polypeptide with long flexible side-chains terminating in benzyl groups. Work on poly-β-benzyl-L-aspartate[8] has shown that under suitable conditions the benzyl groups in this polypeptide will interact strongly enough to distort the backbone into the ω-form. It therefore seemed likely that in the PBLG " complex " phase the benzyl groups were interacting to produce a regular side-chain arrangement. Since the side-chains are flexible this

arrangement need not necessarily have the same symmetry as the backbone configuration.

Parry and Elliott[6] suggested a possible model for this phase consisting of straight α-helices (not coiled-coils) arranged in a unit cell as shown in Fig. 3. Fig. 4 shows a radial projection from the helix axis of the residues of an α-helix and also some of the other

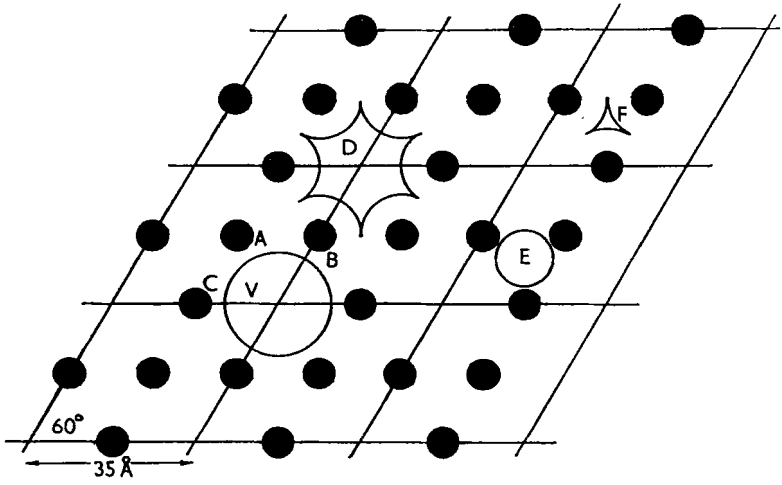

Figure 3. The unit cell of the " complex " phase reproduced from Parry and Elliott[6] by kind permission of the editors of the *Journal of Molecular Biology*.

helices which can be drawn through these residues. The α-helix is the only single helix which can pass through all the residues shown. It has a pitch length of 5.4 Å and is shown as a dotted line in Fig. 4. Other helices can pass through only a fraction of the residues. For example the lines of alternate long and short dashes in Fig. 4 show helices of pitch length 27 Å. Three such helices are required to pass through all the residues. The bold lines in Fig. 4 show helices of even longer period (189 Å) of which seven are required to pass through all the residues. Parry and Elliott found that benzyl groups along adjacent long-period helices of pitch 189 Å could be paired off to form short stacks of benzene rings. A similar stack could be produced in an equiva-

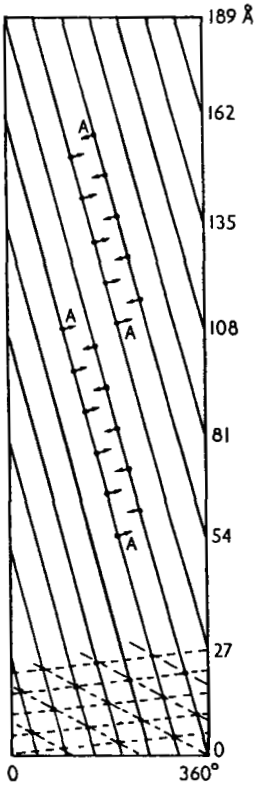

Figure 4. The radial projection of an α-helix showing the genetic helix and other helices of longer period. Reproduced from Parry and Elliott[6] by kind permission of the editors of the *Journal of Molecular Biology*.

lent position 55 Å up or down the molecular axis direction (see Fig. 4). This arrangement is shown in Fig. 5 which is a model of the PBLG molecule made by the present authors. The stacks formed by the benzyl groups can be clearly seen and in the region where the side-chains have been removed the long-period helices from which the stacks are derived are visible. It was found that several such stacks on adjacent molecules could join together to form longer strands of benzyl groups. Figure 6 shows how " stacks " on neighbouring molecules will link up. The " stacks " on the six molecules in equivalent positions around the vacant

