Acta Cryst. (1953). **6**, 724

An X-ray Diffraction Investigation of Poly-ε-carbobenzoxy-L-lysine and a Complex Form of Poly-γ-methyl-L-glutamate*

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(*Received* 16 *March* 1953)

X-ray diagrams from oriented fibers of poly-ε-carbobenzoxy-L-lysine and a new form of poly-γ-methyl-L-glutamate have been obtained. Spacing calculations and rough equatorial intensity measurements from the poly-ε-carbobenzoxy-L-lysine photographs indicate that the molecules of this substance have the 3·60-residue α-helix configuration with one molecule in a simple hexagonal unit cell, $a_0 = 16{\cdot}69$ and $c_0 = 26{\cdot}90$ Å. The interpretation of the poly-γ-methyl-L-glutamate photographs is more difficult, but seems to show that a monoclinic unit cell, $a = 29{\cdot}22$, $b = 8{\cdot}39$, $c = 26{\cdot}84$ Å, and $\beta = 110{\cdot}0°$, is the only one that satisfactorily explains both the X-ray data and the observed density of the fibers. The structure of the molecules in this form is probably based on the 3·60-residue α-helix, but there is some evidence which indicates that the helical configuration may be distorted slightly.

Introduction

Interest in the observation and interpretation of the X-ray diffraction patterns given by oriented fibers of synthetic polypeptides has been heightened by the several models for the folded polypeptide chain which have been proposed recently. The most detailed investigations which have been reported are those dealing with fibrous poly-γ-methyl-L-glutamate and poly-γ-benzyl-L-glutamate (Bamford, Hanby & Happey, 1951; Yakel, Pauling & Corey, 1952). In the case of poly-γ-methyl-L-glutamate, the evidence seems to show that the molecules have the configuration of the 18-residue 5-turn α-helix (Pauling & Corey, 1951) or the very similar 29-residue 8-turn α-helix (Bamford, Brown, Elliott, Hanby & Trotter, private communication).

In this paper we report the results of an X-ray diffraction investigation of oriented fibers of poly-ε-carbobenzoxy-L-lysine and of a new complex modification of poly-γ-methyl-L-glutamate, and attempt to explain the results in terms of helical structures.

Poly-ε-carbobenzoxy-L-lysine

Oriented specimens of poly-ε-carbobenzoxy-L-lysine were prepared by casting a film of a concentrated solution of the peptide in dimethyl formamide on glass. The film was then dried, stripped from the glass, and rolled into a fiber. An X-ray photograph taken with a camera of 10-cm. radius showed a fair degree of orientation, not comparable, however, with the orientation obtained earlier with poly-γ-methyl-L-glutamate (Bamford *et al.*, 1951; Yakel *et al.*, 1952). Spacing measurements (Table 1) were made from a photograph directly calibrated with sodium chloride.

Table 1. *X-ray data for poly-ε-carbobenzoxy-L-lysine*

Hexagonal unit cell, $a_0 = 16{\cdot}69 \pm 0{\cdot}15$, $c_0 = 26{\cdot}90 \pm 0{\cdot}30$ Å

		Equator			Probable error in
HK.L	sin θ/λ	d_o (Å)	d_c (Å)	I_o	σ_{d_o} (Å)
10.0	0·035	14·40	14·45	*vvs*	0·12
11.0	0·060	8·31	8·34	*vs*	0·08
20.0	0·069	7·23	7·24	*s*	0·08
21.0	0·091	5·49	5·46	*w*	0·05
30.0	0·107	—	4·82	—	—
22.0	0·120	—	4·17	—	—
31.0	0·125	—	4·01	—	—
40.0	0·138	3·67	3·61	*vvw*	0·03
32.0	0·151	3·35	3·32	*vvw*	0·03
		Fifth layer-line			
10.5	0·099	5·04	5·04	*m*	0·04
11.5	0·111	4·51	4·52	*s*	0·04
		Eighteenth layer-line			
00.18	0·334	1·500	1·495	*m*	0·010

The interplanar distances are compatible with a hexagonal unit cell whose dimensions are

$$a_0 = 16{\cdot}69 \pm 0{\cdot}15,\ c_0 = 26{\cdot}90 \pm 0{\cdot}30 \text{ Å}.$$

