

Anomalous properties of poly(γ -benzyl L-glutamate) film composed of unusual 7/2 helices

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(Received 6 February 1984; revised 1 May 1984)

Poly(γ -benzyl L-glutamate) film composed of unusual 7-residue 2-turn (7/2) helices was prepared by slow casting from chloroform solution. The 7/2 helical conformation was irreversibly transformed at 84°C to the normal 18/5 conformation. In d.s.c. thermograms the transformation appeared as an endothermic first order transition with an enthalpy change of 3.4 cal g⁻¹. The viscoelastic and dielectric measurements indicated that the loss peak due to the side chain motion in this film was reduced to a great extent compared to the film of 18/5 helices. The dielectric relaxation strength dictated that there was a motional restriction in three quarters of the side chains. By comparison to a racemic mixture of poly(γ -benzyl glutamate), these anomalous properties were attributed to the stack of terminal benzyl groups of side chains (formed in an alternating manner between neighbouring chains). The first order transition arises from the cooperative fusion of the stack and the side chains with their benzyl groups which are restricted in thermal motion. Such stacks may be facilitated by the precise 'knob (side chain) and hole (space between side chains)' packing which can be attained between neighbouring 7/2 helices.

(Keywords: poly(γ -benzyl L-glutamate); 7/2 helical conformation; side chain motion; stack of benzyl groups; dielectric measurement)

INTRODUCTION

Poly(γ -benzyl L-glutamate) (PBLG) film composed of α -helices has two typical modifications, B and C, which depend on the casting solvents^{1,2}. Form B, prepared from dimethyl formamide, showed Bragg reflections on the layer lines as well as on the equator. The spacings were assigned to a monoclinic unit cell ($a=29.0$ Å, $b=13.2$ Å, $c=27.2$ Å, $\alpha=\gamma=90^\circ$, and $\beta=96^\circ$) with two chains of opposite chain directions. Another form C from chloroform solution displayed continuous scattering on the layer lines and reflections on the equator. This form, therefore, is a nematic-like paracrystal in which the packing of α -helices is periodic in the direction perpendicular to the chain axis ($a=14.8$ – 15.2 Å, $b=14.3$ – 14.8 Å and $\gamma=118^\circ$ – 120°), but the relative levels of α -helices along the chain axes are displaced together with the randomness of chain direction.

Apart from such variety of packing structure of α -helices^{1,3-5}, considerable detailed information concerning the possible helical conformations adopted by the synthetic polypeptide chains has been gathered⁶⁻¹¹. The PBLG as well as other poly(L-glutamates), has been found to take up the normal right handed α -helix with 18-residue 5-turn (18/5) whereas the different ω -helical and π -helical forms have been indicated for poly(L-aspartates)^{7,8}. However, Samulski and Tobolsky¹² have reported that a helix distorted from the normal α -helix, is produced in oriented PBLG film prepared from chloroform solution when a strong magnetic field is applied. X-ray observation of this film showed that the helix was of 7-residue 2-turn (7/2) type. According to the formulas by Sugeta and Miya-

zawa¹³, which are able to transpose between helical parameters due to X-ray data and internal coordinates of main chain helix, we elucidated that 7/2 helix had a set of torsion angles ($\phi=-53.0^\circ$ around N-C' bonds, $\psi=-50.0^\circ$ around C $_{\alpha}$ -C' bonds) and normal 18/5 helix has the coordinates (-52.7° , -51.4°)¹⁴. The difference in torsion angles between them is more or less a few degrees and so 7/2 helix lies close to the region of allowed conformation (right handed 18/5 helix) when the torsion angles are plotted on an energy contour map of poly(L-alanine)¹⁵. Despite this fact, the reason why PBLG assumes the unusual 7/2 helical conformation should be presented.

In this paper, we have extended the investigation of this distorted 7/2 helix of PBLG by observing the viscoelastic and dielectric properties with respect to the side chain motion and shown that the 7/2 helix is favoured by a cooperative stacking arrangement of benzene rings at the end of side chains.

EXPERIMENTAL

Materials

PBLG ($M_v=80000$) was synthesized by the NCA method using triethylamine as an initiator. The film composed of the 7/2 helices was prepared by slow casting of a solution in chloroform during two or three months on a glass plate. The film thus obtained was opaque and brittle, different from the transparent film of normal 18/5 helices which can be prepared at usual casting rates over one or two days.

Methods

Wide angle X-ray patterns were recorded with flat plate and cylindrical cameras, using a Rigaku-Denki X-ray generator with Ni-filtered $\text{CuK}\alpha$ radiation. The camera distance was calibrated using Si powder coated on the samples. Thermal measurements were made on a differential scanning calorimeter (Perkin Elmer DSC II). Viscoelastic measurements were performed with a Vibron viscoelastomer (Toyo Baldwin Co., Ltd.), in the temperature range from -50°C – 200°C at 110 Hz. Dielectric measurements were conducted with a mutual inductance bridge (TR-1C), a detector (BAD-18) and an oscillator (WBG-3B) manufactured by Ando Electric Co., Ltd., over a frequency range from 30 Hz to 1 MHz. For this measurement, samples were painted with conductive silver paste in order to assure complete contact with the electrodes.

