

NOTES

## Helix-Coil Conformational Change Accompanied by Anisotropic–Isotropic Transition

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Polypeptides, particularly poly( $\gamma$ -benzyl-L-glutamate) (PBLG) and other glutamic acid ester polymers, have the ability to exist in  $\alpha$ -helix, a well defined chain conformation of long chain order and retain such a structure in numerous organic solvents that support intramolecular hydrogen bonding.<sup>1,2</sup> If PBLG and related polypeptides are dissolved in a binary solvent mixture containing a non-helicogenic component such as dichloroacetic acid (DCA) or trifluoroacetic acid (TFA), they can undergo a conformational change from the  $\alpha$ -helix to random coil form due to the changes in solvent composition, temperature or both.<sup>3,4</sup> The helix-coil transition has been extensively investigated and still is an important subject of studies by far. Among the glutamic acid polymers, PBLG is the most widely studied example. Early studies are focused on the conformational change of PBLG in the dilute binary solvent systems.<sup>5–8</sup> However, studies of the concentrated solution are limited. Above a critical polymer volume fraction, the PBLG/CHCl<sub>3</sub>/DCA or PBLG/CHCl<sub>3</sub>/TFA solutions can form a liquid crystal phase with cholesteric texture.<sup>9–11</sup> Cooling down such an anisotropic solution, the denaturant solvent may disrupt the hydrogen bond network of the helical conformation, and leading to a helix-coil conformational transition. Since the random coil polymer chain is geometrically inconsistent with the long-range orientational order of the liquid crystal, an anisotropic to isotropic transition, so called reentrant isotropic transition, can be observed at lower temperatures.<sup>12–15</sup>

Such an anisotropic–isotropic transition, which is induced by the helix-coil conformational change, may also take place in the acid mediated liquid crystal solution, when the acid content is increased. By far,

limited study of this acid induced anisotropic–isotropic transition has been reported.<sup>13,14</sup> The precise molecular mechanism of the transition remains to be determined.

In the present work, the texture changes in the acid-induced anisotropic–isotropic transition were studied by microscope observations and the corresponding molecular conformational changes were followed by IR measurements. The effect of the polymer concentration was studied. According to the information gained through the experiment, the molecular mechanism with respect to the acid-induced helix-coil conformational change was suggested.

### EXPERIMENTAL

The PBLG sample was prepared by a standard *N*-carboxy- $\gamma$ -benzyl-L-glutamate anhydride (NCA) method.<sup>16</sup> The molecular weight determined from the intrinsic viscosity in DCA was 146000.<sup>17</sup>

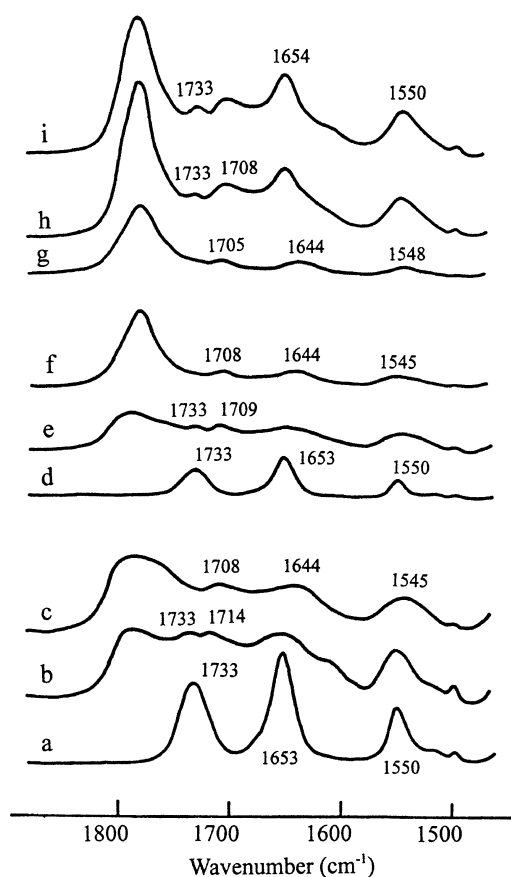
Appropriate amount of PBLG was dissolved in the CHCl<sub>3</sub>/TFA mixed solvents at various acid contents. Then the sample was sealed in the capillary tube for the microscope observation. The texture changes of the PBLG solutions were observed on a Leitz–Ortholux II Polarized Light Microscope. The IR spectra were measured at room temperature using a Nicolet Magna-IR 550 spectrometer.