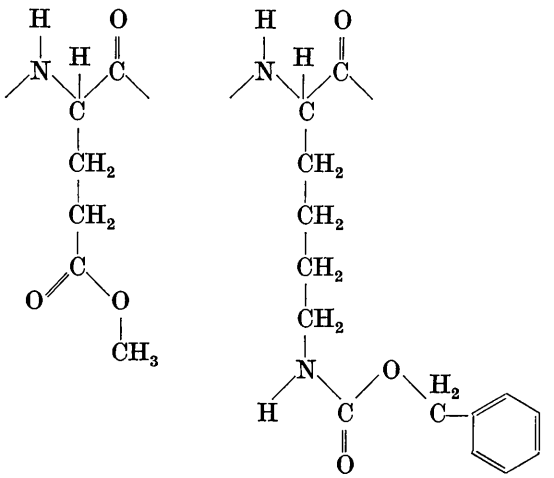
The indices assigned to the observed reflections on the basis of these cell constants, and the calculated spacings, are included in Table 1. It may be observed that the reflections given by this polypeptide have the same indices as those observed for poly-γ-methyl-L-glutamate (Pauling & Corey, 1951). In fact, except for changes arising from the differences in lattice parameters, the two patterns are almost identical.

The lengthening of a_0 from $11{\cdot}58 \pm 0{\cdot}10$ Å in poly-γ-methyl-L-glutamate to $16{\cdot}69 \pm 0{\cdot}15$ Å in poly-ε-

* This work was done under Contract NOnr-220(5) between the California Institute of Technology and the Office of Naval Research.

† Contribution No. 1782.

carbobenzoxy-L-lysine can be interpreted as due to the larger size of the side-chains in the latter compound (see Fig. 1). The difference in the c_0 fiber axis repeat



Poly-γ-methyl-L-glutamate residue. Poly-ε-carbobenzoxy-L-lysine residue.

Fig. 1.

is much smaller (26·75±0·30 Å in poly-γ-methyl-L-glutamate and 26·90±0·30 Å in poly-ε-carbobenzoxy-L-lysine), but none the less real, as a visual comparison of photographs shows.* If one assumes that the basic molecular structure in both cases is helical, this change is not surprising. It can be demonstrated that reasonably small distortions of a helical structure such as the 3·60-residue α-helix in directions parallel to the fiber axis do not seriously weaken intra-chain hydrogen bonds or bend the bonds about the α-carbon atoms. As Edsall (1952) suggests, differences in the repeat distance along the fiber axis, depending on the previous history of the fiber, should be the rule and not the exception.

The calculated density of poly-ε-carbobenzoxy-L-lysine, with the assumption of one 18-residue 5-turn α-helix per unit cell, is 1·21±0·04 g.cm.$^{-3}$. The experimentally observed density is 1·22±0·01 g.cm.$^{-3}$, in fair agreement with the predicted value.

The intensity distribution along the equator of poly-ε-carbobenzoxy-L-lysine photographs supports the conclusion that the molecules have the 3·60-residue α-helix structure, or some configuration closely related to that structure. Fig. 2 shows the approximate relative amplitudes of these reflections plotted on the X-ray scattering curve for the equatorial reflections of the 3·60-residue α-helix given by Pauling & Corey (1951). With allowance for a temperature or disorientation

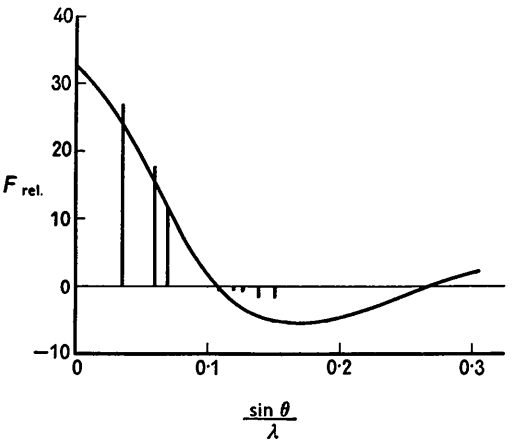


Fig. 2. Calculated scattering curve for equatorial reflections for the 3·60-residue α-helix. The vertical lines represent the visually estimated intensities of equatorial reflections appearing on an X-ray photograph of an oriented poly-ε-carbobenzoxy-L-lysine fiber.

factor, the agreement between the observed data and the curve is good. Although the poor degree of orientation prevents reliable estimation of intensities on the fifth layer-line, the observations are consistent with the calculated intensity curve for the fifth layer-line reflections of the 3·60-residue α-helix (Yakel *et al.*, 1952). The fact that the zero, 5th, and 18th layer-lines are by far the strongest observable on the diffraction diagrams also confirms this structure.