RESULTS

X-ray diffraction pattern

Figure 1(a) shows the X-ray pattern of the specimen from the partly oriented portion, where the X-ray beam was irradiated parallel to the film surface [edge viewed (EV)]. The Figure indicates the characteristic layer line pattern consisting of streaks alone. The spacings and intensities of layer line streaks are listed in Table 1. A set of layer lines are equally spaced about $1/10.5 \text{ \AA}^{-1}$ apart and

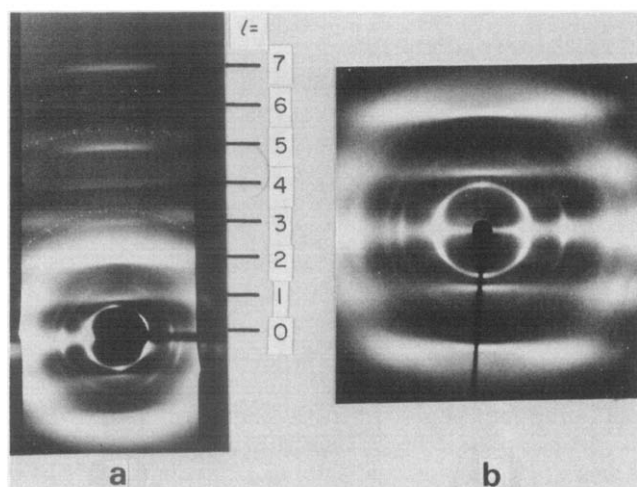


Figure 1 X-ray (edge viewed) photographs of the C(7/2) form. Photographs (a) and (b) were taken by cylindrical and flat cameras, respectively. In (a), the oriented films were set on the sample holder so that the chain axes are tilted 30° normal to the beam. In (b) an elongated view of the low angle region in (a) is shown

the meridional reflection with the spacing of 1.5 \AA , corresponding to the residue height of the helix, arises on $l=7$. The helix, thus, appears to be of a $7/2$ type, with a repeat of pattern of 10.5 \AA . In Table 1, the observed spacings are compared with those of a $7/2$ helix with a repeat of 10.5 \AA , and the agreement is very good. This assignment of $7/2$ helical conformation is also supported by the selection rule for Bessel function order, n , where the lower value of $|n|$ contributes to the higher intensity of layer line¹⁶. In Table 1, the values of n based on the $7/2$ helical conformation are listed. The observed intensity distribution qualitatively corresponds to this rule; the intense layer lines appear on $l=2$ and 5 having $|n|=1$, whereas the weak layer lines arise on $l=1$ and 6 with $|n|=3$.

In contrast to the layer line appearance with streaks, several reflections are observed on the equator (see Figure 1(b)). As found in Table 1, they are assigned to the lateral packing structure with a hexagonal unit cell of $a=b=14.8 \text{ \AA}$ and $\gamma=120^\circ$. Thus, this film has the same nematic-like packing structure as observed previously in form C composed of $18/5$ helices¹. As a consequence, only a chain conformation distinguishes the present film from the previous form C. For brevity, both films were designated by form C(7/2) and form C(18/5).

D.s.c. thermograms

The C(7/2) form is characterized by the irreversible first order transition at 84°C as seen in the d.s.c. thermogram of Figure 2. The enthalpy change, ΔH , is 3.4 cal g^{-1} . On this transition, the brittle and opaque film changed to the transparent one with some elasticity. In addition, the X-ray observation displayed the clear conformational change so that the $7/2$ helical conformation was transformed to $18/5$ conformation. The recovery to $7/2$ helix has never been achieved even by the slow cooling of $0.01^\circ\text{C min}^{-1}$, signifying that the $7/2$ helical conformation is metastable as commented by Samulski and Tobolsky¹².

Viscoelastic and dielectric properties

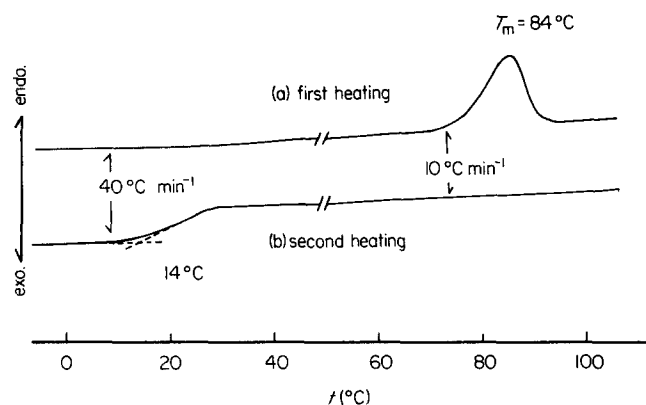
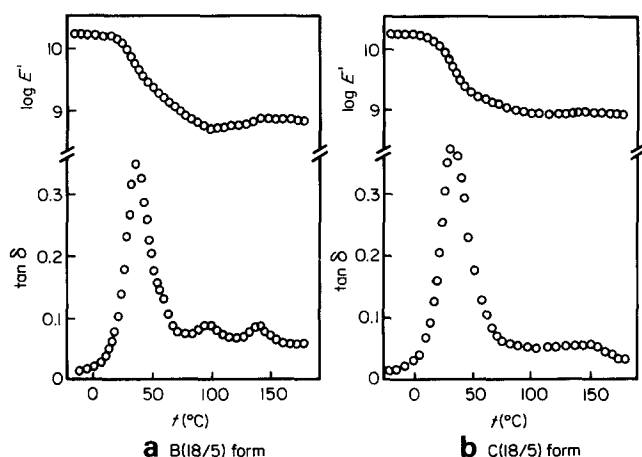
Figures 3(a) and 3(b) show the viscoelastic data for forms B(18/5) and C(18/5), respectively, where the data were obtained at the frequency of 110 Hz. As can be seen in these Figures, the viscoelastic properties of PBLG are described by the remarkable loss peak ($\tan \delta=0.35$) around 40°C accompanied with a decrease in Young's modulus (E') of almost one decade and the maximum excursion in a higher temperature range above 100°C . The former is attributed to the onset of side chain motion^{17,18} and the latter may be due to some kinds of motion with respect to α -helices¹⁹. No definite difference is noticed

