

# Circular dichroism of poly( $\beta$ -benzyl-L-aspartate) films in the $\alpha$ , $\beta$ and $\omega$ conformations

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The conformations of poly( $\beta$ -benzyl L-aspartate) (PBLA) in film were studied by means of circular dichroism (c.d.). Films of PBLA show c.d. which is related to the benzyl chromophores of this polypeptide. The magnitude and sign of the c.d. of the benzyl chromophores depend on the conformation i.e.  $\alpha$ -helix,  $\omega$ -helix or  $\beta$ -form. The aromatic c.d. of the left-handed  $\alpha$ -helix or the left-handed  $\omega$ -helix is negative in sign, and that of the  $\beta$ -form is positive in sign. The absolute magnitude of the c.d. of PBLA is much larger in the  $\omega$ -helical conformation than in the  $\alpha$ -helical or the  $\beta$ -conformations. In the left-handed  $\alpha$ -helical PBLA ( $\alpha$ -PBLA), the positive dichroism band of the  $n-\pi^*$  peptide electronic transition is observed at about 226 nm. When the  $\alpha$ -PBLA is converted to the left-handed  $\omega$ -helical PBLA ( $\omega$ -PBLA), the band at 226 nm shifts to 224 nm, and its magnitude becomes much smaller. In the  $\beta$ -form PBLA ( $\beta$ -PBLA), this band is negative in sign and observed at 223 nm. The absolute magnitude of the c.d. relative to this electronic transition follows the order:  $\beta$ -form <  $\omega$ -helix <  $\alpha$ -helix.

## INTRODUCTION

The application of infra-red absorption, X-ray diffraction, optical rotatory dispersion (o.r.d.), and c.d. has led to the conclusion that many L-polypeptides have a helical conformation with a right-handed screw sense<sup>1,2</sup>. An exception to this general rule is PBLA which appears to exist in a conformation having left-handed screw sense. It has been shown that PBLA forms left-handed  $\alpha$ -helices in chloroform solution<sup>3,4</sup> and left-handed  $\omega$ -helices in films dried quickly from chloroform solution. It is also well known that in PBLA film the  $\alpha$ -helical conformation is converted into the  $\omega$ -helical conformation or the  $\beta$ -form by heating<sup>5,6</sup>. On the other hand, the helix sense of poly(aspartate esters), as has already been pointed out<sup>7-13</sup>, depends subtly upon the nature of the ester groups and other conditions such as solvent and temperature. The conformation of poly( $\beta$ -alkyl aspartates) in solid state has been studied by means of infra-red absorption, X-ray diffraction, and electron diffraction<sup>14,15</sup>. The conformation in solution has also been studied by means of o.r.d., c.d., and n.m.r.<sup>7-13,16</sup>.

Consequently it has been established by many workers that PBLA assumes a helical conformation in helix promoting solvents and a disordered conformation in coil promoting solvents<sup>3,4</sup>. Studies on the film, however, have been rather few. There appears to be no report that deals with the c.d. of the  $\omega$ -PBLA in solid film. This may be due to the fact that in solution the PBLA has not been found in the  $\omega$ -helical conformation. In this report, some c.d. results concerning  $\omega$ -PBLA in film will be given and compared with those of  $\alpha$ -PBLA and  $\beta$ -PBLA.

## EXPERIMENTAL

The PBLA used was obtained from Pilot Chemicals, Inc; the mean molecular weight was 210 000.

C.d. and infra-red measurements were made with a ORD/UV-5 spectropolarimeter with a c.d. attachment and

with a DS-301 spectrophotometer, respectively, both manufactured by the Japan Spectroscopic Co., Ltd.