Poly-γ-methyl-L-glutamate

In a previous investigation of poly-γ-methyl-L-glutamate the author reported results obtained with extruded fibers (Yakel *et al.*, 1952). Oriented samples of this polypeptide have recently been prepared by the method outlined above for poly-ε-carbobenzoxy-L-lysine. X-ray diffraction photographs show that these fibers are decidedly less well oriented than the extruded fibers and that the basic unit cell is larger than that of the extruded fibers.

Spacings measured from photographs taken with a helium-filled camera of 10-cm. radius are listed in Table 2, together with the corresponding data for the simpler hexagonal form obtained in these laboratories. The films were calibrated directly with sodium chloride. The reported spacings were indexed on the basis of a monoclinic unit cell with

$$a = 29{\cdot}22\pm0{\cdot}30,\ b = 8{\cdot}39\pm0{\cdot}06,\ c = 26{\cdot}74\pm0{\cdot}30\ \text{Å},$$
$$\text{and}\ \beta = 110{\cdot}0°\pm1{\cdot}0°\ \text{(angle between } a \text{ and } b).$$

With two 3·60-residue α-helices per cell, these parameters give a calculated density of 1·39±0·04 g.cm.$^{-3}$,

* A recent redetermination of the spacing of the 1·5 Å meridional reflection for well oriented poly-γ-methyl-L-glutamate fibers has given the value 1·489±0·010 Å instead of 1·472 Å as reported previously (Yakel *et al.*, 1952). This figure is in good agreement with the value reported by Bamford *et al.* (1951). The value for c_0 in this compound has been recalculated to be 26·75±0·30 Å. The value 11·58 Å for a_0 was not affected by the new measurements. The previously advanced argument concerning the density of poly-γ-methyl-L-glutamate remains valid, since the calculated density is lowered only to 1·38±0·04 g.cm.$^{-3}$, which is still about 5% higher than the directly measured value, 1·31 g.cm.$^{-3}$.

Table 2(*a*). *X-ray data for complex form of poly-γ-methyl-L-glutamate*

Monoclinic unit cell, $a = 29{\cdot}22 \pm 0{\cdot}30$, $b = 8{\cdot}39 \pm 0{\cdot}06$ $c = 26{\cdot}74 \pm 0{\cdot}30$ Å, $\beta = 110{\cdot}0° \pm 1{\cdot}0°$ (angle between a and b)

		Equator			Probable error in
hkl	$\sin\theta/\lambda$	d_o (Å)	d_c (Å)	I_o	d_o (Å)
100	0·018	27·35	27·46	*vs*	0·30
200	0·036	13·87	13·73	*s*	0·15
010	0·064	7·86	7·88	*s*	0·06
310	0·068	7·37	7·34	*m*	0·06
020	0·127	3·94	3·94	*s*	0·04
810	0·139	3·61	3·63	*w*	0·04
910	0·154	3·24	3·24	*w*	0·03
$\bar{7}10$	0·161	3·11	3·11	*w*	0·03
030	0·190	2·63	2·63	*m*	0·03
11,0,0	0·202	2·48	2·50	*w*	0·03
10,3,0	0·215	2·33	2·34	*w*	0·03
11,3,0	0·224	2·23	2·23	*w*	0·03
		Fifth layer-line			
305	0·107	4·65	4·62	*s*	0·05
$\bar{2}15$	0·127	3·94	3·99	*s*	0·05
		Eighteenth layer-line			
0,0,18	0·336	1·488	1·486	*m*	0·010

Table 2(*b*). *X-ray data for simple hexagonal form of poly-γ-methyl-L-glutamate*

