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Structural Study of Poly(β-Benzyl-L-Aspartate) Monolayers at Air-Liquid Interfaces

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ABSTRACT As normally studied, in the solid state or in solution, poly(β-benzyl-L-aspartate) (PBLA) differs from the other helical polyamino acids in that its α-helical conformation is most stable in the left-handed rather than in the right-handed form. The slightly lower energy per residue for the left-handed form in PBLA is easily perturbed, however. The helical screw sense can be inverted in a polar environment and, upon heating above 100°C, a distorted left-handed helix or ω-helix is irreversibly formed. From external reflectance Fourier transform infrared measurements at the air-water interface, the conformation of PBLA in the monolayer state is directly established for the first time. The infrared frequencies of the amide bands suggest that right-handed α-helices are formed on the surface of water immediately after spreading the monolayers and independently of the polypeptide conformational distribution in the spreading solution. The right-handed helical form prevails throughout the slow compression of the Langmuir monolayers to collapsed films. The helical screw sense can be reversed by lowering the polarity of the aqueous phase. In addition, an alternate conformation similar to the ω-helix forms on addition of small amounts of isopropanol to the aqueous subphase, and appears to be an intermediate in the helix–helix transition.

INTRODUCTION

Poly(β-benzyl-L-aspartate) (PBLA) as well as poly(β-methyl-L-aspartate) are unusual in that their α-helical (3.6_{13} helix) conformation is left-handed (Bradbury et al., 1960; Karlson et al., 1960) in the solid state and in solutions of chloroform or trifluoroethanol, while the larger aliphatic and *para*-substituted benzyl esters of poly-L-aspartic acid form right-handed α-helices (Bradbury et al., 1968b; Goodman et al., 1963). Conformational energy calculations for isolated (single-stranded) helices of poly(L-aspartate esters) performed in vacuum (Yan et al., 1968) have been used to determine the relative stability of the right- and left-handed α-helical forms. This was investigated by minimizing the total energy, consisting of contributions from torsional, nonbonded, hydrogen bonded, and electrostatic interactions, with respect to the backbone and side chain dihedral angles. It was found that the left-handed form was slightly more stable than the right-handed form, and that the contribution from the electrostatic energy term appeared to determine the relative stability of these α-helices. These energy calculations have indicated that the interaction of the dipole of the side chain ester group with the dipole of the backbone amide group in both PBLA and poly(β-methyl-L-aspartate) is more repulsive in the right-handed than in the left-handed α-helix, thus destabilizing the right-handed form (Ooi et al., 1967; Yan et al., 1968). Recently there have been ^{2}H-NMR and molecular dynamics simulation studies showing that the conformational entropy of the side chains may be the key in determining helical sense (Abe, 1992, 1997; Okamoto et al., 1995).

Environmental parameters, such as temperature, organic solvent, and aqueous medium, were not taken into account in these calculations. These factors play an extremely important role in determining the conformations of lowest total energy for polypeptides in solution, in films, or in the solid state. Later investigations of the influence of temperature on intermolecular potential energies for several poly(L-aspartate esters) have predicted a reversible helix sense inversion, changing from the right-handed to the left-handed α-form upon increasing the temperature. This was found to agree with experimental results in all cases but one. When heating solid films of PBLA to ~150°C, the left-handed α-helix was irreversibly transformed to a distorted left-handed helix or ω-helix (4.0_{13} helix) (Bradbury et al., 1962). In addition, the presence of water is expected to weaken the dipole–dipole interactions between backbone and polar side chains, thereby reinforcing the right-handedness. From electron diffraction and infrared spectroscopy measurements, Malcolm first reported the presence of some right-handed α-helices in collapsed monolayers of high molecular weight PBLA that were formed on the surface of water (Malcolm, 1970).

The geometric constraints imposed on polymer chains at an interface are quite different from those encountered in bulk or in solution. The helical screw sense and conformational stability of several polyamino acids that have been studied so far in the bulk state may then be very different in nearly two-dimensional ultrathin films such as membranes and monolayers. Insoluble polypeptide monolayer films present at the air-water interface have been more recently utilized as model compounds for biological systems by a number of authors (Bradbury et al., 1960; Cheesman and Davies, 1954; Kawai et al., 1981; Loeb and Baier, 1968; Malcolm, 1973a, b) in order to investigate chain folding,

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molecular orientation, and domain formation. Very little is known about the conformational state and stability of these polypeptides in the monolayer state at the air-water interface, where specific interactions with the polar aqueous phase may result in different equilibrium microstructures. In addition, the arrangement and interfacial conformation of the polypeptide chains in this asymmetric environment may be modified upon slow compression of Langmuir films on the surface of water. The effect of spreading solvent, and thereby of the conformation in solution, on the film conformation at the liquid interface has already received some attention (Baglioni et al., 1988; Gabrielli and Davidson, 1975), since different macromolecular forms may be induced either by changing the liquid substrate or by modifying the spreading solvent composition. Other factors such as the pH, polarity, and temperature of the liquid substrate, and the degree of film compression, may affect the stability and the packing of the microstructures in the monolayer state.

When monolayers of PBLA were first spread on the surface of water, the investigations of collapsed films removed from the liquid surface showed that right-handed α-helices were present in the dried films. Since no technique was available at the time to probe the conformation of these monolayers in situ at the liquid surface, and since the left-handed α-helix is more stable than the right-handed one in the solid state, it was suggested that right-handed α-helices could not have been formed during the deposition and drying processes, but were already present in the monolayer films at the air-water interface (Malcolm, 1970). The different investigations were not conclusive regarding the conformational change, whether it took place during the spreading of the monolayer or whether it was pressure-induced during the compression of the monolayer to a collapsed film, or even induced during both stages. To summarize, the conformational stability of most natural and synthetic polypeptides in the monolayer state remained speculative and determination of the chain conformation in situ was limited by the available techniques.