Table 1 X-ray data of form C(7/2)

$d_{\text{obs.}}$ (Å)	$d_{\text{calc.}}$ (Å) ^a	layer line streaks			n	equatorial reflections			
		layer line ^a	intensity			$d_{\text{obs.}}$ (Å)	$d_{\text{calc.}}$ (Å) ^b	h	k
10.53	10.50	1	w		12.81	12.82	1	0	0
5.27	5.25	2	vs	–3	7.37	7.40	1	1	0
3.51	3.50	3	m	1	6.41	6.41	2	0	0
2.64	2.63	4	w	–2	4.84	4.84	2	1	0
2.12	2.10	5	s	2	~4.2	4.27	3	0	0
~1.8	1.75	6	vw	–1	–	–	–	–	–
1.50	1.50	7	s	3	–	–	–	–	–
				0	–	–	–	–	–

^a Calculated spacings and indices are based on $7/2$ helix with a repeat of 10.50 \AA

^b Calculated spacings and indices are based on the hexagonal unit cell with dimensions of $a=b=14.8 \text{ \AA}$ and $\gamma=120^\circ$


Figure 2 D.s.c. thermograms of form C(7/2)

Figure 3 Temperature dependence of E' and $\tan \delta$ for: (a) B(18/5) form; (b) C(18/5) form. The data were obtained at a frequency of 110 Hz

between both forms, exhibiting that the dissimilarity in the packing structure, as presented in a previous paper¹, does not significantly reflect the type of molecular motion.

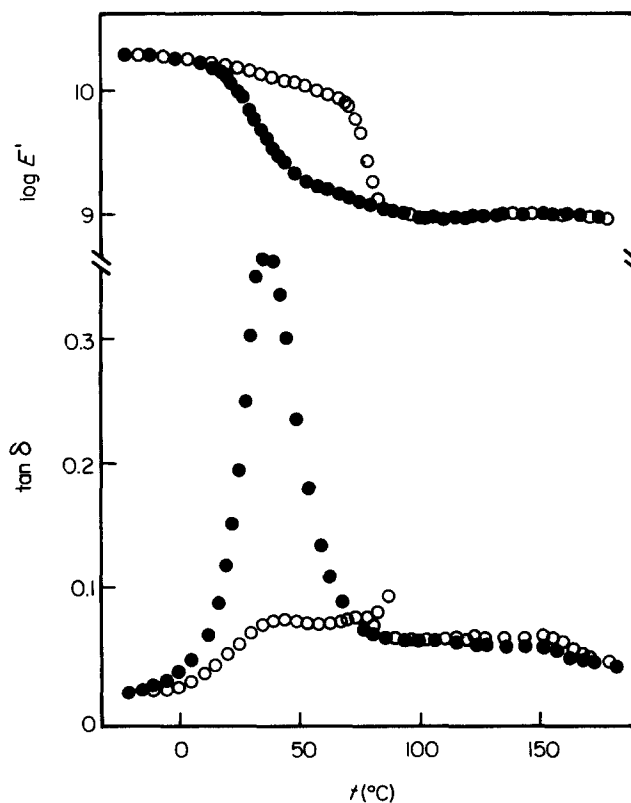
On the other hand, form C(7/2) shows anomalous viscoelastic properties (as shown by the open circles in Figure 4). The loss peak ($\tan \delta = 0.07$) at 40°C, due to the side chain motion, is negligible compared with those of the two previously mentioned forms, and no significant drop of E' is observed at the same temperature. The abrupt drop of E' occurs at around 80°C (corresponding to the d.s.c. transition temperature). As is expected from the d.s.c. curves of Figure 2, this drop takes place irreversibly. The same property as that of form C(18/5) is obtained on the second heating (see solid circles of Figure 4). The results confirm the X-ray observation that the transformation from form C(7/2) to form C(18/5) occurs irreversibly at 84°C. It is interesting that there is a great reduction of the side chain motion in form C(7/2).