Hexagonal unit cell, $a_0 = 11{\cdot}58 \pm 0{\cdot}15$, $c_0 = 26{\cdot}75 \pm 0{\cdot}30$ Å

		Equator			Probable error in
HK.L	$\sin\theta/\lambda$	d_o (Å)	d_c (Å)	I_o	d_o (Å)
10.0	0·050	10·02	10·03	*vvvs*	0·12
11.0	0·087	5·77	5·78	*vs*	0·05
20.0	0·098	5·09	5·02	*w*	0·06
21.0	0·132	3·78	3·79	*s*	0·04
30.0	0·149	3·34	3·34	*m*	0·03
22.0	0·173	2·89	2·90	*w*	0·03
31.0	0·180	2·77	2·78	*w*	0·03
		Second layer-line			
20.2	0·094	5·31	5·31	*w*	0·08
		Third layer-line			
10.3	0·075	6·65	6·66	*w*	0·06
		Fifth layer-line			
10.5	0·107	4·69	4·72	*vvs*	0·05
11.5	0·127	3·95	3·93	*vs*	0·05
20.5	0·137	3·65	3·66	*w*	0·04
		Eighth layer-line			
10.8	0·159	3·15	3·17	*w*	0·05
11.8	0·174	2·87	2·89	*m*	0·04
20.8	0·181	2·76	2·78	*m*	0·04
21.8	0·200	2·50	2·51	*w*	0·03
		Eighteenth layer-line			
00.18	0·336	1·489	1·486	*m*	0·010

which compares satisfactorily with the observed density of $1{\cdot}35 \pm 0{\cdot}02$ g.cm.$^{-3}$.

A unit cell with orthorhombic symmetry,

$$a = 27{\cdot}46 \pm 0{\cdot}30,\ b = 7{\cdot}88 \pm 0{\cdot}06, \text{ and}$$
$$c = 26{\cdot}74 \pm 0{\cdot}30 \text{ Å},$$

also accounts for the observed X-ray data to within their experimental accuracy. With two 3·60-residue α-helices per cell, the above dimensions lead to a calculated density of $1{\cdot}48 \pm 0{\cdot}04$ g.cm.$^{-3}$. This value is so high compared with the observed density that the structure may be eliminated. It should also be noted that the a and b orthorhombic axes given above are in the ratio $2\sqrt{3}:1$, so that a hexagonal unit cell with $a_0 = 31{\cdot}55 \pm 0{\cdot}30$ and $c_0 = 26{\cdot}74 \pm 0{\cdot}30$ Å will also fit the observed data. This cell must also be rejected on the basis of calculated densities of 1·30, 1·48, and 1·67 g.cm.$^{-3}$ for 7, 8, and 9 3·60-residue α-helices per cell, respectively, all of which are incompatible with the observed density.

The differences in the structures of the simple hexagonal form of poly-γ-methyl-L-glutamate may be explained to some extent by changes in the packing of the glutamic ester side-chains. If the assignment of a monoclinic unit cell is correct, the diameter of the polypeptide molecules must be about 8·4 Å in the b direction, as compared with 11·6 Å in the simple hexagonal form. While the reduction in size is very large, it seems possible that the bulky side-chains might be reoriented so as to shrink the molecules in one direction, b, while extending them in other directions. It is possible that the helical shape of the polypeptide chains of the molecules is distorted. Some evidence for distortion can be found in the relative intensities of the equatorial reflections from these fibers, which do not follow the predicted scattering curve for the 3·60-residue α-helix; the discrepancies may be due, however, to the effect of the side chains. The fact that the zero, 5th, and 18th layer-lines are the most intense found on X-ray diagrams of this form indicates that the departure from the 3·60-residue α-helix configuration cannot be great.

The presence of the strong 27·35 Å spacing on the equator also shows that the monoclinic unit cell is not end-centered; the molecules must lie in some sort of double layer parallel to the fiber axis.

The only difference between the two modifications other than the method of their preparation was the lengths of time which the solutions of poly-γ-methyl-L-glutamate in dimethyl formamide were allowed to stand before preparation of the fibers. The complex form was found only in fibers prepared from the older solutions. Since a slow polymerization reaction probably occurs in solution, it seems likely that this modification contains longer polypeptide chains than the simpler form. Longer average chain length may account for the relatively poor orientation of the fibers, but it is difficult to see how it could be responsible for

the variation in side-chain or main-chain configuration.