Only recently has external reflectance infrared spectroscopy been used to acquire structural information at the molecular level at air-liquid interfaces, especially to characterize in situ molecular chains adsorbed at the air-water interface. This technique has been applied in particular for the determination of chain orientation, chain conformation, and packing density of small molecules such as phospholipids (Dluhy et al., 1988a, b), fatty acids (Gericke and Huehnerfuss, 1993), and fatty alcohols (Buontempo and Rice, 1993; Ren et al., 1994) on the surface of water, and more recently of macromolecular systems (Ren et al., 1995; Riou et al., 1997). Vibrational spectroscopy, a nondestructive technique, is particularly successful in the determination of conformational order in alkyl chains (e.g., *trans/gauche* ratio) as well as in the evaluation of helical or extended conformations in polyamino acids. The presence of different helical forms in polypeptide systems can be easily detected by infrared measurements and the associated conformationally sensitive amide bands. Hashimoto and Arakawa (1967) and Bradbury et al. (1968b) have shown from infrared transmission studies of homopolymers and copolymers of poly(L-aspartate esters) that there is a correlation between the helix screw sense and the frequencies of the amide I and amide II bands. The frequencies for the right-handed α-helix form were found to lie between 1656 and 1659 cm^{-1} for the amide I and 1552–1555 cm^{-1} for the amide II, while for the left-handed form they range between 1664 and 1668 cm^{-1} and 1555–1561 cm^{-1}, respectively. For PBLA, the ω-helix formed at high temperatures gave very different results, with the amide I exhibiting a band around 1675 cm^{-1} and the amide II a band around 1536 cm^{-1}.

In this work monolayers of PBLA have been studied through the use of the combined methods of external reflectance infrared spectroscopy and surface tensiometry. The measurements were performed at the air-water interface using an apparatus described earlier consisting of a homebuilt Langmuir trough equipped with an electrobalance and coupled to a Fourier transform infrared spectrometer (Riou et al., 1997). Monolayer films of polypeptides were formed on an aqueous subphase by spreading from solution. After solvent evaporation, surface pressure–area isotherms were recorded upon slow compression of the films. The infrared frequencies of the amide I and amide II bands that characterize the conformational state of the polypeptides were observed in situ. Pressure-induced conformational changes or formation of alternative conformations according to the spreading conditions and subphase composition have been examined. In this paper we describe unprecedented conformational states of PBLA macromolecules assembled at the air-liquid interface.

EXPERIMENTAL

Materials

PBLA with molecular weights of 14,100 and 39,000 (viscosity measurements) were purchased from Sigma (St. Louis, MO) and used without further purification. The solvents used for spreading the monolayers on the surface of water were used as received. Solutions of PBLA were prepared from a 99:1 (v/v) mixture of chloroform (99.0+%, Fisher Scientific, Pittsburgh, PA) and dichloroacetic acid (99.0+%, Fisher Scientific) at 0.1 mg/ml PBLA. To assure complete solubilization of the polymer, PBLA was first dissolved in dichloroacetic acid and chloroform was then added. Solutions were stored at +5°C and allowed to equilibrate at room temperature before use. Distilled water was further treated by filtration through a Milli-Q purification system (Milli-Q, Marlborough, MA) to give deionized water of nominal resistivity (18.2 $M\Omega\ cm^{-1}$).

Instrumentation and methods

Infrared transmission measurements were performed using a Bruker Model 98 FTIR spectrometer equipped with a liquid nitrogen-cooled wide-band mercury-cadmium-telluride (MCT) detector (Bruker, Bellerica, MA). Films were cast onto calcium fluoride windows and infrared spectra were recorded in the mid-infrared region by co-adding 512 scans with a resolution of 2 cm^{-1}. The temperature-dependent infrared spectra were obtained using a homebuilt heating cell controlled by a Watlow temperature controller (Watlow, Ft. Lee, NJ) and a copper-constantan thermocouple. The temperature values were approximated to ±1°C.

Circular dichroism (CD) spectra were recorded using an Aviv 62DS spectrometer (Aviv, Lakewood, NJ). A rectangular quartz liquid cell with a 0.01 mm pathlength was used for the CD measurement of PBLA solutions. The temperature was maintained constant at +22°C.

External reflectance infrared spectroscopy and surface pressure measurements were performed at the air-water interface using an apparatus described earlier in more detail (Riou et al., 1997), consisting of a home-built Langmuir trough equipped with a Cahn electrobalance and coupled to a Fourier transform infrared spectrometer. The monolayer films were spread on the surface of water using a Hamilton microsyringe by allowing the drops to touch the surface of water. Particular care was taken in the film spreading on isopropanol-containing aqueous subphases. In order to ensure complete evaporation of the solvent and film spreading, sufficient time was allowed before starting the measurements. To ensure complete solvent evaporation, characteristic infrared bands of the solvents used were monitored until disappearance was complete. Monolayer films were then slowly compressed and infrared reflectance spectra acquired as a function of molecular area and surface pressure.

The surface pressure measurements were obtained by the Wilhelmy plate method using a modified Cahn electrobalance. The surface pressure values were approximated to ±0.2 mN m^{-1} due to the variation of the water level over the time frame of the experiments and the surface area values were approximated to ±0.5 $Å^2$/residue. The isotherms were recorded by continuously compressing the monolayer films at the compression rate of 3.0 cm^2 min^{-1}. The temperature of the liquid subphase was maintained constant at +20°C with an accuracy of ±0.2°C using a refrigerated bath circulator. The incident infrared beam was brought to a focus at the air-liquid interface at an angle of incidence of 30 ± 4° and redirected to a narrow-band MCT detector cooled with liquid nitrogen. The external reflection-absorption infrared spectra were obtained by typically co-adding 512 scans with a resolution of 4 cm^{-1} and 0.2 cm^{-1} data interval. A gain of 8 was employed to compensate partially for the weak reflectance from the air-water interface. The absorbance units of the spectra were defined as $-\log(R/R_0)$, where R_0 and R were the reflectivities of pure and polypeptide film-covered liquid surfaces, respectively. In the spectral region between 1400 and 1800 cm^{-1}, the amide I, amide II, and carbonyl ester stretch vibrations, which are conformationally sensitive, overlap strong water vapor bands. These water vapor bands could be reduced and, in some cases, almost totally compensated by carefully regulating the humidity level in the compartment enclosing the Langmuir trough.