D.s.c. measurement offers other evidence for the restricted side chain motion in form C(7/2). In the d.s.c. thermogram, the onset of side chain motion can be detected in the similar fashion to the glass transition behaviour appearing as a discontinuous change in a specific heat. It should be noted that such a glass transition-like behaviour for PBLG side chains has also been observed by the dynamic mechanical measurement²⁰. A typical example of this transition is shown for form C(18/5) in curve b of Figure 2, at a temperature of 14°C, which is lower than 40°C (detected by dynamic viscoelastic measurement) as one might expect. By con-

trast, no corresponding transition is found in form C(7/2) in the temperature range from -50°C-50°C as seen in curve a of Figure 2.

The further examination with respect to the side chain motion was performed by the dielectric measurement. Figure 5 shows the variation of dielectric constant and loss factor, ϵ' and ϵ'' , with temperature at 1 KHz and 300 KHz. The solid and dashed curves show the first and second heating data, respectively. At each frequency, ϵ' on first heating was found to increase gradually with increasing temperature until a temperature of first order transition, where an abrupt increase in ϵ' occurs irreversibly like a drop of Young's modulus in Figure 4. Above this transition temperature, ϵ' follows the path of the second heating. On the other hand, the loss factors, ϵ'' , on first heating display the fairly small peak of 0.04 around 40°C at 1 KHz. This loss peak corresponds to the mechanical $\tan \delta$ peak observed around 40°C in Figure 4 and so it can be attributed to the side chain motion. When applying the higher frequency of 300 KHz, the corresponding loss peak is shifted to appear around 70°C. At that frequency, a peculiar phenomenon is observed in the vicinity of d.s.c. first order transition temperature (around 80°C) so that another large peak with an abnormal shape appears after the above mentioned peak at 70°C. In comparison of the first and second heating data, it can be realized that this abnormal peak is caused by the abrupt transformation of small ϵ'' value to remarkable ϵ'' value of the second heating. This observation, together with the temperature dependence of dielectric strength shown later in Figure 7, dictates that the first order transition should be associated with the release of the restriction of side chain motion.

The frequency dependence of the ϵ'' peak temperature varied little for the C(7/2) and C(18/5) forms. Both forms have similar activation energies for the rotational motion


Figure 4 Temperature dependence of E' and $\tan \delta$ of C(7/2) form obtained at 110 Hz. (O): First heating; (●): second heating

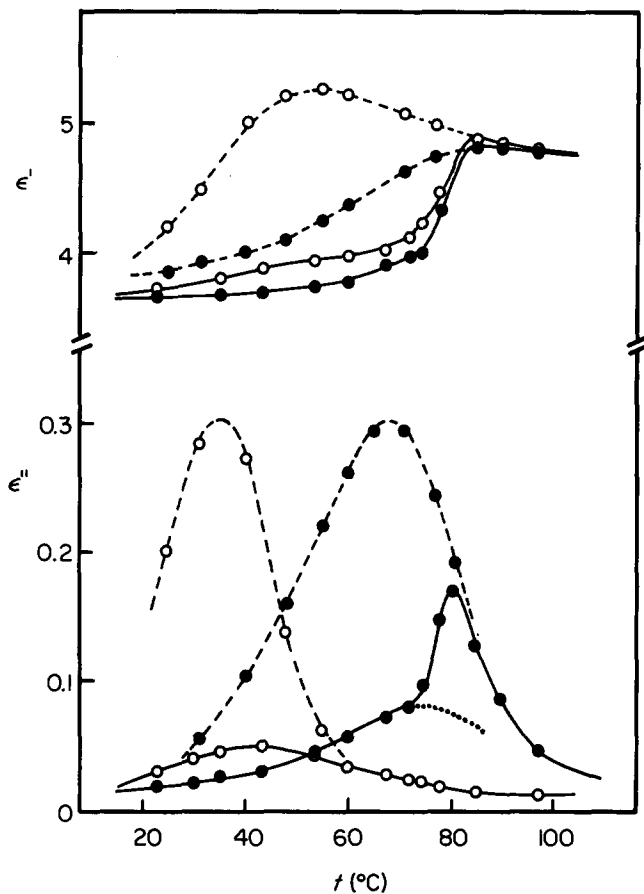


Figure 5 Temperature dependence of ϵ' and ϵ'' obtained (O) at 1 KHz and (●) 300 KHz for form C(7/2). The solid and broken lines indicate the first and second heating data, respectively. The dotted curve shows the tentative peak which would appear unless the first order transition takes place at 84°C (see text)

of side chain (40 kcal/mol of repeat unit for form C(7/2) and 42 kcal mol⁻¹ of repeat unit for form C(18/5)). The similarity in activation energy assumes that in form C(7/2), the side chain rotational mode may be similar to that in form C(18/5) and the remarkable reduction of loss peak height may be associated with the considerable decrease in the number of side chains which are not able to move freely. If this is the case, the dielectric strength, $\Delta\epsilon (= \epsilon'_0 - \epsilon'_\infty)$, evaluated from Cole-Cole plots on the basis of the frequency dependence of ϵ' and ϵ'' , allows the elucidation of the number of such unmoving side chains, according to the well known equation²¹