Films were prepared on quartz windows from chloroform solutions by evaporating off the solvent. The  $\alpha$ -helical films were prepared from solutions having polymer concentrations 0.5–2 wt% by quick drying at room temperature. These films were heated for about 1 h at 120°C to obtain  $\omega$ -helical films<sup>5</sup>. The  $\beta$ -form films were produced by heating the  $\alpha$ -helical films for about 1 h at 160°C<sup>5</sup>. The thickness of a film was determined from its weight and dimensions by assuming a density of 1.3 for the polypeptide<sup>17</sup>. The concentration of the film was taken as 6.34 mol/l.

The polypeptide film was mounted on a holder that could be rotated in a plane perpendicular to the incident beam<sup>18</sup>. The c.d. measurements of the film specimen were carried out every 30° and the c.d. was taken as the average of the twelve curves in order to counterbalance the effect of birefringence of the film on the c.d. value. To further minimize experimental errors, the c.d. measurements of the film were the average of duplicate runs, the cell being reversed in the second run. The c.d. results were the averages of at least three samples of specimens.

All the data were expressed in terms of molecular ellipticity,  $[\theta]$ .

## RESULTS AND DISCUSSION

### *Infra-red absorption*

It has been reported that infra-red spectroscopy offers considerable potential as a tool for the estimation of the conformation of PBLA<sup>14,15</sup>. In this study, the conformations of the films such as the  $\alpha$ -helix, the  $\omega$ -helix or the  $\beta$ -form were judged from their amide I and II bands. *Figure 1* shows typical infra-red spectra of the films of PBLA in various conformations. The films in the  $\alpha$ -PBLA have amide I band at about 1665 cm<sup>-1</sup> and amide II band at 1555 cm<sup>-1</sup>

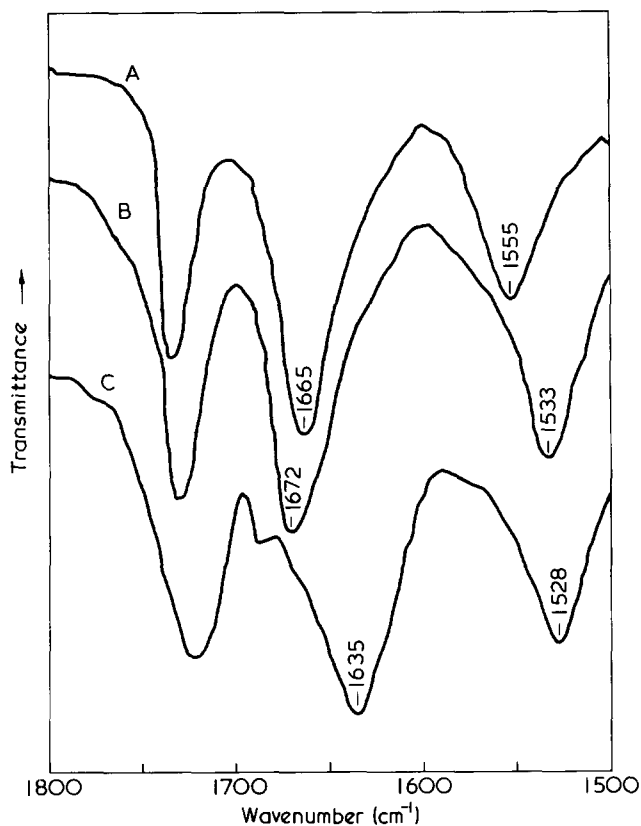


Figure 1 Infra-red absorption spectra of poly( $\beta$ -benzyl L-aspartate) films. Measured at 22°C. A,  $\alpha$ -helix; B,  $\omega$ -helix; C,  $\beta$ -form

Table 1 Characteristic absorption bands of poly( $\beta$ -benzyl-L-aspartate) films at 22°C

Assignment	Wavenumber (cm <sup>-1</sup> )		
	$\alpha$ -Helix	$\omega$ -Helix	$\beta$ -Form
Amide I	1665–1667	1670–1673	1635–1637
Amide II	1555–1557	1530–1533	1525–1528

When the  $\alpha$ -helix  $\rightarrow$   $\omega$ -helix transition is complete, the wavenumber of the amide I band increases and the amide II band decreases. When the  $\alpha$ -helix  $\rightarrow$   $\beta$ -form transition occurs, both the amide I and the amide II decrease in wavenumber. These results are summarized in Table 1.

