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ELECTRON-DIFFRACTION STUDY OF POLY-γ-METHYL-*L*-GLUTAMATE

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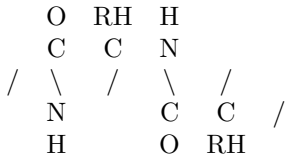
The electron-diffraction method, which has been successfully used for the analysis of the structure of various polymers ([1]), has not until now been applied to the study of synthetic polypeptides, substances that model the structure and properties of proteins. The structure of synthetic polypeptides has been studied mainly by means of X-ray analysis.

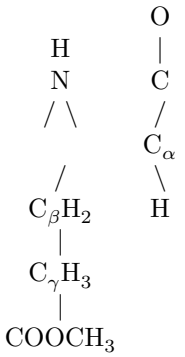
Native proteins are built on the basis of the polypeptide chain



where each amino-acid residue is characterized by one or another side radical R, the number of which, as is known, exceeds 20–25. The individuality of a protein is determined by its primary structure, i.e., by the sequence of R along the chain; by its secondary structure—the mode of spatial organization of the polypeptide chain as such, for example into a helix or a flat extended chain; and by its tertiary structure—the packing of the chain into a globular or fibrillar molecule.

Synthetic polypeptides ([2]) can so far be synthesized with one, more rarely two or three, types of R. These substances, which exhibit many properties of proteins, are the most convenient for the study of secondary structure, i.e., the spatial arrangement of the chain, determined primarily by the pattern of closure of hydrogen bonds NH ... O within the chain or with neighboring chains.

In the present work, poly-γ-methyl-*L*-glutamate, synthesized in the laboratory of K. Bamford ([2]), was investigated by electron diffraction. The use of modern experimental techniques and methods of structural electron diffraction, in particular the construction of Fourier series of the potential ([3]), made it possible to obtain new data on this already classic object.



(1)

(1) is the formula of its residue.

For preparing specimens for electron-diffraction study, the polypeptide was dissolved in chloroform with a small addition of dichloroacetic acid. One or two drops of the solution were lowered onto the surface of distilled water. The film that formed was picked up on a special frame, stretched on it, dried, and placed in the electron-diffraction apparatus. Stretching, which reached 1000% and more, oriented the polymer. The instrument was a horizontal EG electron-diffraction camera ([4]). The accelerating voltage was ~ 55 kV.

From the electron-diffraction patterns of various types obtained, for detailed analysis of the structure photographs with sharp reflections in the form of arcs located on the equator and meridian, as well as along well-expressed–

layer lines. On photographs of this type, 41 reflections were recorded. In X-ray studies [(5)], 32 reflections were obtained, 4 of which could not be indexed.

The presence of a clearly expressed 1.49 Å reflection on the meridian, as well as a number of other features of the electron diffraction pattern, indicated that in our specimens the polypeptide had the secondary structure of the Pauling-Corey α-helix [(6)], in which there are approximately 18 residues per 5 turns (18/5 helix). Each amino group is linked by a hydrogen bond to the third amino group along the chain. The residues lie on a helix whose pitch is ~ 5.4 Å; the axial translation of successive residues is ~ 1.5 Å.

The hexagonal unit cell found from the electron diffraction patterns,

$a = 11.96$ Å, $c = 26.8$ Å,

is close to the cell proposed on the basis of X-ray analysis:

$a = 11.98$ Å, $c = 27.0$ Å [(7)].

As is known [(8)], the intensities of reflections from helical structures are determined by Bessel functions J_n, so that those of these functions which obey the selection rule contribute to a layer line of number l:

$$\frac{l}{c} = \frac{n}{P} + \frac{m}{p}. \tag{2}$$

Here c is the full period of the helix, P is its pitch, and p is the projection of the distance between residues on the axis. For the 18/5 α-helix of poly-γ-methyl-L-glutamate we have:

$P = 5.4$ Å, $p = 1.5$ Å, $P/p = 18/5$, $5n + 18m = l$; $m = 0, \pm 1, \pm 2, ...$

The reflections observed on the electron diffraction patterns generally obey the selection rule. However, there is one serious violation of this rule (2). According to it, nonzero intensity values for meridional reflections are possible only when, for a given l, $n = 0$, since all the other Bessel functions $J_{n\neq 0}$ have zero value on the meridian. Thus, all meridional reflections except 00.18, 00.36, etc., are "forbidden." However, we observed a number of $00l$ reflections, for example 006, 007, 008, etc. The strongest of them is the (006) reflection, with interplanar spacing $d = 4.47$ Å. A similar reflection was also observed in X-ray patterns. Below we shall briefly consider the reasons for the appearance of these reflections.

The structure of the polypeptide was analyzed by constructing a Fourier projection of the potential of this structure onto the basal plane, i.e., along the helix axis. This projection is completely determined by equatorial reflections. We recorded 15 such reflections and estimated their intensities I, combining visual and microphotometric estimation. The transition from intensities to the moduli of structural amplitudes was made according to the formula

$$|\Phi| = \sqrt{I/pd^2}, \tag{3}$$

where p is the multiplicity factor.