$$\Delta\epsilon \approx 4\pi N\mu^2/3kT \quad (1)$$

where N is the number of side chains in a unit volume contributing to the dielectric relaxation, μ the dipole moment of carbonyl group of side chain, k the Boltzmann's constant, and T the temperature in Kelvin. In Figures 6a and 6b the Cole-Cole plots of sets of ϵ' and ϵ'' obtained at around 40°C for forms C(7/2) and C(18/5), respectively, are shown. The $\Delta\epsilon$ evaluated from these plots are shown against temperature in Figure 7. They are listed together with $\Delta\epsilon$ for other kinds of polyglutamates in Table 2. When compared at around 40°C, the $\Delta\epsilon$ of 0.38 for form C(7/2) is approximately one quarter of $\Delta\epsilon$ of 1.45 for form C(18/5) or form B(18/5). The latter value of $\Delta\epsilon$ is similar to the value of 1.3 obtained for poly(γ -methyl D-glutamate)²². This is to be expected when all of the side chains contribute to the dielectric relaxation. Con-

sequently from the inspection of equation (1), there is a motional restriction for three fourths of the overall side chains in form C(7/2) and, as evidently found in Figure 7, such a restriction is imposed until the d.s.c. first order transition temperature.

DISCUSSION

Similarity in properties between form C(7/2) and the PBG racemic form

The anomalous properties of form C(7/2) are interestingly compared with those for a racemic mixture of L and D of PBG. Uematsu *et al.*^{23,24} found that the racemic form

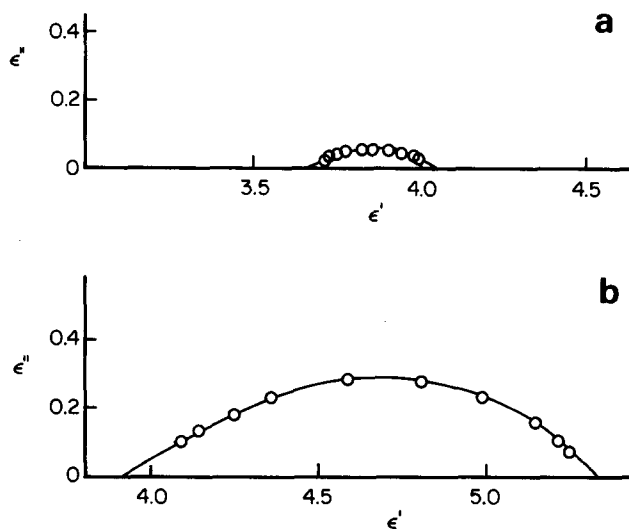


Figure 6 Cole-Cole plots of ϵ' and ϵ'' obtained at around 40°C for: (a) C(7/2) form at 43.4°C; (b) C(18/5) form at 40.3°C

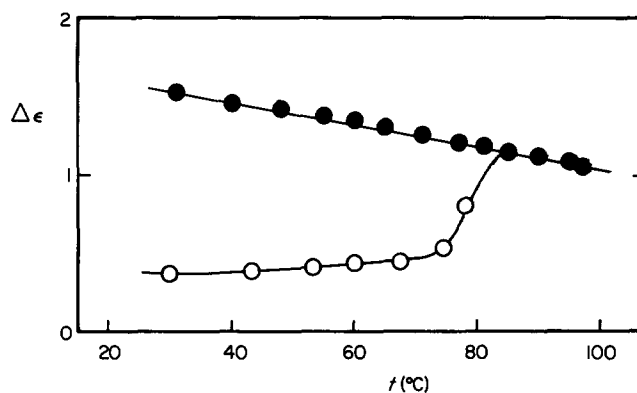


Figure 7 Temperature dependence of dielectric strength, $\Delta\epsilon$, for form C(7/2) (O) and form C(18/5) (●)

Table 2 Dielectric strength and degree of stack for various kinds of polyglutamate films

Polymer	$\Delta\epsilon$ at 40°C	degree of stack, pd
PBLG form B (18/5)	1.45	0
PBLG form C (18/5)	1.45	0
PBLG form C (7/2)	0.38	0.74
PBG racemic form	0.90 ^a (1.0 ^b)	0.38
Poly (γ -methyl D-glutamate)	1.3 ^c	—

^a data of ref. 25; ^b data of reference 27; ^c data of ref. 22

^d the degree of stack was estimated from $\Delta\epsilon$ using the equation (2) (see text)

showed a first order transition at 95°C in d.s.c. and dilatometric measurements. We observed that the enthalpy change, ΔH , for this transition is 3.6 cal/g²⁵, which value is similar to that of form C(7/2). In addition, viscoelastic and dielectric measurements indicated that the relaxation strength corresponding to the side chain rotation in the racemic form is markedly smaller than that in enantiomorphic PBLG^{23,26,27}. On heating, such a restriction imposed on the side chain motion is removed at the d.s.c. transition temperature (mentioned above). A sharp drop in Young's modulus and an abrupt increase in the dielectric constant takes place simultaneously. The dielectric strength was found to be around 0.9²⁵. This small value is again similar to that for form C(7/2). The racemic form, thus, has similar properties to form C(7/2), except that the first order transition and the restriction of side chain motion are reversibly observed on both heating and cooling.

