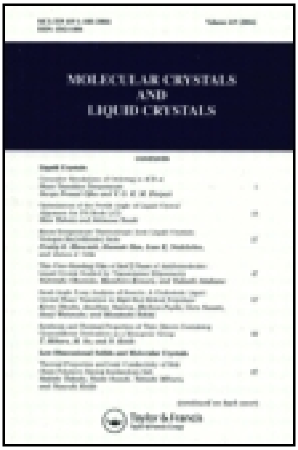

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Formation of Anisotropic Phase of Low-molecular-weight Poly(γ-benzyl-L-glutamate)s in Solution

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Low-molecular-weight poly(γ-benzyl-L-glutamate)s having degrees of polymerization (D. P.) 4 and 8 give an anisotropic phase in ethylene dichloride, if the concentration is appropriately chosen and the solution is incubated at a suitable temperature range. The optimum condition of concentration and temperature is more restricted for D. P. 8 than for D. P. 4. Ethylene dibromide solutions of the D. P. 4 specimen also give an anisotropic phase at some conditions. Infrared spectra and x-ray diffraction together with polarized microscopic observations suggest that the anisotropic phase consists of the β-structure of antiparallel arrangement of extended polypeptide chains; it has regular sequences of chains in the main chain direction and a stack of β-sheets in the side chain direction.

INTRODUCTION

Low-molecular-weight poly(γ-benzyl-L-glutamate)s having the average degree of polymerization from 3 to 8 consist of residues in either of the two conformations, i.e., the σ- or β-form, in some organic solvents.[1-3] The σ-form has a solvated peptide group and is free from hydrogen bonding,[4] whereas the β-form is hydrogen-bonded with the other through peptide groups and form polypeptide chains of the β-conformation having antiparallel chains. The polypeptide molecules are in the σ-conformation at infinite dilution, but the residues gradually transform from the σ-form to the β-form with increasing concentration. Consequently, the polypeptide molecules are associated with one another in solution, except at infinite dilution where all the residues are in the σ-form.

The content of β-form and the degree of association of the polypeptides could be determined at different concentrations by means of infrared spectra and vapor pressure osmometry, and it was found that both of them increase with concentration in the solvents of following increasing order: chloroform, dioxane, ethylene dichloride and extensively in ethylene dibromide.

In the present work we will investigate the properties of concentrated solutions of these low-molecular-weight poly(γ-benzyl-L-glutamate)s, mainly of the degree of polymerization, D. P. 4, in organic solvents and examine whether they can form an anisotropic or a birefringent phase such as a lyotropic liquid crystal in solution.

The dioxane solution of the polypeptide was clear and fluid at low concentrations but it separated precipitates at high concentrations. The chloroform solution of the polypeptide gave a viscous and somewhat turbid solution at 50 (w/w) % concentration. These solutions did not yield any anisotropic liquid phase at the conditions examined.

We have found, however, that the ethylene dichloride solutions of the polypeptides having D. P. 4 and 8 form optically anisotropic phases if the concentration is appropriate and the solution has been properly incubated. The ethylene dibromide solution of the polypeptide of D. P. 4 also gives an anisotropic liquid phase after incubated.

In this article we report the results of our observations on ethylene dichloride and ethylene dibromide solutions of these low-molecular-weight poly(γ-benzyl-L-glutamate)s, using the methods of polarized microscopy, infrared spectra and x-ray diffraction. Throughout this article we will represent the polypeptide concentration by *w*/*w* %.

EXPERIMENTAL

The samples of poly(γ-benzyl-L-glutamate)s of D. P. 4 and 8 are the same as previously used.[1-3] They were prepared by the polymerization of the corresponding N-carboxyamino acid anhydride initiated by n-hexylamine at the anhydride-to-initiator molar ratios, 4 and 8, and were partially fractionated by the treatment with formic acid. Consequently, they have an n-hexylamino group at the carboxyl end and a formic acid group at the amino terminal. Their number-average degree of polymerization, D. P., was found to be approximately equal to the anhydride-to-initiator molar ratio.

Ethylene dichloride and ethylene dibromide were purified as previously described.[1,3]

Solutions were prepared in a sealed glass tube (6 mm ϕ) or in a sealed quartz rectangular cell (40 × 10 × 1 mm), and they were incubated in a water bath regulated at a desired temperature within ±0.1°C. Solutions were usually matured for a week or two at a definite temperature, when anisotropic phases showing interference color under polarized microscope was developed. Solid films were obtained by spontaneous evaporation of solvents from the incubated solutions.