RESULTS AND DISCUSSION

Solutions and cast films

Fig. 1 illustrates the CD absorption spectrum for the n-π* transition around 200–240 nm measured at +22°C for the PBLA (M_W = 39,000) sample in chloroform solution. Since chloroform readily absorbs the ultraviolet light below 240 nm, a quartz liquid cell of 0.01 mm pathlength was used. The sign and position of the maximum centered around 223 nm coincide with those reported for polypeptides in the α-helical conformation, where the positive sign indicates that the helix has a left-handed screw sense.

A typical transmission infrared spectrum of a PBLA (M_W = 39,000) film cast on a calcium fluoride disk from a 99:1 (v/v) chloroform-dichloroacetic acid solution at room temperature is shown in Fig. 2 (*thick line*). Characteristic vibrational bands arising from the N—H stretch, carbonyl ester stretch, amide I, and amide II are located at ~3301, 1734, 1666, and 1558 cm^{-1}, respectively. These frequencies are consistent with the left-handed α-helix, the amide band frequencies falling within the ranges given earlier by Elliott and Ambrose (1950).

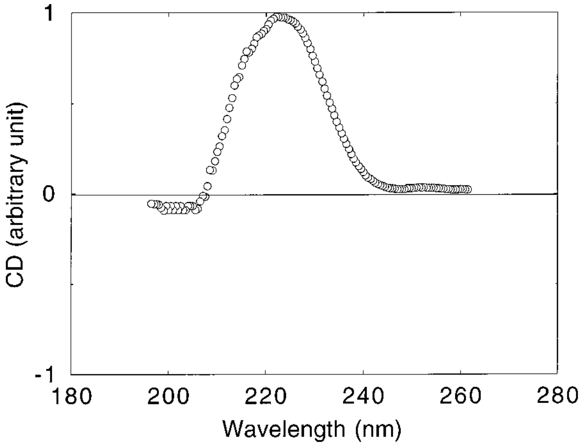

FIGURE 1 Circular dichroism spectrum obtained for a 99:1 (v/v) chloroform-dichloroacetic acid solution of PBLA (M_W = 39,000) at room temperature.

Raising the temperature from 20°C to above 100°C results in frequency shifts in the infrared spectrum, as shown

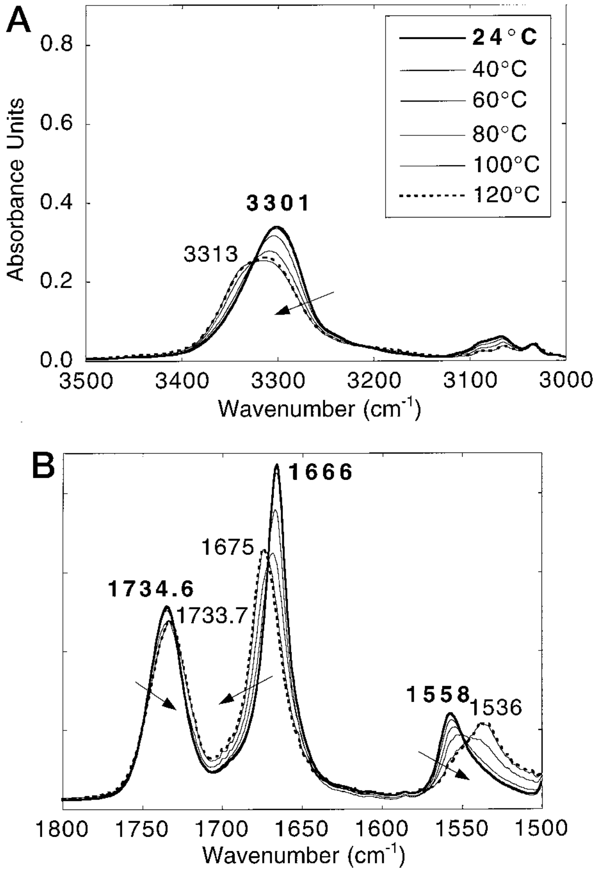

FIGURE 2 Effect of increasing the temperature on the Fourier-transform transmission spectrum of a PBLA (M_W = 39,000) film cast on a CaF_2 disk from a 99:1 (v/v) chloroform-dichloroacetic acid solution. (*A*) N—H stretching region; (*B*) amide I and II region.

in Fig. 2. These changes are accompanied by a decrease in peak intensity and band broadening of each vibrational band. The frequency shifts of each characteristic band for PBLA (M_W = 39,000) upon heating above 100°C and subsequent cooling to room temperature are summarized in Fig. 3. Upon raising the temperature to ~105°C, frequency shifts to higher values of 9 cm^{-1} for the amide I band and 12 cm^{-1} for the N—H stretching band are observed, while the amide II band and the carbonyl ester stretching band are shifted 22 cm^{-1} and ~1 cm^{-1}, respectively, below their values at room temperature. The frequencies of the amide I and amide II bands at 1675 cm^{-1} and 1536 cm^{-1}, respectively, observed at 120°C are characteristic of the left-handed ω-helix conformation. This is consistent with the findings first made by Bradbury et al. (1962), where a transition from the α-helix to the ω-helix was induced on heating solid films of PBLA to 140°C. The frequency shifts induced on heating the solid films and shown in Fig. 3 are accompanied by a transition region between ~80°C and 100°C. This is in good agreement with the results from NMR measurements (Happey et al., 1971) and differential thermal analysis (Watson et al., 1966) that indicated transitions from the α-helix to the ω-helix form at 90°C and 95°C, respectively. On cooling the films to room temperature, this temperature-induced transition is irreversible, as illustrated by the frequencies of the filled circles in Fig. 3. The frequency changes which occurred in the amide bands and ester carbonyl stretching vibration on transforming from the left-handed α-helix to the left-handed ω-helix form at room temperature are summarized in Table 1. In this table are also included the literature results from infrared measurements of the left-handed α-helical PBLA (Bradbury et al., 1962) and right-handed α-helical homopolymers and copolymers of poly(L-aspartate esters) (Bradbury et al., 1968a).