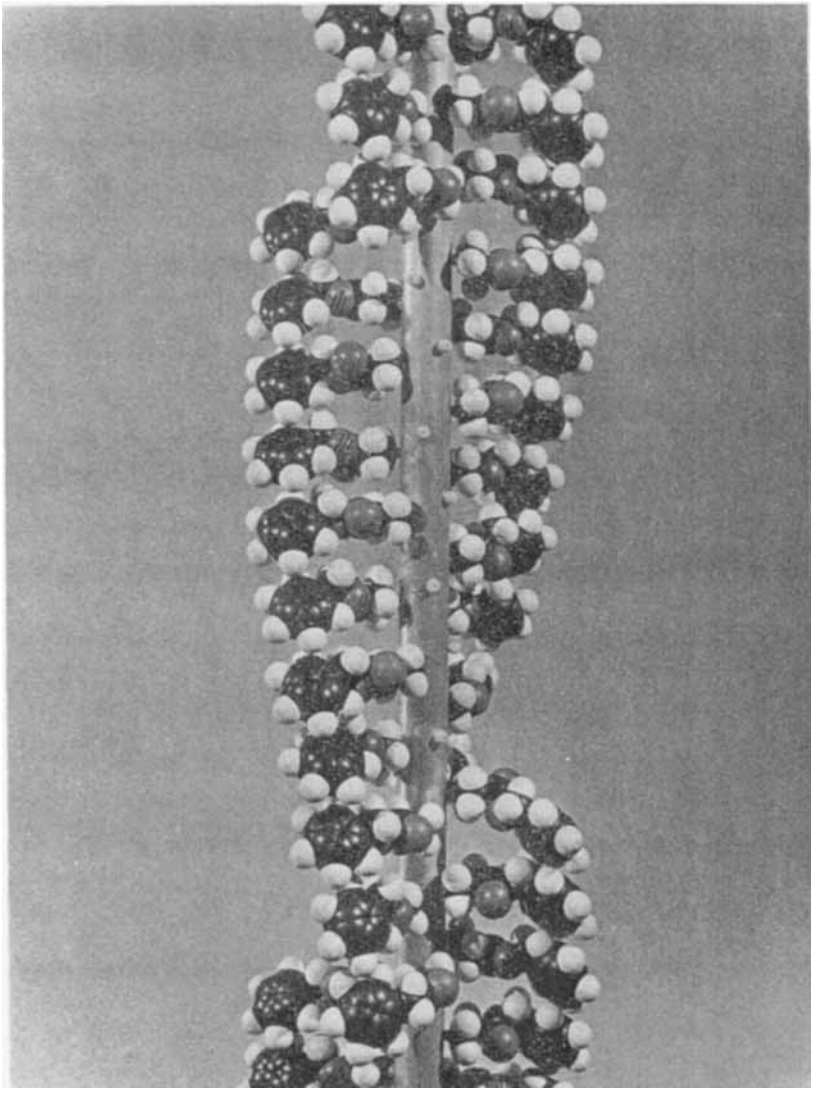

Figure 5. Model of the "complex" phase of PBLG showing the short benzene stacks on one molecule and the long-period helices from which the stacks are derived. The metal tube represents the α-helical backbone out to the α to β carbon bond.