The author wishes to express his gratitude to Dr Mark A. Stahmann of the College of Agriculture at the University of Wisconsin for making available a sample of poly-ε-carbobenzoxy-L-lysine for use in this investigation and to Profs. Linus Pauling and Robert B. Corey of the California Institute of Technology for their many helpful criticisms.

References

BAMFORD, C. H., BROWN, L., ELLIOTT, A., HANBY, W. E. & TROTTER, I. F. Private communication.
BAMFORD, C. H., HANBY, W. E. & HAPPEY, F. (1951). *Proc. Roy. Soc.* A, **205**, 47.
EDSALL, J. T. (1952). *Nature, Lond.* **170**, 53.
PAULING, L. & COREY, R. B. (1951). *Proc. Nat. Acad. Sci., Wash.* **37**, 241.
YAKEL, H. L., JR., PAULING, L. & COREY, R. B. (1952). *Nature, Lond.* **169**, 920.

Acta Cryst. (1953). **6**, 727

A Simple but Versatile Strip Technique for Calculating Structure Factors

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(*Received* 6 *February* 1953 *and in revised form* 25 *March* 1953)

Two new sets of strips especially designed for the rapid calculation of structure factors are described. The smaller set, consisting of but 150 basic strips, requires that the parameters be expressed with two-place accuracy, as in the earlier stages of a structure analysis. The second set consists of 1500 basic strips and provides for the calculation of exact structure factors for parameters accurate to 0·001. These new strips overcome several weaknesses inherent in the application of Beevers–Lipson 3° Fourier strips to structure-factor calculations. Either set permits the calculation of structure factors involving terms of the important product type, $\sin 2\pi hx . \sin 2\pi ky$, etc., at the rate of 20–25 per hour in typical cases.

1. Introduction

Beevers & Lipson (1952) have recently shown how a set of standard Fourier strips at 3° intervals (Beevers, 1952) can be applied to the calculation of structure factors. Although their procedure represents a definite step forward in reducing the labor of such calculations, it nevertheless lacks the desired degree of versatility in several respects. The method is chiefly of value in evaluating simple structure factor formulas consisting of terms of the types $\sin 2\pi hx$ or $\cos 2\pi hx$. The frequently occurring formulas involving product terms, such as $\sin 2\pi hx . \sin 2\pi ky$ for example, cannot be handled in such a straightforward manner. Instead, they must be solved by first computing numerically and accurately the sine or cosine functions corresponding to the larger of the two cell dimensions involved, say, the function of y, after which these factors are made the amplitudes of a set of strips used to compute the final sum by way of a summation in x, the parameter corresponding to the shorter cell dimension. This hybrid method of computation is not only cumbersome and inherently unsystematic in character, but it still leads to undesirably large errors if the shorter dimension is much larger than 6 Å. In view of the large unit-cell dimensions encountered in many present-day structural investigations, particularly among organic compounds, this represents a rather severe limitation of the method.

Beevers & Lipson (1952) also describe a modified procedure for increasing the accuracy, but it more than doubles the computational work besides increasing the likelihood of errors. A less serious drawback of both these methods for calculating structure factors is that no strips are available for direct calculations at parameters exceeding 0·25, with the result that special rules must be observed governing the choice of strips and changes in sign in the range 0·25–1·00. The several objections just cited combine to make the computational procedures far from routine. Surely a high degree of routineness should be a feature of any really valuable aid to structure factor calculations, because the probability of errors occurring is more or less proportional to the extent to which the human element must be reckoned with in the operations.

2. New structure-factor strips

Most of the above objections stem from the fact that the Beevers–Lipson method seeks to apply standard Fourier strips to the solution of a problem for which they were not primarily designed. To be specific, Fourier strips must bear values of $A \sin nh\theta$ and $A \cos nh\theta$ corresponding to variations in $n\theta$ of from 0 to $\pi/2$ radians and to variations in h of from 1 to at least 30, which means that a net range in the argument $nh\theta$ of about 0 to 15π radians is needed. For 6°