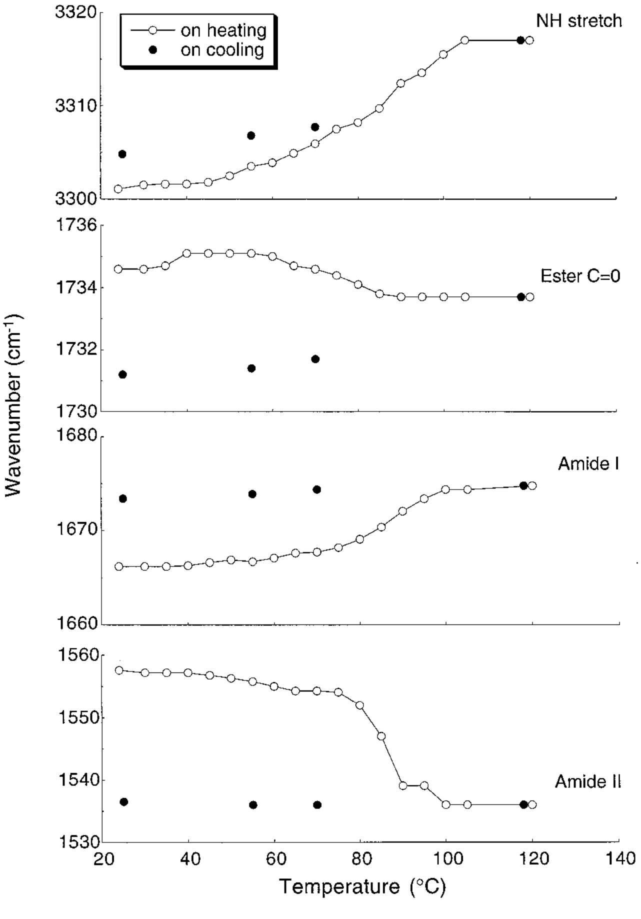

FIGURE 3 Effect of temperature on the frequency positions of the amide and carbonyl ester bands for a PBLA (M_W = 39,000) cast film.

External reflectance infrared spectra

External reflectance infrared spectra were measured during the slow continuous compression of the monolayers. A careful humidity control above the surface of water was necessary to observe the characteristic spectral features associated with the carbonyl ester vibration and the conformationally sensitive amide I band that overlap strong water vapor bands in the range 1600–1800 cm^{-1}. Fig. 4 shows a typical reflection-absorption infrared spectrum of a PBLA (M_W = 39,000) monolayer in the mid-infrared region obtained by co-adding 512 scans at 4 cm^{-1} resolution. The infrared data were not smoothed and show only residual water vapor fluctuations. The shift in the baseline at ~1650 cm^{-1} is caused by the presence of the strong change in the refractive index of liquid water in this spectral region. Another remarkable feature is the large upward band in the 3000–3700 cm^{-1} region arising from the O—H stretching vibration of liquid water that is caused by the displacement of water molecules from the interface.

Surface pressure–area isotherms

Monolayers of PBLA on the surface of water were obtained by spreading the polypeptide from chloroform solutions containing 1% by volume of dichloroacetic acid at an area of ~23 $Å^2$/residue. After the solvent had evaporated, the monolayers were continuously compressed at 0.22 $Å^2$ min^{-1}/residue. The surface pressure–area isotherms obtained at room temperature (+19.5°C) for both molecular weights (M_W = 14,100 and 39,000) of PBLA were almost identical (Figs. 5 *A* and 6 *A*). The first rise in surface pressure occurred at a surface area of ~21 $Å^2$/residue and the height of the plateau was ~12 mN m^{-1}. The plateau is characteristic of α-helical polypeptides and has been interpreted as a transition from a monolayer to a bilayer before the collapse of the film (Malcolm, 1973b).

In Figs. 5 *B* and 6 *B* are represented reflectance infrared spectra of PBLA monolayers for M_W = 14,100 and 39,000, obtained at various cross-sectional areas. The frequencies of the ester band, the amide I, and the amide II band that lie approximately at 1740, 1660, and 1553 cm^{-1}, respectively, were measured at the different cross-sectional areas marked by the labeled arrows drawn above the isotherms in Figs. 5 *A* and 6 *A*. The frequency of the N—H stretching vibration, which appeared as a notch at ~3296 cm^{-1} in the strong

TABLE 1 Band positions for left- and right-handed helical forms of poly(β-benzyl-L-aspartate)

Band Assignment	Frequencies (cm^{-1})					
	α_L-helix*	Cast Films#	ω-Helix*	Heated Films§	α_R-Helix¶	Monolayers‖
NH stretch	3302 ± 3	3301	3296 ± 5	3305	3296 ± 3	3290
Ester C═O	1735 ± 2	1735	1731 ± 2	1731	1741 ± 2	1739–1741
Amide I	1666 ± 2	1666	1675 ± 2	1674	1659 ± 2	1659–1661
Amide II	1557 ± 2	1558	1536 ± 2	1536	1553 ± 2	1553–1555

*Frequencies for the left-handed α-helix (α_L) and ω-helix in poly(β-benzyl-L-aspartate) films obtained by Bradbury et al. (1962).

#Films cast from chloroform solution at room temperature.

§Cast films heated to 120°C, then cooled to room temperature.