#### Circular dichroism

*C.d. spectra between 245 and 280 nm.* It is well known that poly( $\gamma$ -benzyl glutamate) in a poor solvent such as chloroform is in the  $\alpha$ -helical conformation and has its characteristic c.d. spectrum below 240 nm<sup>19</sup>. In a dilute solution (polymer concentration below 0.2 mol glutamyl residue/l) the benzyl groups of this helical polypeptide do not have optical activity. However, the films and liquid-crystalline solutions of the polypeptide show aromatic c.d. bands between 245 and 280 nm. According to Iizuka *et al.*<sup>18</sup> the sign of these bands is positive in the L-isomer (PBLG) and negative in the D-isomer. The induced c.d. has been interpreted as due to the following hypothesis that the benzene rings in the side chains of the polypeptide associate with each other to enhance the intermolecular interactions and induced optical activity. Tsuchihashi *et al.*<sup>20</sup> reported

the c.d. of concentrated solutions of PBDG in methylene chloride under the influence of a magnetic field. The aromatic c.d. of PBDG was positive in sign, which contrasted with the results by Iizuka *et al.*

Figure 2 shows typical c.d. of PBLA in various conformations. The sign and the magnitude of the c.d. differ markedly depending on the conformation. The multiple c.d. bands in  $\alpha$ -PBLA are negative in sign, and have magnitude less than  $-1000$ . The c.d. of the  $\omega$ -PBLA is also negative in sign, and has magnitude about  $-7000$ . The c.d. of  $\beta$ -PBLA is positive in sign, and has magnitude comparable with that of  $\alpha$ -PBLA.

These aromatic c.d. appear to be related to the  $\pi$ - $\pi^*$  vibrational allowed transition of the phenyl groups of the polypeptide, and their sign and magnitude depend on the helix sense and conformation. As may be seen from Figure 2, magnitude of the c.d. in the  $\omega$ -PBLA is much larger than that of the  $\alpha$ -helical and the  $\beta$ -conformation. This may indicate that in the  $\omega$ -helical conformation interactions between the side chains are much stronger because of closer stacking of the benzyl groups in the side chains of PBLA molecules.

*C.d. spectra below 245 nm.* In order to clarify the c.d. related to the  $n$ - $\pi^*$  peptide electronic transition, c.d. measurements of the films were carried out in the wavelength region below 245 nm. Figure 3 shows the c.d. bands below 245 nm for various conformations of PBLA. In  $\alpha$ -PBLA, a positive c.d. is observed at 226 nm and has magnitude  $[\theta]_{226} = +36\,000$ . PBDG in dilute solution has a positive c.d. band at 222 nm, reflecting the presence of the left-handed  $\alpha$ -helices, whereas  $\alpha$ -PBLA in solid film has a positive c.d. band at 226 nm. This result is similar to that obtained for liquid crystals and films of poly( $\gamma$ -benzyl glutamate)<sup>18,20</sup>. The band at 226 nm is to be assigned to the  $n$ - $\pi^*$  peptide electronic transition, and the  $\alpha$ -PBLA would be of the left-handed type. In the  $\omega$ -PBLA, this c.d. shifts to 224 nm and has magnitude smaller than that in the  $\alpha$ -helical conformation ( $[\theta]_{224} = +28\,000$ ). When in the  $\beta$ -