With the observation of these properties, simple but important characteristics of the racemic form have been found from the X-ray diffraction diagram. The 'extra' 10.5 Å meridional reflection in the diagram cannot be explained simply in terms of the α -helical main chain conformation^{28,29}. Squire and Elliott²⁸ proposed a structural model in which the benzyl groups of side chains protruding from two adjacent α -helical molecules are piled up in an alternating fashion to form a regular stack of aromatic rings, which is independent on the main chain conformation. The regular stack is regarded as the origin of the 'extra' reflection.

Such a stacking arrangement of benzyl groups offers a reasonable explanation for the anomalous properties commonly observed in the C(7/2) form and the racemic form. The first order transition arises from the cooperative melt of the regular stack of benzyl groups (the motion of the benzyl groups in the stacks are rotationally restricted). It should be noted here that, more recently, new evidence of the regular stack in the racemic form has been obtained from the analysis of ¹³C-n.m.r. and fluorescence emission spectra³⁰.

Stacking model based on radial projection

It can be understood from the radial projection of helix that the stacking arrangement of benzyl groups occurs in the racemic form. Figure 8 shows part of the radial projection of the 18/5 helix with a repeat of 27 Å and radius of 7.4 Å (half the intermolecular distance). In this model the side chains are envisaged as knobs of uniform size and shape (represented by circles) and we will try to examine the pattern they make in space. When two helices are packed together parallel to the helix axis, two regions of radial projection come into contact as shown in Figure 8a. In the plane of contact, part of the radial projection of 18/5 helix of opposite screw sense has been superimposed in such a manner that the knobs (side chains) in L-helix mesh with the holes (spaces between side chains) in the other D-helix; i.e., every seventh knob of one chain will fall into every seventh hole in the other. If the circles in the Figure are replaced by the benzyl groups with rectangular shape, it is easy to envisage that the benzyl groups of two helices with opposite sense can be stacked next to each other in an alternating fashion²⁸. Along such a stack, the repeat length is to be about 10.5 Å (= 7 × 1.5 Å), since every second benzyl group comes from the same molecule. This length corresponds to the spacing of the previously mentioned 'extra' reflection.

In the enantiomorphic form of 18/5 helices, the same type of knob and hole packing cannot be achieved in the parallel contact of helices. Instead, such a packing is possible if the axes of helices were mutually inclined at an appropriate angle (see Figure 8b). This management leads to the well known prediction of the coiled coil rope in which α -helices strand next to each other to give the knob and hole packing over the whole length of chain³¹. Parry and Suzuki³² calculated the interaction energies for various coiled coil rope models of poly(L-alanine) and found that they were generally more stable than the corresponding straight chain assemblies. So far, however, PBLG, like other synthetic homopolypeptides has never been prepared in a coiled coil conformation even if it could be energetically favoured by the possible stacking