¶Frequencies for the right-handed α-helical form (α_R) of poly(L-aspartate esters) films (Bradbury et al., 1968a).

‖Monolayer films spread from chloroform solution at the air-water interface.

upward O—H stretching band, was a further check at high surface concentrations to discriminate between the right-handed and the left-handed α-helical forms. The results are summarized in Fig. 7. The frequencies are in good agreement with the values obtained for the right-handed helical form of poly(L-aspartate esters) (Bradbury et al., 1968a), and are quite different from those of the left-handed α-helices present in the cast films of PBLA (Table 1). Upon compression of the molecular films on the surface of water, only slight shifts in the frequencies are identified. One can fairly say that the spectra were independent of the degree of compression, even through the plateau region and the film collapse that follows. These results are thus consistent with Malcolm's early findings showing the presence of right-handed α-helices in collapsed films of PBLA (Malcolm, 1970). We find in addition that the right-handed helical conformation is formed immediately on spreading the polypeptide solution on the surface of water, since it is found before the film was compressed at large molecular areas. The right-handedness probably arises from the presence of water, which weakens the side chain-backbone interactions. The conformational analysis of poly(L-aspartate esters) has already shown that the side chain conformation is sensitive both to the proximity of the ester group to the backbone and to the dielectric constant of the environment (Ooi et al., 1966, 1967). The entropy of the side chain may also influence the helical sense of the main chain (Abe, 1992, 1997; Okamoto et al., 1995).

Effect of pH changes in the subphase

Monolayers of PBLA (M_W = 39,000) were spread on various aqueous phases with different pH values. The sur-

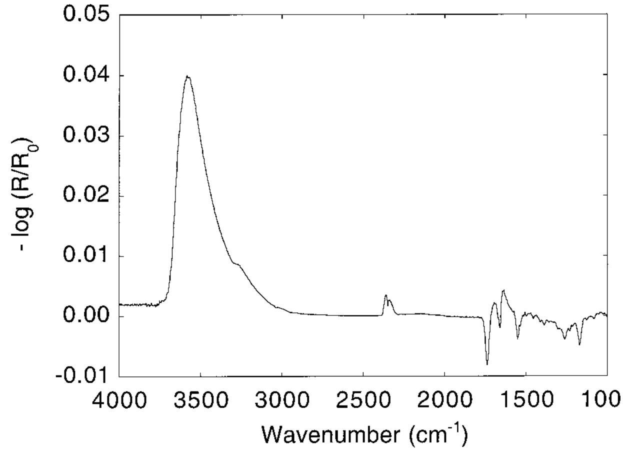

FIGURE 4 Infrared reflection-absorption spectrum at the air-water interface of a PBLA (M_W = 39,000) monolayer obtained at room temperature and a surface pressure of 42 mN m^{-1}.

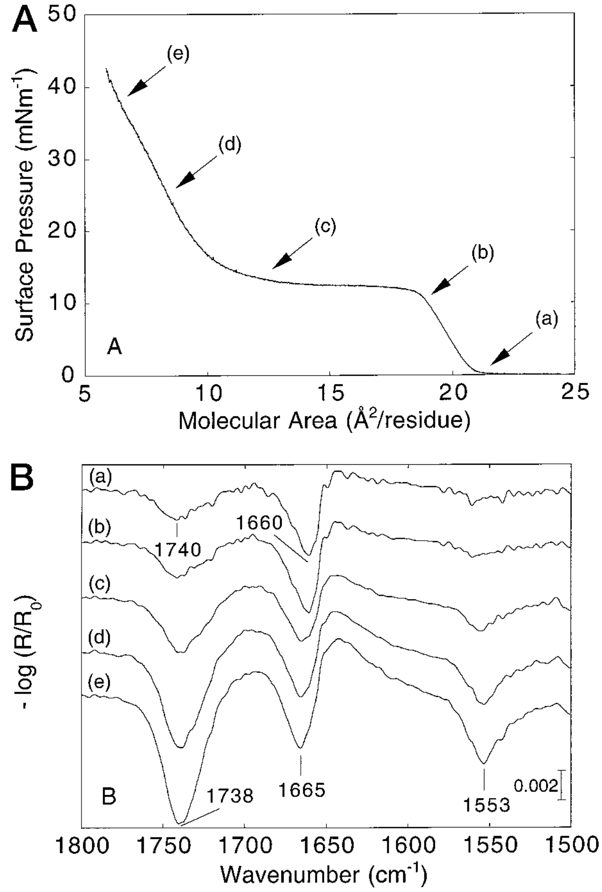

FIGURE 5 (*A*) Surface pressure–area isotherm of a PBLA (M_W = 14,100) monolayer spread from a 99:1 (v/v) chloroform-dichloroacetic acid solution on the surface of water. (*B*) Infrared reflection-absorption spectra at the air-water interface of the corresponding monolayer for different molecular areas per residue.