### RESULTS AND DISCUSSION

#### *Acid Induced Helix-Coil Transition in Anisotropic and Isotropic Solutions*

Liquid crystal phase was observed above a critical volume fraction, when the PBLG was dissolved in a CHCl<sub>3</sub>/TFA mixed solvent. If the acid content was in-

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**Figure 1.** IR spectra of the PBLG/CHCl<sub>3</sub>/TFA solutions: (a) PBLG/CHCl<sub>3</sub> liquid crystal solution with  $v_p = 0.199$ , (b) PBLG/CHCl<sub>3</sub>/TFA biphasic solution with  $v_p = 0.199$  and  $f_{TFA} = 0.340$ , (c) PBLG/CHCl<sub>3</sub>/TFA isotropic solution with  $v_p = 0.199$  and  $f_{TFA} = 0.710$ ; (d) PBLG/CHCl<sub>3</sub> isotropic solution with  $v_p = 0.081$ , (e) PBLG/CHCl<sub>3</sub>/TFA isotropic solution with  $v_p = 0.081$  and  $f_{TFA} = 0.195$ , (f) PBLG/CHCl<sub>3</sub>/TFA isotropic solution with  $v_p = 0.081$  and  $f_{TFA} = 0.397$ ; (g) PBLG/CHCl<sub>3</sub>/TFA isotropic solution with  $v_p = 0.035$  and  $f_{TFA} = 0.400$ , (h) PBLG/CHCl<sub>3</sub>/TFA isotropic solution with  $v_p = 0.116$  and  $f_{TFA} = 0.400$  (f) PBLG/CHCl<sub>3</sub>/TFA liquid crystal solution with  $v_p = 0.228$  and  $f_{TFA} = 0.400$ .

creased, the phase separation of a small portion of an isotropic phase appeared. Further increase in acid content resulted in a complete extinction of the anisotropic phase.

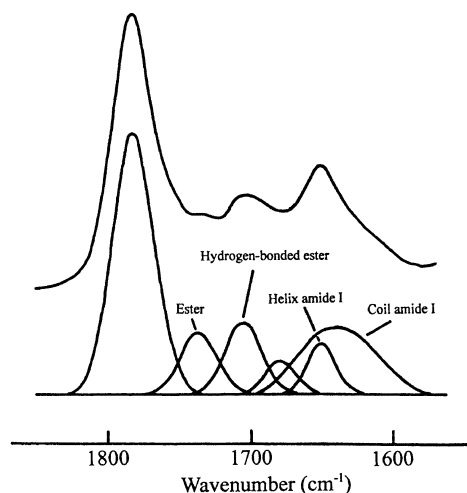
To obtain the information regarding the conformation change in the acid-induced anisotropic-isotropic transition, IR measurements were carried out. Typical IR results of the concentrated PBLG anisotropic and isotropic solutions with the PBLG volume fraction ( $v_p$ ) of 0.199 are shown by curve *a*, *b*, and *c* in Figure 1. Curve *a* shows the absorption bands of the PBLG/CHCl<sub>3</sub> sample. Strong amide I and II bands respectively at 1653 cm<sup>-1</sup> and 1550 cm<sup>-1</sup> are observed, indicating that PBLG molecules are in the helical form.<sup>18,19</sup> The absorption band at 1733 cm<sup>-1</sup> is assigned to the free ester group in the side chain.<sup>20</sup> When small amount of TFA is present, the side chain ester

absorption band becomes broader and a shoulder peak at 1714 cm<sup>-1</sup> appears. The shoulder peak is the absorption band of hydrogen-bonded ester carbonyl.<sup>20</sup> Further increasing the acid concentration, the intensity of the shoulder peak increases and the one at 1733 cm<sup>-1</sup> decreases. When the acid mole fraction  $f_{TFA}$  is increased to 0.340 as shown by curve *b*, the amide I and II bands are broader which suggests some disruption of helical structure in the main chain.<sup>21</sup> The association of the acid molecule with the PBLG side chain and the disruption of the helical main chain must give rise to an increase in the overall chain flexibility. Accordingly, an anisotropic-isotropic phase transition takes place, as the chain rigidity tends to be unable to support the orientational ordering. When the acid mole fraction is increased to 0.710, the absorption band of amide I shifts to a lower frequency at 1644 cm<sup>-1</sup> as shown by curve *c*. At the same time, the band of amide II shifts from 1550 cm<sup>-1</sup> to 1545 cm<sup>-1</sup> and the hydrogen-bonded ester carbonyl band appears at 1708 cm<sup>-1</sup>. The presence of the amide I band at 1644 cm<sup>-1</sup> shows that the PBLG molecules completely turn into the random coil structure.<sup>19</sup>

Curves *d*, *e*, and *f* in Figure 1 present the infrared spectra of the dilute PBLG isotropic solutions with  $v_p = 0.081$ . Curve *d* is that for the PBLG/CHCl<sub>3</sub>. It shows the same characteristics as curve *a*, indicating that the PBLG molecules adopt the helix conformation in the dilute CHCl<sub>3</sub> solution. When the acid is introduced, the helix to coil transition takes place as indicated by the broader amide I and II bands shown by curve *e*. At the acid mole fraction of  $f_{TFA} = 0.397$ , the PBLG molecules turn into coil structure, as the amide band I and amide II bands appear at 1644 cm<sup>-1</sup>, and 1545 cm<sup>-1</sup> respectively (shown by curve *f*).