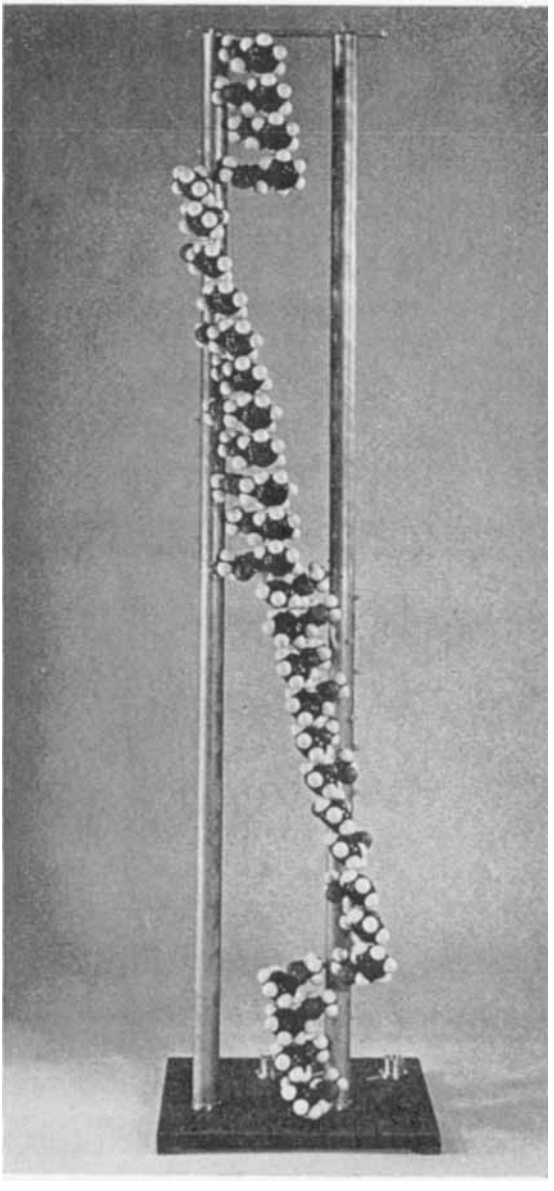

Figure 6. Model of the " complex " phase of PBLG showing the linking of benzene stacks on adjacent molecules (the other side-chains have been removed for clarity).

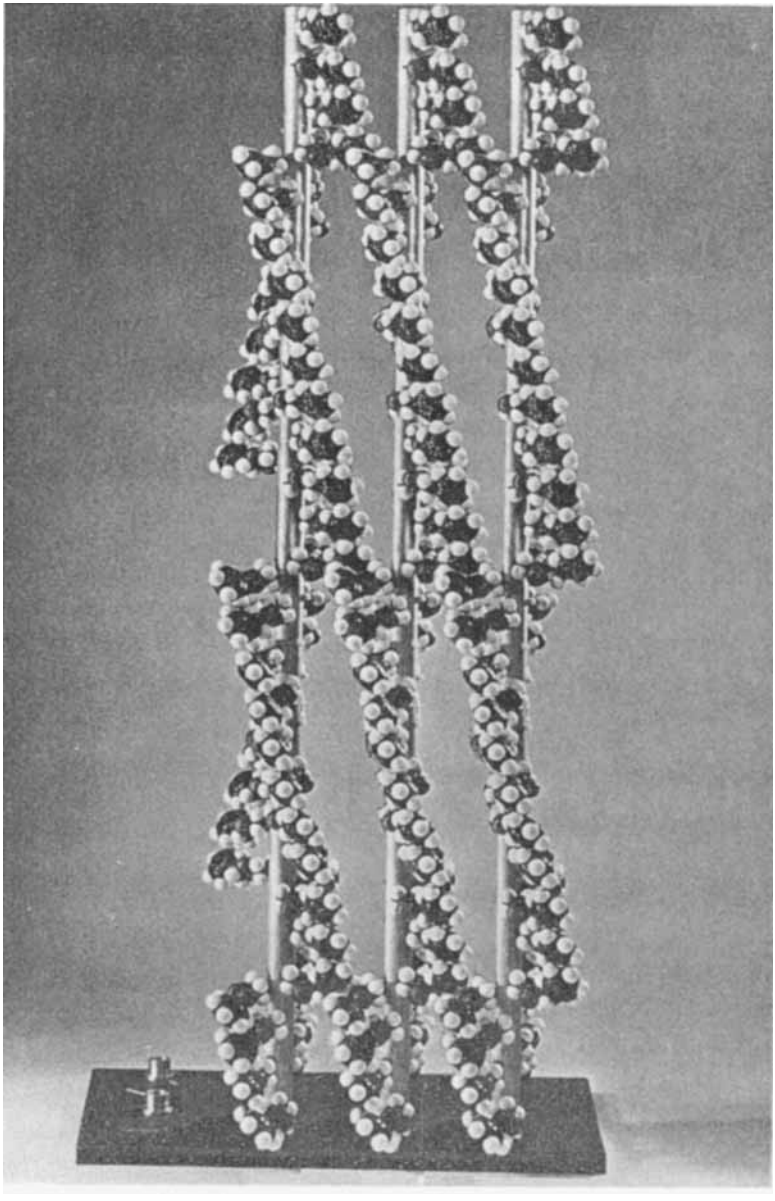

Figure 7. Model of the " complex " phase of PBLG showing how several strands of benzene stacks can form across three molecules.