Infrared spectra of an incubated solution were taken by putting it between two KBr window plates. For the x-ray diffraction an incubated solution was sucked very slowly into a Lindemann glass capillary tube of 1.5 mm ϕ, and it was left without being sealed in until some amounts of solvent evaporate, since the scattering of x-ray from the solvent was very strong in the presence of the solvent.

An oriented film for x-ray diffraction was prepared from a concentrated chloroform solution by unidirectional stroking on a glass plate and peeling after dried.

The optical anisotropy of the solutions was examined by an Olympus BH microscope with a crossed nicol at room temperature (25 ± 2°C). The microscopic observation was performed within ten seconds after the specimen was pulled out from the incubation bath, in such a way that temperature lowering did not affect the state of phase. The infrared absorption spectra were recorded on a Jasco A-3 Infrared Spectrophotometer. The x-ray diffraction patterns were photographed with either an evacuated cylindrical camera of a diameter of 7 cm or a flat camera, using Ni-filtered Cu-Kα radiation, and their spacings were calibrated by referring to those of NaCl powder.

RESULTS

Formation of anisotropic phase of the polypeptide of D. P. 4

When incubated at a constant temperature, the concentrated solution of poly(γ-benzyl-L-glutamate) of D. P. 4 in ethylene dichloride changed from a fluid liquid to a clear jelly via a thixotropic stage, provided that concentration and temperature were appropriate. The jelly was more readily formed with solutions of higher concentrations and of lower temperatures. The bright interference color showing the formation of an anisotropic phase was mostly observed in the region of clear jelly. For the development of such an anisotropic phase it took from a few hours to several days, depending on the concentration and temperature. For example, the 28 and 34% solutions at 50°C gave interference color within a few hours, but with an 11% solution at 25°C the color was developed only partly after 5 days and spread over a wide region after 9 days.

The formation of anisotropic phase was reproducible, as far as a fresh solution of appropriate concentration was kept at an appropriate temperature. However, a solution that had been incubated at a certain other temperature often produced the optical anisotropy to a different extent from a freshly prepared solution or sometimes did not give any anisotropic phase, even if it was brought to the same temperature. The temperature and time for the first incubation influenced the final state of anisotropy considerably, that is, the development of anisotropic phase showed hysteresis. Furthermore, no anisotropic phase was formed when a solution was stirred vigorously or heated at too high temperatures. Such a solution yielded fine opaque gels or separated transparent gels, both of which were isotropic. It was also observed that no anisotropic phase was developed in a cell having a width less than 1 mm.

In a few cases the interference color faded after a too long incubation, and translucent gels were formed, which were sometimes as large as 1 mm in diameter. Fading of the interference color was also accelerated by shearing stress such as stirring.

Microscopic observation

The appearance of interference color observed by the polarized microscope can be roughly classified into three types: the first is blueish, the second is partly blue and partly colored, and the third is very colorful. These types

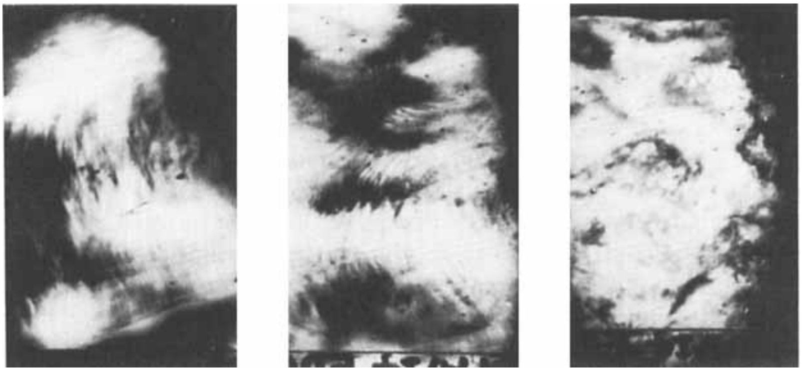

FIGURE 1 Polarized micrographs of anisotropic phases formed in ethylene dichloride solutions of poly(γ-benzyl-L-glutamate) of D. P. 4 at 43°C. Left: 5% (the light parts are blueish), Middle: 11%, Right 18%. — indicates a length of 100 μ.