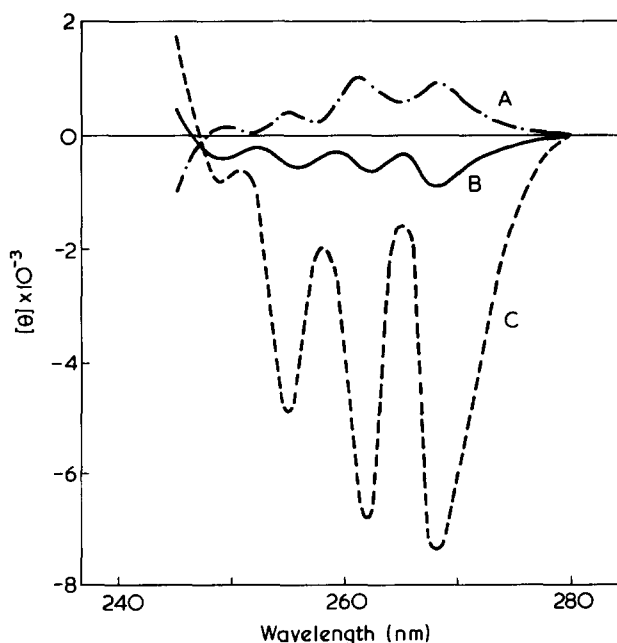


Figure 2 Circular dichroism spectra of poly( $\beta$ -benzyl L-aspartate) films in the wavelength region between 245 and 280 nm. Measured at 22°C. A,  $\beta$ -form; B,  $\alpha$ -helix; C,  $\omega$ -helix

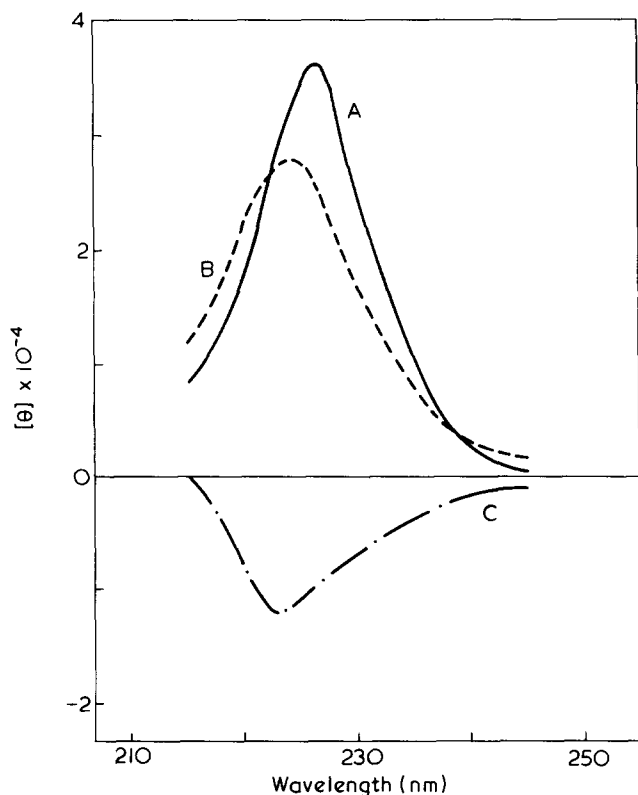


Figure 3 Circular dichroism spectra of poly( $\beta$ -benzyl L-aspartate) films in the wavelength region below 245 nm. Measured at 22°C. A,  $\alpha$ -helix; B,  $\omega$ -helix; C,  $\beta$ -form

PBLA, the c.d. is negative in the sign, is found at 223 nm, and has magnitude  $[\theta]_{223} = -12\,000$ .

As has been mentioned, in the aromatic region the c.d. of the PBLA film depends on the helix sense and polypeptide conformation, the sign, position and magnitude of the  $n-\pi^*$  peptide electronic transition also show a marked dependence in the wavelength region below 245 nm. This dependence could be due to the helix sense and interactions of the peptide groups in the backbones of polypeptide molecules.

Thus, the c.d. has been proved to be a useful technique for the study of the conformation of the  $\omega$ -PBLA that is realized only in the solid state so far.

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