site marked *D* in Fig. 4 could join up to form a continuous quasi-helical side-chain configuration which in projection would roughly follow the cusped figure shown around *D*. Fig. 7 shows how several strands of benzyl groups can be formed in this case across three molecules. The whole arrangement over the six molecules at *D* in Fig. 4 is a six-stranded benzene quasi-helix with a pitch length of 330 Å. Each short stack is about 55 Å long in an axial direction.

We have studied similar " complex " phases of PBLG in acetophenone and benzene (Fig. 2) and the results of Parry and Elliott have been confirmed. Certain new features in the diffraction pattern can also be accounted for in terms of their model. Other observations support the suggestion by Parry and Elliott that the six-strands of the quasi-helix are not identical (J.M.S. unpublished results). However the proposed structure accounts very satisfactorily for all the features of the observed diffraction pattern. The molecular model shown in Figs. 5, 6, 7 was made and studied by the present authors and the stereochemistry of the structure proposed for the PBLG " complex " phase was found to be very satisfactory.

It is reasonable to conclude that the presence of a near-equatorial streak in α-protein diffraction patterns does not necessarily demand the presence of coiled-coil structures. Indeed Elliott *et al.*[9] have shown that other features of the X-ray diffraction patterns from certain molluscan muscles cannot be interpreted in terms of a simple coiled-coil model. It is likely that whatever conformation the backbone adopts an ordered arrangement of side-chains occurs which contributes significantly to the diffraction patterns observed.

The Racemic Form of Poly-γ-benzyl-glutamate

Parry, Vibert and Elliott (unpublished) have found an interesting liquid crystalline phase of the racemic form of poly-γ-benzyl-glutamate (PBG) in dimethylformamide. Racemic PBG is a mixture of equal parts by weight of poly-γ-benzyl-L-glutamate

and poly-γ-benzyl-D-glutamate. We have studied this phase (obtained from oriented fibres of racemic PBG wetted with DMF) using X-ray diffraction techniques. The medium-angle diffraction pattern from this phase is shown in Fig. 8. Certain features of this pattern together with a 1.5 Å meridional reflection (not shown)

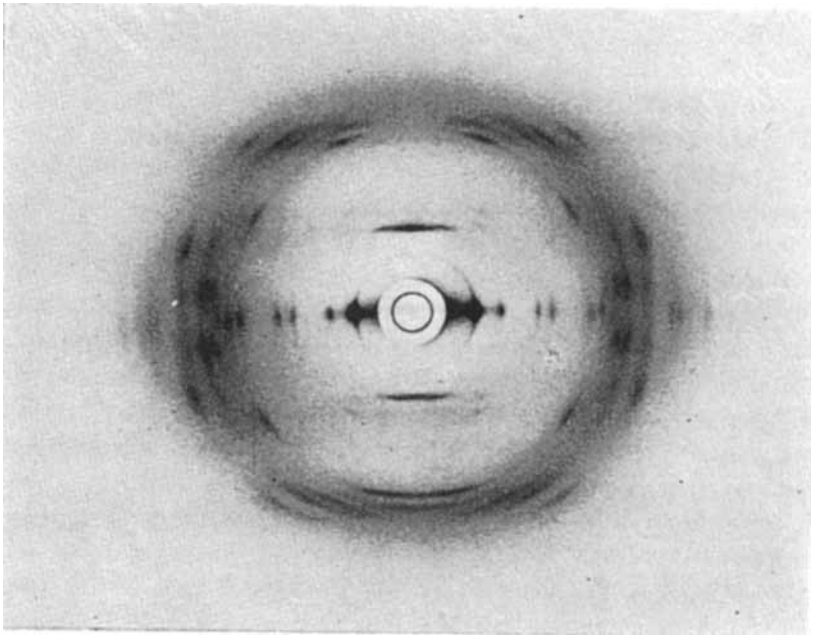

Figure 8. X-ray diffraction pattern from a paracrystalline phase of racemic PBG in dimethylformamide (flat film: fibre tilted 5° from normal to beam).