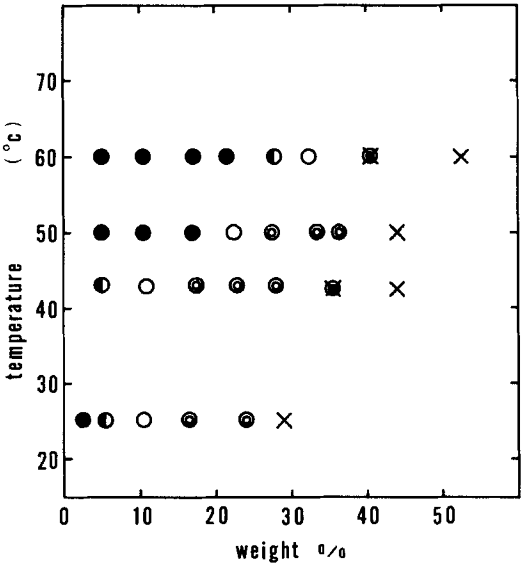

FIGURE 2 Temperature-concentration relation for the states of poly (γ-benzyl-L-glutamate) of D. P. 4 in ethylene dichloride. ●, isotropic solution; ◐, anisotropic solution having blueish parts; ○, anisotropic solution having blueish and colored parts; ◎, anisotropic solution having colored parts; ×, insoluble.

are reproduced in the order of increasing polypeptide concentration, as illustrated in Figure 1.

The diagram shown in Figure 2 gives the relation of the incubation temperature for the development of interference color with the concentration of poly(γ-benzyl-L-glutamate) of D. P. 4 in ethylene dichloride solution. It can be seen that for the formation of anisotropic phase a concentrated solution higher than about 5% has to be incubated at higher temperatures, but some optimum region of concentration and temperature exists: the polypeptide sometimes became insoluble when the concentration was higher than 25%.

Figure 3 shows a similar relation of the incubation temperature for the formation of anisotropic phase with the concentration of the same polypeptide in ethylene dibromide solution. The optimum range for the anisotropy formation is shifted to lower polypeptide concentrations, and it is narrower in ethylene dibromide than in ethylene dichloride.

The anisotropic phase formed by poly(γ-benzyl-L-glutamate) of D. P. 4 can be characterized by a stripe-like texture, as shown in Figure 4. The texture can be seen even under unpolarized light, and the stripes are not separated by equal spacing. The texture is more predominant when the

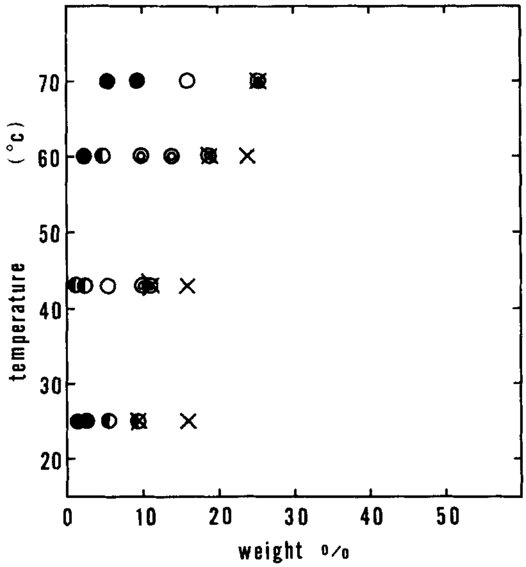

FIGURE 3 Temperature-concentration relation for the states of poly (γ-benzyl-L-glutamate) of D. P. 4 in ethylene dibromide. ●, isotropic solution; ◐, anisotropic solution having blueish parts; ○, anisotropic solution having blueish and colored parts; ◎, anisotropic solution having colored parts; ×, insoluble.