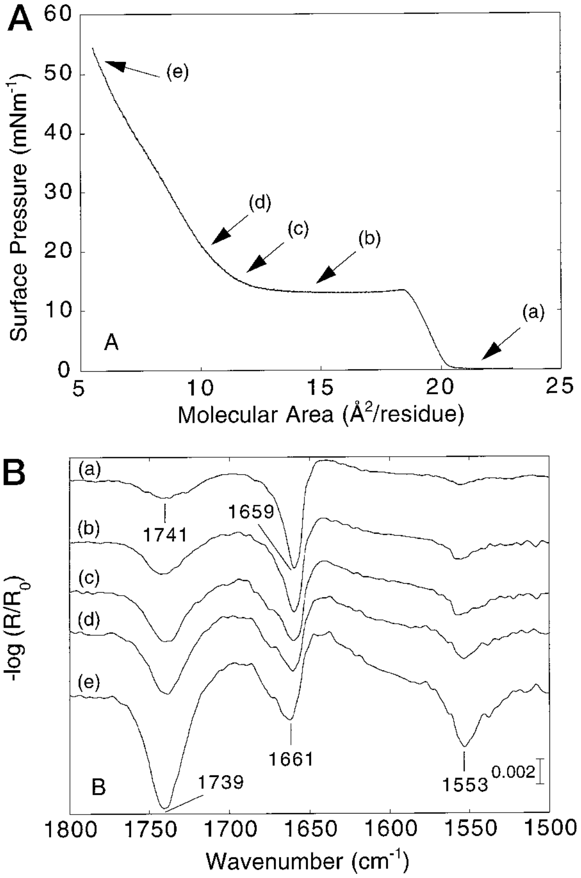

FIGURE 6 (*A*) Surface pressure–area isotherm of a PBLA (M_W = 39,000) monolayer spread from a 99:1 (v/v) chloroform-dichloroacetic acid solution on the surface of water. (*B*) Infrared reflection-absorption spectra at the air-water interface of the corresponding monolayer for different molecular areas per residue.

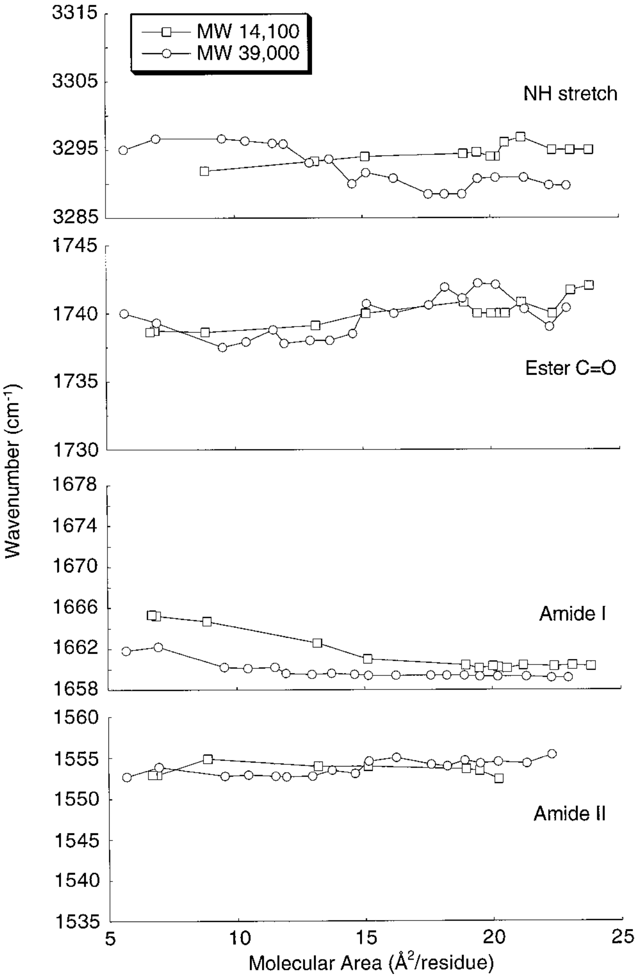

FIGURE 7 Frequency positions of the N—H stretch, ester C═O, amide I and amide II bands for PBLA (M_W = 14,100 and 39,000) monolayers at the air-water interface as a function of molecular area per residue.

face pressure–area isotherms obtained on pure water (pH = 6) and on acidic substrate (pH = 2) showed no appreciable difference. The isotherms obtained on supports at pH > 7 show a gradual rise of the flat region and a shift toward smaller areas with increasing pH (Fig. 8). This can be attributed to the increasingly hydrophilic nature of the side chains and a partial solubilization into the subphase. Although the monolayers should become more expanded when the side chains are ionized, the slow dissolution into the subphase causes the molecular areas to decrease. At pH 12 no pressure rise is recorded upon compression, which is probably due to the complete denaturation of the polypeptide at this pH value (Baglioni et al., 1982; Malcolm, 1973b).

The external reflectance infrared spectra that were recorded on the different substrates at pH 2, 6, 8, and 10 for different molecular areas all have vibrational bands characteristic of the right-handed α-helix. No sign of polymer in the infrared spectra was visible at the air-liquid interface for the substrate at pH 12, consistent with complete polypeptide desorption from the interface. Since all monolayer films were compressed at a relatively slow rate (0.22 Å^2 min^{-1}/residue), allowing unstable films to desorb during the course of the experiment, a more rapid compression rate (3.05 Å^2 min^{-1}/residue) was used to compress a PBLA film spread on an aqueous substrate of pH 12. After cessation of compression, the surface pressure was found to decrease rapidly in the next hour, reaching zero values ~12 h after the compression step. External reflectance infrared spectra were obtained at different pressure values as indicated in Fig. 9. The intensity of all the vibrational bands decreased slowly with the change in surface pressure as molecules desorbed from the interface. The frequencies, however, remained unchanged and are characteristic of the right-handed α-helical conformation such as the conformation found in monolayers spread onto more acidic substrates. A broadened band for the carbonyl ester stretching vibration to lower frequencies (~1720 cm^{-1}) suggests the hydrolysis of the side chains directed into the water phase and confirms the denaturation of the polypeptide taking place.

Effect of isopropanol in the subphase

When monolayers were spread on an aqueous subphase containing small amounts of isopropanol (up to 1% v/v), the

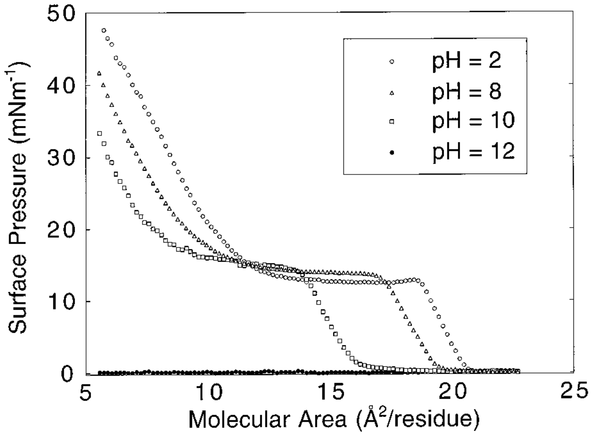

FIGURE 8 Surface pressure–area isotherms of PBLA (M_W = 39,000) monolayer films spread on various aqueous subphases with different pH values.