In order to obtain the variation of the hydrogen-bonded side chain ester and amide I at 1644 cm<sup>-1</sup> in the helix-coil transition process, the observed ester and amide I bands were deconvolved and curve-fitted to the superposition of respective Gauss functions. The fitting procedure was repeated until the residue of the root-mean-square becomes less than 10<sup>-4</sup>. Shown in Figure 2 is a typical example of the deconvolved IR spectrum and corresponding resolving curve of a PBLG/CHCl<sub>3</sub>/TFA solution in which the PBLG molecules are in the helix-coil transition range.

Figure 3a shows the obtained result for the acid-content dependence of the hydrogen-bonded side chain ester fraction and the fraction of the amide I at 1644 cm<sup>-1</sup> in the concentrated solution. Figure 3b is that for the dilute isotropic solution. The solid circle indicates the ratio of the hydrogen-bonded side chain ester peak area to the total area of the side chain ester



**Figure 2.** IR spectrum and resolving curve of a PBLG/CHCl<sub>3</sub>/TFA solution with  $\nu_p = 0.116$  and  $f_{\text{TFA}} = 0.400$ . Upper lines: the deconvoluted IR spectrum, Lower lines: the curve-fitted components.

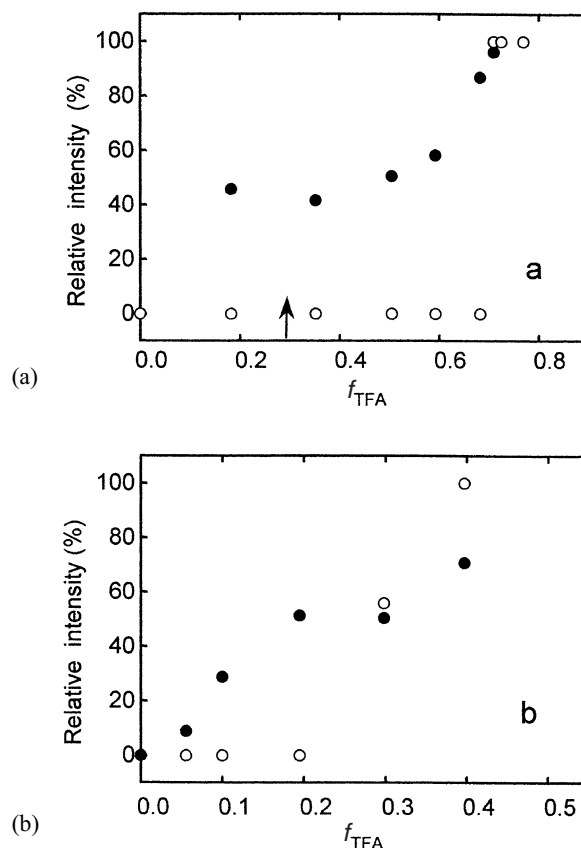
peak. The open square indicates the ratio of the peak area of amide I at  $1644\text{ cm}^{-1}$  to the total peak area of the amide I. As it can be seen, at the beginning, the free ester groups of the side chain gradually hydrogen-bond with the TFA molecules, while the amide I remains unchanged. Further increasing the acid content to a high concentration, the main chains rapidly convert into the random coil structure, especially in the concentrated solution. The comparison between Figures 3a and 3b shows that the helix-coil conformational change occurs at a lower acid concentration in the dilute isotropic solution.

Based on the experimental results presented above, the helix-coil transition process may consist of two steps. First, the acid molecules interact with the side chain ester by hydrogen-bonding with the ester carbonyl. With increasing the acid content in the system, the acid molecules start disrupting the intramolecular hydrogen bonds within the PBLG backbone. The breaks rapidly propagate along the backbone and a random coil structure finally forms.

The comparison between Figures 3a and 3b also shows that the helix-coil transition is more cooperative in the concentrated liquid crystal solution than that in the dilute isotropic solution. This implies that the initiation of a helical sequence after a run of coil segments is more difficult in the concentrated situation. The polypeptide chain tends to take either helical form or random coil form.