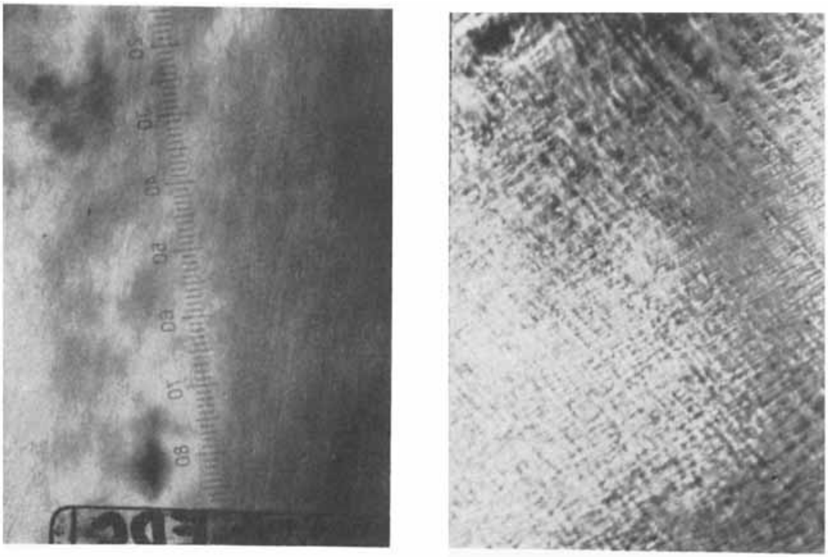

FIGURE 4 Stripe-like textures on an anisotropic phase in ethylene dichloride solution prepared at 37% and 50°C (left) and on a solid film (right) of poly(γ-benzyl-L-glutamate) of D. P. 4. ———— indicates a length of 100 μ.

solution is prepared in a rectangular cell and under a very low shear or flow. Then it is found that the direction of the stripes is parallel to the flow line.

When the solvent was evaporated from a fully matured anisotropic solution, the resulting film was highly birefringent and the stripe-like texture was left on the dried film. Figure 4 also gives a photograph of the texture on the solid film. In contrast, a fresh solution that had not exhibited any interference color did not yield any birefringent film when the solvent was evaporated.

Infrared spectra

The infrared spectra of anisotropic solutions of poly(γ-benzyl-L-glutamate) of D. P. 4 in ethylene dichloride obtained after maturation at 43°C were compared with those of isotropic solutions before incubation. It was found that the anisotropic solutions of concentrations, 5, 11, 18 and 28 %, reduced their interference color when they were transferred from an incubation cell to an infrared cell having KBr windows. This must have been caused by the effect of shear or flow on transferring the solution. Nevertheless, it was confirmed that the anisotropic solutions contained the polypeptide having the β-conformation of antiparallel chains in a similar way that the isotropic solutions had, since the infrared spectra gave strong absorption bands at 1632 and 1734 cm^{-1} and a weak band around 1700 cm^{-1}.[5,6]

X-ray diffraction

The anisotropic solutions formed in glass tubes were carefully transferred into Lindemann tubes with the lowest shearing stress and were subjected to the x-ray analysis. After confirming the conservation of the interference color under the polarized microscope, we have assumed that the direction of the stripes of the texture in the anisotropic phase is aligned along the direction of capillary tube.

The anisotropic solutions of poly(γ-benzyl-L-glutamate) of D. P. 4 in ethylene dichloride or in ethylene dibromide failed to give any clear diffraction pattern because of strong scattering from the solvent. Consequently, the solvent was gently evaporated from the anisotropic solution in the capillary tube, and the x-ray diffraction was measured with the almost dried specimen. We have further assumed that even in the almost dried specimen the conformation of the polypeptide and the orientation of the main chain in the anisotropic solution are preserved.

Figure 5 shows the x-ray diffraction pattern of the almost dried specimen regenerated from an anisotropic solution of the polypeptide in ethylene dichloride. There are observed arcs corresponding to five spacings, 25.7,

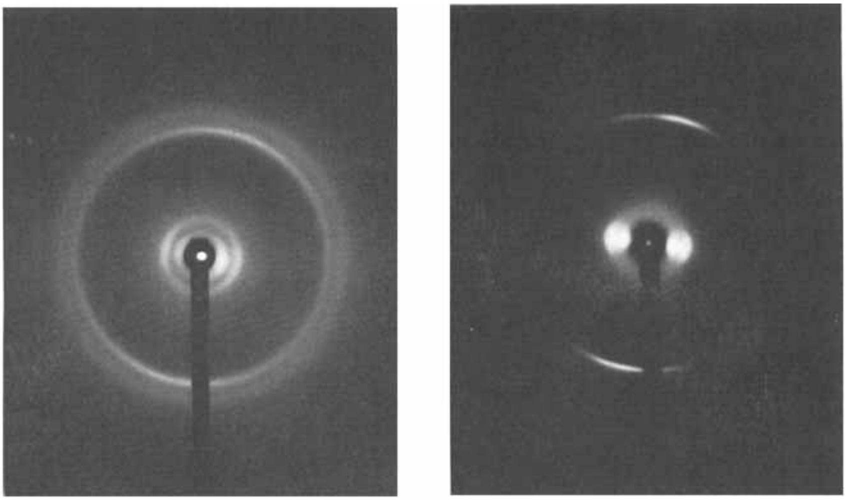

FIGURE 5 X-ray diffraction photographs of an almost dried specimen obtained from an anisotropic solution (left) and an oriented solid film (right) of poly(γ-benzyl-L-glutamate) of D. P. 4. Flat camera, Cu-Kα radiation. The specimen-to-film distance was 7.0 cm. The directions of flow and stroking were put vertical.