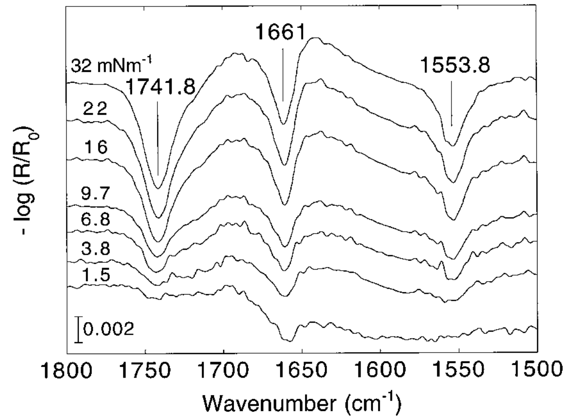

FIGURE 9 Changes in infrared reflection-absorption spectra of a PBLA monolayer rapidly compressed on an aqueous substrate of pH 12 and corresponding surface pressure values.

height of the plateau and the area at which the first rise in surface pressure occurred were both reduced (Fig. 10). When the monolayer was spread on a surface of water containing 1% (v/v) isopropanol, the plateau disappeared and the measured cross-sectional areas were extremely low, suggesting a nonequilibrium surface film present on the liquid surface. A similar contraction of area was observed by Malcolm (1970). Reducing the amount of isopropanol to 0.5% produced isotherms with higher areas at given surface pressures. Intermediate isotherms for various isopropanol content with different limiting areas and plateau lengths have been left out of Fig. 10 for simplicity.

The low values of the limiting areas may be attributed to the loss of polymer from the interface into the subphase due to the solubility of the polypeptide in isopropanol. The poor reproducibility in surface area indicates that the molecules are not fully condensed into an organized continuous film. The lowering of the plateau mainly arises from the reduction of the adhesion between the polypeptide and the aqueous subphase containing isopropanol. It has been shown that the work of adhesion, as calculated from the Young's equation and applied to monolayers, is a function of the surface tension of the liquid subphase with respect to air and the angle of contact of the polymer with the liquid interface (Malcolm, 1968). The surface tensions of isopropanol and water determined at 20°C are quite dissimilar with reported values of 21.7 mN m^{-1} and 72.8 mN m^{-1}, respectively, and the measurement of surface tensions for mixtures of water and isopropanol in the range of 0 to 4% (v/v) isopropanol have given a range of surface tensions from 72.8 mN m^{-1} to 54 mN m^{-1} (Malcolm, 1968). It has been indeed predicted that when the surface tensions of two liquids, such as isopropanol and water, differ appreciably, the addition of small amounts of one liquid with lower surface tension generally results in a marked decrease of that of the other liquid. This effect arises from a selective adsorption of the solvent with lower surface tension at the interface of the mixture. Moreover, the conditions of spreading are modified with the reduction of the angle of contact of the polymer/liquid interface with the addition of isopropanol into the subphase.

External reflectance spectra were obtained for the monolayers spread on the surface of water containing different amounts of isopropanol. In Fig. 11 are represented reflectance infrared spectra of PBLA (M_W = 39,000) monolayers spread on 0, 0.4, 0.5, 0.8, and 1% (v/v) isopropanol contained in the aqueous subphase and obtained at ~40 mN m^{-1} surface pressure. The frequencies of the amide I and II bands, the carbonyl ester band, and the N—H stretching vibration are given in more detail in Fig. 12 for the 0, 0.5, and 1% (v/v) isopropanol compositions as a function of molecular area or degree of compression. The frequencies obtained for the monolayer spread on pure water are consistent with the right-handed α-helix, as shown earlier, whereas for the monolayer spread on an aqueous substrate containing 1% (v/v) isopropanol the frequencies are characteristic of the left-handed form. The latter frequencies are

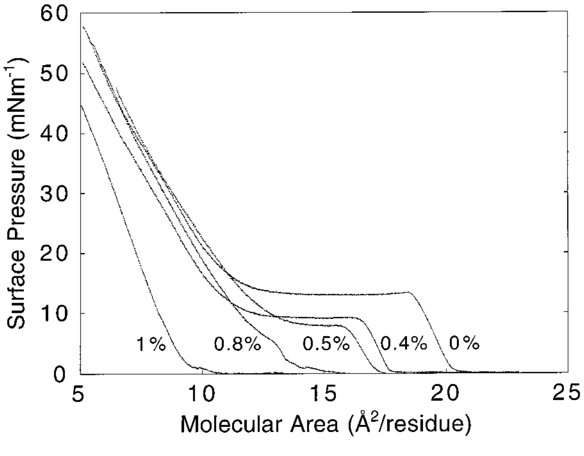

FIGURE 10 Surface pressure–area isotherms of PBLA (M_W = 39,000) monolayer films spread from a 99:1 (v/v) chloroform-dichloroacetic solution on aqueous subphases containing various amounts (% v/v) of isopropanol.