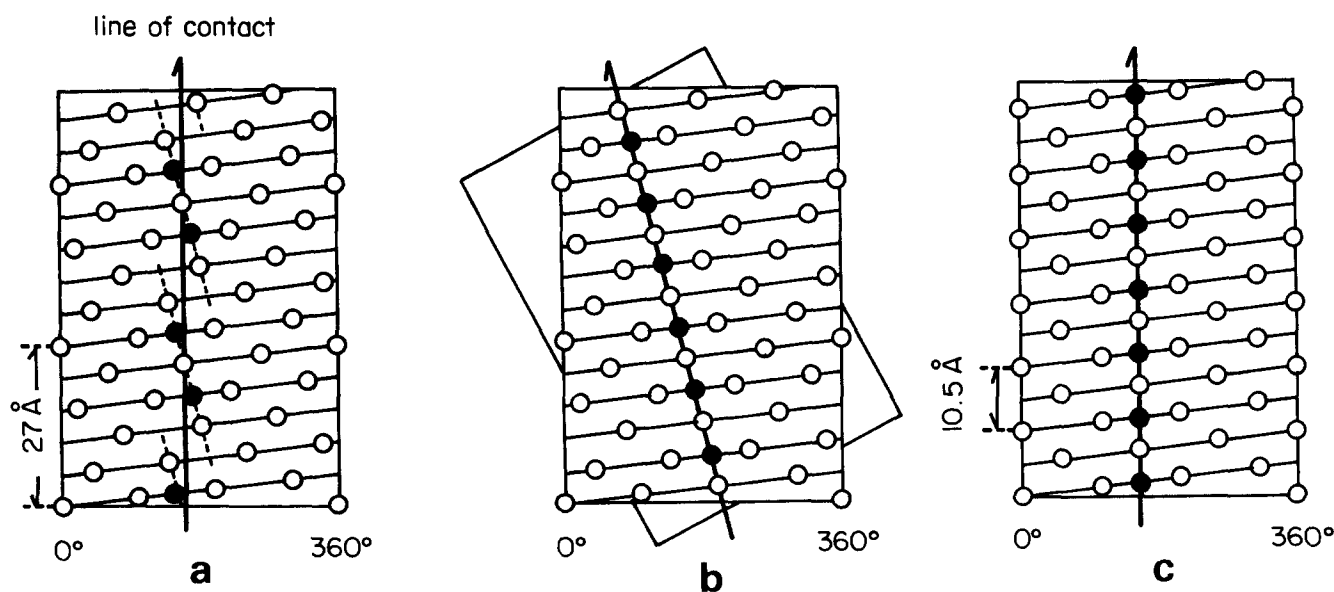


Figure 8 Pattern formed by side chains along the line of contact of two helices. In (a), (b), and (c) the radial projections of two 18/5 helices with opposite screw sense, two 18/5 helices with the same screw sense, and two 7/2 helices with the same screw sense are illustrated. The side chains of one helix are denoted by ○ and ones of the other by ●

arrangement of benzyl groups³³. This stems from the fact that a three dimensionally crystalline arrangement will generally be more stable, since coiled coil ropes do not pack neatly together and so the packing density is very low.

The radial projection of helices with 7/2 conformation is shown in Figure 8c. In this case (different from the case of 18/5 helix) every seventh knob and hole of a chain lie parallel to the chain axis. The precise knob and hole packing, thus, can be attained between 7/2 helices of the same screw sense, with their axes parallel. In the similar manner to the racemic mixture, the regular stack of benzyl groups may be formed in form C(7/2).

Here, it should be noticed that the type of stack appears to be different between the racemic form and the C(7/2) form. In the racemic form, the angle which a set of stack makes with the axis of helix is about 12° as shown by the dashed line of Figure 8a. Therefore, there cannot be more than five or six benzyl groups in a stack if there is to be appreciable overlap between these groups²⁸. In the C(7/2) form, on the other hand, the stack can be formed parallel to the chain axis, which may allow the formation of very long stack over the whole length of chain. In both cases, the benzyl groups in a stack placed 5.25 Å apart to each other, can come into a van der Waals contact of 3.4 Å by tilting so that their normals are inclined at about 65° to the helix axis^{28,33}.

Degree of stack

Side chains with their benzyl groups regulating the stack may take up only a few conformations, since both ends are located in the confined positions. In the stack the rotational motion between conformations needs considerably higher activation energies than that for the side chains which are free from the stack. Thus, it is reasonable to assume that the side chains in the stack may not contribute to the present viscoelastic and dielectric relaxation. Under this assumption, the degree of stack, P , can be estimated from the dielectric strengths using the following equation (based on the equation (1)),

$$P = (N_1 - N_2)/N_1 = (\Delta\epsilon_1 - \Delta\epsilon_2)/\Delta\epsilon_1 \quad (2)$$

where $\Delta\epsilon_1$ is the dielectric strength for the system in which all of the side chains, N_1 , are rotatable and $\Delta\epsilon_2$ for the system including stacks in which the number of rotatable side chains is reduced to be N_2 . Naturally, $\Delta\epsilon_1$ and $\Delta\epsilon_2$ must be chosen which were obtained at the same temperature.

For the racemic mixture, $\Delta\epsilon$ is around 0.9 at 40°C. This compares with 1.45 for B(18/5) and C(18/5) forms which are free from the stack. This value of $\Delta\epsilon$ gives the degree of stack as 0.38 [= (1.45 - 0.9)/1.45] (as listed in Table 2). This degree of stack compares well with that evaluated from the structural model by Squire and Elliott²⁸; the detailed stacking model can be found in Figure 11 of ref. 28. According to this model, the α -helix is very slightly distorted to give a 43/12 conformation. Thirty one benzyl groups of the 43 in a repeat unit of the L-molecule, contribute to a stack, with the six surrounding D-molecules in a hexagonal array. Since the actual possibility in which D-molecules are in the nearest neighbours of L-molecule is 2/3, the degree of stack is 0.48 (= 31/43 \times 2/3). This value is in agreement with the one estimated above, considering that the experimentally observed value is generally smaller than that of the ideal value.

In the C(7/2) form, the dielectric strength of 0.38 gives the degree of stack as 0.74. This value of the degree of stack corresponds approximately to 0.86 which is expected when six side chains of the whole seven ones in a repeat participate a stack. When considering the helical symmetry of a main chain, however, it is somewhat surprising that the benzyl groups in side chains are organized so well so as to give a high degree of stack. Each chain is surrounded by the six closest neighbours but 7/2 helices do not have 6-fold symmetry. Therefore, the same interaction of side chains between one pair of neighbouring molecules can occur between no other pairs. This is shown in Figure 9 which shows the projection of 7/2 helices in a hexagonal array on the plane perpendicular to chain axes. In this system, dense packing would generally disturb the intermolecular order (the stack). In this particular case, however, it seems possible that the stacks may extend for six pairs of neighbours, irrespective of their dissimilarity in the side chain interaction field, since the benzyl groups are located through the five flexible bonds at the end of side chain. The high degree of stack (compared to 0.86) leads to the simple conclusion that each chain offers a stacking site for all six surrounding chains leaving unstacked benzyl groups in one seventh of the side chains. In this way, six sets of stack are formed parallel to the chain axis for all six pairs of neighbouring chains in contact. An example is shown in Figure 9; the side chains in positions a, b, c, d, e and f can help form the stacks, but the side chains in position g are left free from a stack. For an optimum degree of stacking, the rotation and translation of each chain has to be chosen and the conformation of side chains involved in one stack will appear different from that in other stacks. As a conclusion, no simple three dimensional unit cell for this system can be defined and only a favourable stack of benzyl groups is likely to have been constructed along chains. The diffraction pattern from such system would contain layer lines which are composed of a certain amount of streaks. This is precisely what is observed in the diffraction pattern of this film (see Figure 1a).