15.48, 9.0, 4.74 and 4.21 A. The appearance of arcs indicates the preservation of anisotropy in the original solution. Figure 5 also shows the diffraction pattern of an oriented film cast from a chloroform solution by unidirectional stroking. When the direction of stroking of the film is put parallel to that of stripes on the anisotropic phase, it is seen that both diffraction patterns are very similar to each other.

The 4.74 A meridian reflection can be assigned to the distance between extended polypeptide chains in a β-sheet or the repeat distance in the direction of hydrogen bonds in a β-sheet. Then the interchain hydrogen bonds in the β-conformation are oriented parallel to the observed stripes on the anisotropic texture, and the β-conformation in the anisotropic phase may be classified as a cross-β structure.[7]

The 15.48 A equatorial reflection corresponds to the distance between pleated β-sheets stacked together or the side chain spacing. The spacing is almost equal to each other in both the oriented solid film and the specimen regenerated from an anisotropic solution. It is not necessarily certain whether or not such a stack of β-sheet is in the anisotropic phase. A recent light-scattering work[8] has shown that the β-form aggregates of a monodisperse hexa(γ-benzyl-L-glutamate) formed in dilute ethylene dichloride solutions are free from such a stack of β-sheets. The observed value of side chain spacing is in agreement with the reported by Takeda *et al.*[9] for low-molecular-weight poly(γ-benzyl-L-glutamate) in the solid state. Tsuboi[10] proposed a linear relation between the side chain spacing and the number of carbon and oxygen atoms along the side chain for the β-structure of various polypeptides. The observed value, 15.48 A, falls on his straight line if the phenyl ring is counted as three atoms.

Another strong equatorial reflection corresponding to a spacing 25.7 A can be assigned to the whole length of an extended polypeptide chain of D. P. 4, having an n-hexylamino group at the carboxyl end and a formic acid group at the amino terminal, assuming a monodisperse polypeptide. Some fractionation would occur to hold only the chains of equal length together in register.

Thus we may propose a structure of the β-conformation of polypeptide in the anisotropic phase as follows. Extended polypeptide chains are arranged in a head-to-tail array along the main chain direction and are hydrogen-bonded with the neighboring antiparallel polypeptide chains, to form a β-sheet. Such β-sheets are piled up in the direction of side chain to form a stack or the anisotropic phase. The direction of hydrogen bonds can be made parallel to the flow line of a shearing stress, when a shear is applied.

Formation of anisotropic phase of the polypeptide of D.P. 8

In contrast to that of D. P. 4, a 14% solution of poly(γ-benzyl-L-glutamate) of D. P. 8 in ethylene dichloride remained isotropic at 25°C, and even an 18% solution gave only blue interference color portending the incipient formation of anisotropic phase. At higher temperatures the anisotropic phase was readily developed, especially with more concentrated solutions. The 9 and 15% solutions kept at 43°C yielded blue color and had some dark stripes, as was observed with some solutions of the polypeptide of D. P. 4. A 20% solution at 43°C gave anisotropic phase showing interference color together with isotropic gels, although the same solution at 50°C separated only isotropic gels to a large amount without forming any anisotropic phase. The condition for the anisotropy development is much more restricted with the D. P. 8 specimen. Figure 6 shows the relation of the incubation temperature for the development of anisotropic phase with the concentration of polypeptide of D. P. 8 in ethylene dichloride.

The development of an anisotropic phase with the D. P. 8 specimen is different from that with the D. P. 4 specimen. The anisotropic phase appeared as patches held together over a wide region, and sometimes isotropic gels were separated outside the region. Figure 7 illustrates the appearance of the anisotropic region for an 18% solution at 25°C and a 20% solution at 43°C.