The signs of $|\Phi|$ were found by theoretical calculation of the amplitudes. If only the distances r_j of the atoms from the helix axis are specified, then in the first approximation this calculation can be performed by the formula

$$\Phi_{hk0} = \sum_j f_j J_0\{2\pi r_j/d\,(hk0)\}, \tag{4}$$

where f_j is the atomic factor for electron scattering, and J_0 is the Bessel function of zeroth order. By continuously varying the quantity $d = 1/R$, where R is the distance in reciprocal space from the origin, one can obtain an integral continuous curve—the radial component of the Fourier–Bessel transform of a given structure (Fig. 1). Such an analysis for the atoms of the α-helix proper shows [(2)], which was also confirmed by our calculation, that the first three reflections, 100, 110, and 200, have a positive sign, and are followed by a negative region of the transform. The magnitude and sign of more distant reflections depend on the configuration of the atoms of the side radical.

Figure 1

Figure 1: Figure 1

Since the α-helices in our specimens are packed into a crystalline lattice, more exact calculations of the amplitudes Φ_{hk0} are obtained by

formulas for crystallographic structural factors [9]. The projection onto the basal plane, for example, of right-handed α-helices in hexagonal packing is described by the plane symmetry group $P6$; its structural factor has the form:

$$A = -2\{\cos 2\pi(hx + ky) + \cos 2\pi[kx - (h + k)y] + \cos 2\pi[hy - (h + k)x]\}. \quad (5)$$

As independent variables in this formula one must substitute x, y—the coordinates of three residues (1), rotated relative to one another in projection by 20°. The remaining residues are derived from these three by a sixth-order axis.

Using literature data [10] on the structure of L-glutamic acid, we considered, on models, various possible positions of the side radical with the β-carbon atom in position I, which corresponds to a right-handed helix with left-handed amino-acid residues. It turned out that, although there is considerable scatter in the magnitude of the amplitudes for these variants, the signs needed for constructing the synthesis, both in the calculation by formula (4) and by formula (5), change hardly at all. The first three amplitudes and the last three have a plus sign, the remaining ones a minus sign. A comparison of the calculated and computed amplitudes is given in Fig. 1.

Fig. 1. Experimental (a) and theoretical (b) values of the structural amplitudes Φ_{hk0}. The dashed line is the Fourier–Bessel transform line.

Next, a projection of the Fourier potential was constructed (Fig. 2). We note that if one proceeds from the plane group $P6$, which has no symmetry planes, then the reflections $hki0$ and $khi0$ must have different magnitudes. On the other hand, in our samples of poly-γ-methyl-L-glutamate one should assume an equal fraction of right-handed α-helices with the peptide sequence oriented in opposite directions relative to the stretching axis, since there are no reasons that could cause, during crystallization from solution and stretching, the predominance of helices of one sequence. This means that, in projection onto the basal plane, helices occur in equal measure both with the sequence clockwise and counter-clockwise. In other words, a "statistical" plane of symmetry of the projection arises, and the plane group describing such a structure becomes $P6m$ [9]. In this group the reflections $hki0$ and $khi0$ are equal in magnitude, and their signs coincide with the signs found from formula (5) for the enantiomorphic group $P6$.

Fig. 2. Projection of the Fourier potential of poly-γ-methyl-L-glutamate onto the basal plane (in arbitrary units). Dots are projections of atoms of the side

Figure 2

Figure 2: Figure 2

Fig. 3. Structural model

Figure 3: Fig. 3. Structural model

radicals of the α-helix. The shaded region is the region of mutual penetration of atoms of the side chains.

The maxima of the synthesis (Fig. 2) correspond to atoms or groups of atoms. The strong ring of potential with a minimum near the origin is the projection of the atoms of the peptide chain itself that have merged with one another—N, C', O, C_α—of the α-helix. As the distance from the origin increases, ring-like

the regions of large values of the potential begin to break up into individual peaks. As shown in Fig. 2, the protrusions and the first row of maxima are readily identified as the atoms C_β. It is interesting to note that the two following rings of maxima contain 18 peaks each, though not quite equal in magnitude.

Thus, independently of any assumptions, the projection of the potential approximately reveals the 18-fold symmetry inherent in the α-helix. In the first of these rings are located the atoms C_γ, C_O, O_1, and in the second O_2 and CH_3 (see the diagram in Fig. 2). The choice of the best variant for the arrangement of the side radicals, presented in the figure, was made on the basis of the Fourier projection obtained and of bringing the theoretical amplitudes into agreement with the experimental ones. The possibility of mutual packing of α-helices was checked on models.

Fig. 3. Structural model

The coordinates x and y and the distances r from the helix axis for one chain of the model found are given in Table 1. The atoms of the remaining chains are obtained from these by rotation through 20°.

The structural model is shown in Fig. 3. This model, as follows from the already discussed presence of forbidden reflections $00l$, of which the strongest is 006, is idealized. If all the side radicals were oriented in exactly the same way, only the reflections 00.18, 00.36, etc. would be observed. Another confirmation of the fact that the "attachment" of the side radicals to the polypeptide chain is not quite identical is the circumstance that in both rings of the potential projection, containing 18 peaks each and indicating (pseudo)symmetry of order 18, these peaks are not quite equal in magnitude. The true symmetry of these rings is sixfold, which correlates with the presence of a strong 006 reflection. Thus, deviations in the orientation

Table 1

Atom	x	y	r	Atom	x	y	r
C_α	0.170	0.203	2.29	C_O	0.492	0.316	5.20
C_β	0.300	0.247	3.34	C_M	0.533	0.140	5.70
C_γ	0.417	0.362	4.75	O_2	0.596	0.413	6.40
O_1	0.459	0.193	4.80				

of the side radicals appear both in the projection onto the helix axis and in the projection along this axis—onto the basal plane.

Thus, the electron-diffraction method has made it possible to substantially supplement the data on the α-helical structure of poly-γ-methyl-L-glutamate obtained by X-ray analysis ([2]).

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