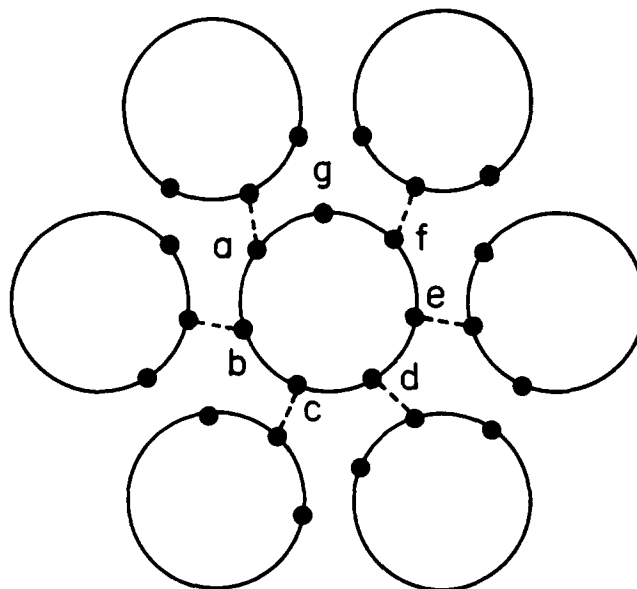


Figure 9 Projection of 7/2 helices in a hexagonal array on the plane perpendicular to the chain axis. The seven side chains in a unit are situated at positions a, b, c, d, e, f, and g. Six sets of the stack are considered to form between side chains linked by the dashed line (see text)

The degree of stack would be also reflected in the enthalpy change of first order transition which is attributable to the cooperative transformation of regular stack. For the racemic form, the observed ΔH of 3.6 cal/g leads to 2.1 kcal/mol of benzyl groups in a stack, based on the observed degree of stack of 0.38. This enthalpy change agrees approximately to that in fusion of crystals of analogous low molecular weight compounds such as benzene (2.35 kcal/mol) and benzyl alcohol (2.15 kcal/mol). Such agreement confirms the crystal-like arrangement of benzyl groups in a stack. In the C(7/2) form, the combination of the degree of stack (0.74) and ΔH (3.4 cal/g) gives 1 kcal/mol of benzyl groups in the stack. This is rather small compared to 2.1 kcal in the racemic form. Possibly, it may be partly decreased by the enthalpy change of the opposite sign due to the simultaneous conformation change of main chain from the metastable 7/2 helix to the most stable 18/5 one and partly by some irregularity of stack which might be caused by the difference between packing symmetry and the chain-fold symmetry.

CONCLUSIONS

PBLG film composed of unusual 7/2 helices shows anomalous behaviour with respect to the side chain motion. This anomaly is caused by the cooperative stacking of benzyl groups at the end of side chains. The fusion of the stack takes place at 84°C, accompanied with the change of main chain conformation of the 7/2 helix to the normal 18/5 helix. The lack of reformation of the stack on cooling indicates that the stacking of benzyl groups has been produced at the expense of distortion of main chain conformation. The dielectric strength due to the side chain motion indicates that the degree of stacking is 0.74, corresponds approximately to the value of 0.86 expected if six side chains of the seven ones in a repeat unit participate to form the stacks. A model using the radial projection of helix indicates that the precise 'knob and hole' packing can be attained between two 7/2 helices in contact with their axes parallel. This occurs in the racemic form where the stack can be formed in an alternating fashion between benzyl groups protruding from two neighbouring chains. The observed degree of stack can be explained so that each chain makes six sets of stack with six surrounding chains in a hexagonal array. At the present time, we have no explanation of how the stack in the solid film was produced during the evaporation of the solvent. However, it can be promoted by slow casting or by applying the magnetic field.

Such self-organization of aromatic rings at the end of side chains suggests the possibility that an organic semiconductor could be produced from this type of polypeptide. One of the best of these is a charge-transfer complex formed by the interaction of electron donors with electron acceptors. In general, aromatic donor and acceptor crystallize in an alternating manner in stacks with the planes of their aromatic rings parallel³⁴ (as observed in this paper). Thus, it is likely that the charge-

transfer complexes will be obtained in polypeptides, if the aromatic donor and acceptor can be properly attached to the side chains^{35,36}. The remarkable advantage of using polypeptides for this purpose is that their rigid chain with high axial ratio, would allow easy orientation by drawing the concentrated solutions in liquid crystals³⁷, causing a high electrical anisotropy.

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