The formation of anisotropic phase with a 27% solution at 50°C was unique. At first it gave colored anisotropic patches and blue spherulites, and then both patches and spherulites coalesced separately with one another, forming colored region such as shown in Figure 7. The spherulite of the low-

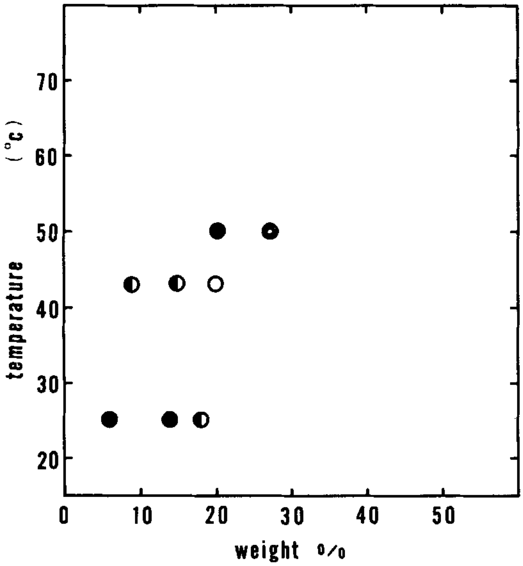

FIGURE 6 Temperature-concentration relation for the states of poly(γ-benzyl-L-glutamate) of D. P. 8 in ethylene dichloride. ●, isotropic solution; ◐, anisotropic solution having blueish parts; ○, anisotropic solution having blueish and colored parts; ◎, anisotropic solution having colored parts; ×, insoluble.

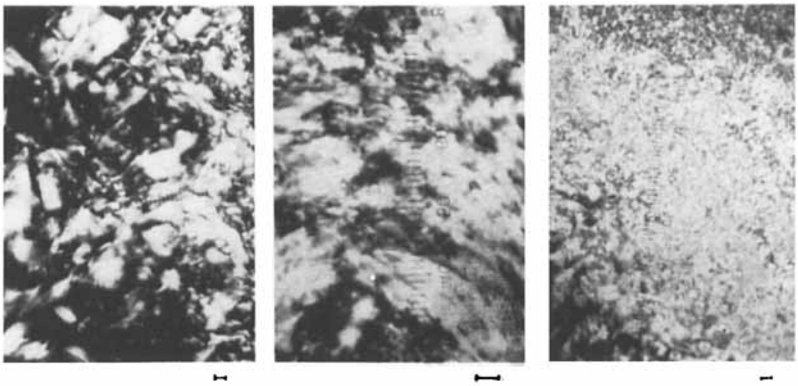

FIGURE 7 Polarized micrographs of anisotropic phases formed in ethylene dichloride solutions of poly(γ-benzyl-L-glutamate) of D. P. 8. Left: 18% at 25°C, Middle: 20% at 43°C, Right: 27% at 50°C. The bars indicate length of 100 μ.

molecular-weight polypeptide is distinguished from that formed by the α-helical polypeptide[11,12] in two respects. The spherulite of the β-form polypeptide is not colored but only blueish, and it does not have any concentric stripes.

DISCUSSION

We have demonstrated the formation of anisotropic phase of low-molecular-weight poly(γ-benzyl-L-glutamate)s in some organic solvents. Whereas high-molecular-weight poly(γ-benzyl-L-glutamate)s can form cholesteric liquid crystals in helix-forming solvents, when the concentration exceeds some critical value,[11–14] it was shown that low-molecular-weight poly(γ-benzyl-L-glutamate)s can form anisotropic phases only in ethylene dichloride or ethylene dibromide. The reason for its formation in the two solvents is mostly due to more complete formation of the β-conformation. We have verified the presence of the β-conformation of antiparallel chain arrangement in both isotropic and anisotropic solutions of these polypeptides.

The formation of anisotropic phase of the β-form polypeptide can be realized only in relatively restricted conditions of temperature and concentration. Since there are suitable solvent and optimum ranges of degree of polymerization, concentration and temperature, it can be considered that some appropriate content of β-form is initially required for the maturation and development of an anisotropic phase. In contrast to the nearly instantaneous formation of cholesteric phase of the α-helical polypeptide, it takes some hours or days for the development of anisotropy in the concentrated solutions of β-form polypeptides.

These various restrictions in the formation of anisotropic phase of the β-form polypeptide would be caused by complication arising from some conformational change and intermolecular association through hydrogen bonding and side chain or terminal group interaction. A stacked arrangement of β-sheets having head-to-tail sequences of antiparallel hydrogen bonded chains has been shown to form eventually in the anisotropic phase.

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