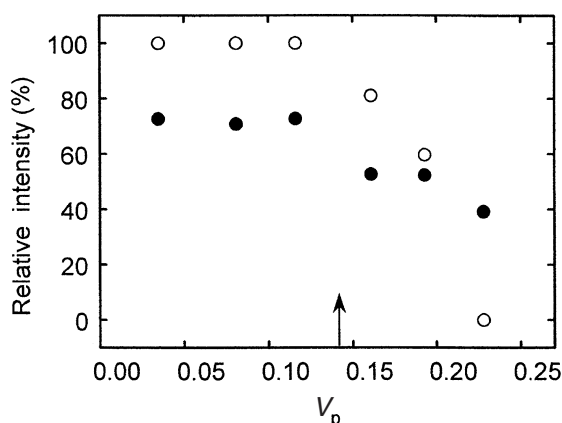
#### *Effect of Polymer Concentration on the Helix-Coil Transition*

A small quantity of the organic acid in the PBLG solution can interact with the amino acid residue. Thus, it



**Figure 3.** Acid-content dependence of the hydrogen-bonded side ester fraction and the fraction of the amide I at  $1644\text{ cm}^{-1}$  for the PBLG/CHCl<sub>3</sub>/TFA concentrated solutions with  $\nu_p = 0.199$  (a), and PBLG/CHCl<sub>3</sub>/TFA dilute isotropic solutions with  $\nu_p = 0.081$  (b). Solid circles indicate the relative intensity changes of the hydrogen-bonded side chain ester peak area to the total area of the side chain ester peak. Open circles indicate the relative intensity changes of the  $1644\text{ cm}^{-1}$  amide I peak area to the total peak area of the amide I. Arrow indicates the onset of the anisotropic to isotropic transition.

is important to keep the acid effect invariant in studying the polymer concentration influence. For this purpose, the mole fraction of TFA is fixed at 0.400 throughout the experiment of the study on the polymer concentration effect. Curves *g*, *h*, and *i* in Figure 1 show the IR results of PBLG/CHCl<sub>3</sub>/TFA samples at different polymer concentrations. At lower polymer concentration of  $\nu_p = 0.035$ , PBLG takes random coil form, which is evident by the amide I band at  $1644\text{ cm}^{-1}$  and amide II band at  $1548\text{ cm}^{-1}$  as shown by curve *g*. The ester carbonyl band is at  $1705\text{ cm}^{-1}$ , indicating that the acid molecules are hydrogen-bonded with the side chains. The absorption frequency and intensity of these characteristic bands are changed with the polymer concentration increased as it can be seen from curve *h*. At a higher polymer concentration of  $\nu_p = 0.228$ , the amide I and II bands are shifted to  $1654\text{ cm}^{-1}$  and  $1550\text{ cm}^{-1}$  respectively as shown by curve *i*. This suggests that a coil-helix transition takes place with the polymer con-



**Figure 4.** Concentration dependence of the hydrogen-bonded side ester fraction and the fraction of the amide I at  $1644\text{ cm}^{-1}$  of PBLG/ $\text{CHCl}_3$ /TFA solutions with  $f_{\text{TFA}} = 0.400$ . Solid circles indicate the relative intensity changes of the hydrogen-bonded side chain ester peak area to the total area of the side chain ester peak. Open circles indicate the relative intensity changes of the  $1644\text{ cm}^{-1}$  amide I peak area to the total peak area of the amide I. Arrow indicates the onset of the liquid crystal formation.

centration increasing. The concentration dependence of the hydrogen-bonded side chain ester fraction and the fraction of the amide I at  $1644\text{ cm}^{-1}$  in such a transition is illustrated in Figure 4. Before the PBLG volume fraction increases to 0.116, the fraction of hydrogen-bonded side chain ester and random coil structure remains stable. Further increasing the polymer concentration, the coil chains of PBLG start winding up into the helix as indicated by the decreasing tendency of the hydrogen-bonded side chain ester fraction and the fraction of the amide I at  $1644\text{ cm}^{-1}$ . When the  $v_p$  is increased to 0.140, the liquid crystal formation occurs. The hydrogen-bonded side chain ester fraction and the fraction of the amide I at  $1644\text{ cm}^{-1}$  start to decrease rapidly, suggesting that the coil-helix transition process is accelerated. At  $v_p = 0.228$ , the PBLG backbone adopts a nearly complete helical structure. However, some portions of the side chain esters are still hydrogen-bonded with TFA molecules.

So far less attention has been concerned on the effects of the polymer concentration on the helix-coil transition. A marked concentration effect can be seen from the present experimental results. In an isotropic PBLG solution, the polymer-polymer interaction may increase with the polymer concentration increasing. Such an intermolecular interaction could stiffen the chain rigidity, as manifested by the coil-helix transformation in the present work, especially at higher polymer concentrations. Theory and experiment also bear some additional evidences of this effect.<sup>22</sup> With further increasing the polymer concentration above a critical value, the liquid crystal phase formation takes

place. The coil to helix transition is *promoted* by the anisotropic field due to the conformational ordering effect, *i.e.*, intermolecular orientational order promoting intramolecular flexible to rigid conformational changes.<sup>23, 24</sup>

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