suggest a straight α-helical backbone configuration. But this alone will not account for all of the diffraction pattern. Once again it seems likely that an ordered side-chain arrangement is involved. (See also Mitsui *et al.*[10]) One interesting feature is that the α-helix backbone is probably a 43/12 α-helix (with repeat of 64 Å) and not a simple 18/5 α-helix with a repeat of 27 Å, which is sometimes found. The very slight distortion required to convert one to the other is probably due to the side-chain interactions.

The unit cell of this phase has a square base but contains α-helices and not ω-helices. Saludjan *et al.*[11,12] have suggested that the square-based unit cells found in solutions of both poly-carbobenzoxy-L-lysine and poly-L-glutamic acid in dimethyl formamide indicate that these polypeptides are in the ω-helix configuration which has four-fold helical symmetry. The validity of this deduction now seems in doubt.

The dimensions of the unit cell obtained from a least squares refinement using all of the observed reflections are

$$a = b = 30.03\,\text{Å}\ (\text{E.S.D.} = 0.03\,\text{Å}),\ c = 64.16\,\text{Å}\ (\text{E.S.D.} = 0.07\,\text{Å});$$
$$\alpha = \beta = \gamma = 90°.$$

It is remarkable that a structure with such a high degree of three-dimensional order should occur in a liquid system. This phase occurs at concentrations by weight of about 70% to 80% polymer. A similar phase has been found to occur in solutions of racemic PBG in acetophenone. The unit cell dimensions are

$$a = b = 29.78\,\text{Å}\ (\text{E.S.D.} = 0.03\,\text{Å}),\ c = 64.16\,\text{Å}\ (\text{E.S.D.} = 0.07\,\text{Å}),$$
$$\alpha = \beta = \gamma = 90°.$$

Once again the degree of three dimensional order is remarkable.

In conclusion, it seems likely that studies of liquid crystalline solutions of different polypeptides may lead to a greater knowledge of the structures which occur in fibrous proteins. For example studies of polypeptides with long side-chains of amino acids which actually occur in muscle proteins may be instructive. Such polypeptides are poly-L-glutamic acid and poly-L-aspartic acid. Work on poly-L-glutamic acid has already been started.

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REFERENCES

1. Elliott A., in Poly-α-amino acids, pp. 1–67, ed. G. Fasman (Marcel Dekker, New York, 1967).

2. Crick, F. H. C., *Acta Cryst.* **6**, 685 (1953).
3. Elliott, A., *J. Sci. Instrum.* **42**, 312 (1965).
4. Crick, F. H. C., *Nature* **170**, 882 (1952).
5. Pauling, L., and Corey, R. B., *Nature* **171**, 59 (1953).
6. Parry, D. A. D., and Elliott, A., *J. Mol. Biol.* **25**, 1 (1967).
7. Luzzati, V., Cesari, M., Spach, G., Masson, F., and Vincent, J. M., *J. Mol. Biol.* **3**, 566 (1961).
8. Bradbury, E. M., Brown, L., Downie, A. R., Elliott, A., Fraser, R. D. B., and Hanby, W. E., *J. Mol. Biol.* **5**, 230 (1962).
9. Elliott, A., Lowy, J., Parry, D. A. D., and Vibert, P. J., *Nature* **218**, 656 (1968).
10. Mitsui, Y., Iitaka, Y., and Tsuboi, M., *J. Mol. Biol.* **24**, 15 (1967).
11. Saludjian, P., de Lozé, C., and Luzzati, V., *Comptes Rendus, Ac. Sci., Paris* **256**, 4297 (1963).
12. Saludjian, P., de Lozé, C., and Luzzati, V., *Comptes Rendus, Ac. Sci., Paris* **256**, 4514 (1963).