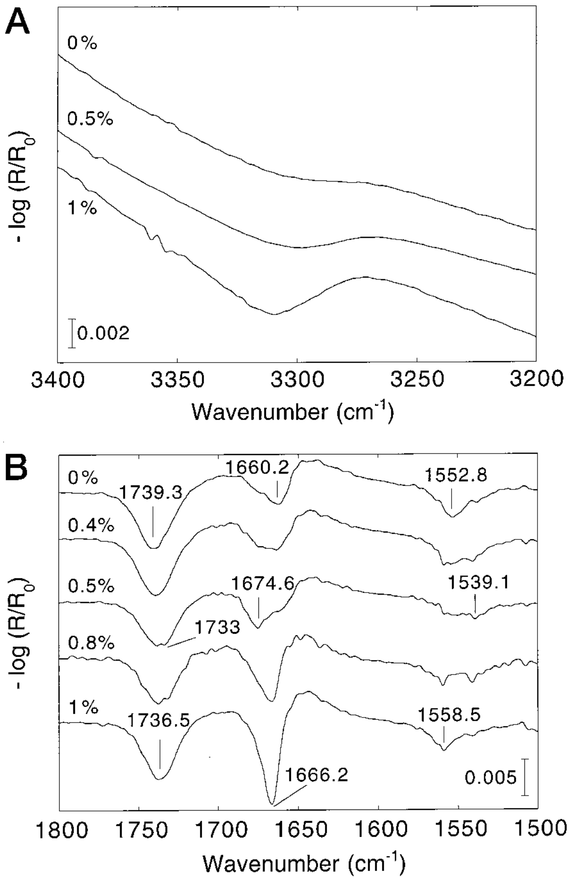

FIGURE 11 Infrared reflection-absorption spectra at the air-liquid interface of PBLA monolayers spread on aqueous subphases containing various amounts of isopropanol and compressed to surface pressures of ~40 mN m^{-1}. (*A*) N—H stretching region; (*B*) amide I and II region.

in good agreement with the ones obtained for the solid cast films (Table 1).

Most interesting are the frequencies found when monolayers were spread on an aqueous subphase containing ~0.5% (v/v) isopropanol. Additional vibrational bands appeared with frequencies quite different from the ones characteristic of the right- or left-handed α-helical forms. In particular, a second band at 1733 cm^{-1} in addition to the 1739 cm^{-1} band appears in the carbonyl ester vibration, a strong band maximum at 1674.6 cm^{-1} dominates the amide I band and an additional band at 1539 cm^{-1} arises in the amide II region (Fig. 11). All these features are in surprisingly good agreement with the presence of the ω-helix conformation normally obtained by heat treatment of either right- or left-handed α-helices of PBLA. Furthermore, reflectance infrared spectra of monolayers spread on subphases of intermediate isopropanol concentrations show a progressive transition from one conformation to the other (Fig. 11). At 0.4% (v/v) isopropanol concentration the amide I band is broadened, suggesting a large distribution of conformations. Due to the high signal-to-noise ratio obtained for this particular spectrum, three distinct bands at frequencies corresponding to the ω-conformation, the right-handed, and the left-handed α-helices appear to contribute to the amide I band. At 0.8% (v/v) isopropanol concentration, band frequencies correspond to the left-handed α-helix with some contribution of the ω-conformation. The frequencies of the characteristic vibrational bands shown in Fig. 12 were quite independent of the molecular area, the different conformations being stable throughout the film compression.

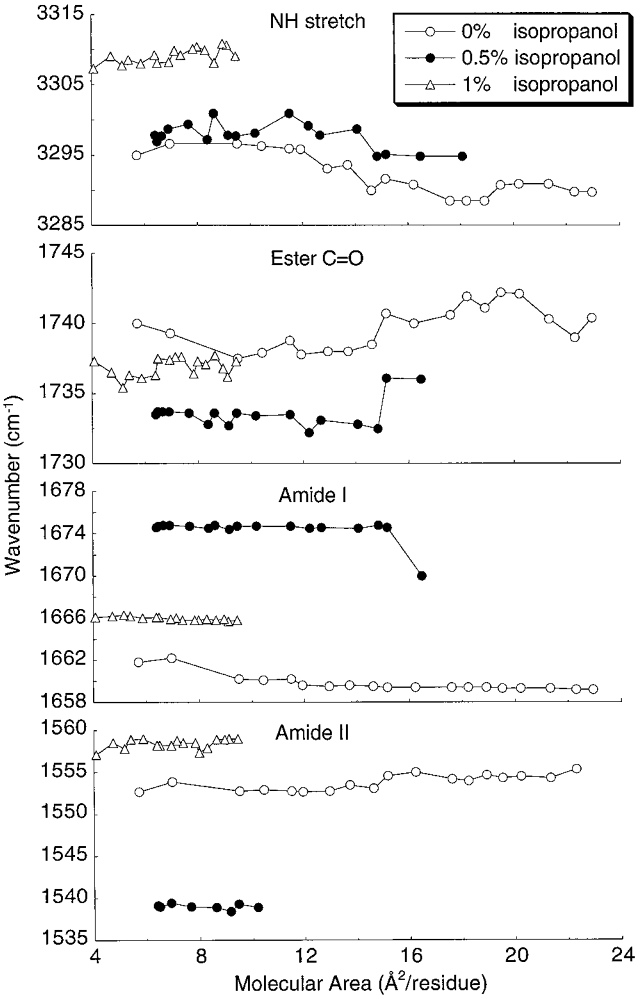

FIGURE 12 Frequency positions of the N—H stretch, ester C=O, amide I, and amide II bands for PBLA monolayers spread on aqueous subphases containing 0, 0.5, and 1% (v/v) isopropanol as a function of molecular area per residue.

As the isopropanol concentration is increased in the aqueous subphase, the infrared spectra clearly show the increase of the left-handed α-helix contribution at the expense of the right-handed helical form. This structural change is to be correlated with the diminution of the limiting areas in the surface pressure–area isotherms. A number of factors may affect the structure and behavior of the monolayer films when isopropanol is added to the subphase. In addition to the fact that the surface tension of the subphase and the contact angle of the polymer at the liquid interface decrease with the presence of isopropanol, the polarity and dielectric constant of the aqueous subphase near the air-liquid interface are lowered. The dielectric constant ϵ for isopropanol measured at 25°C is ~18.3, compared to 78.5 for water. In general, reducing the polarity and the dielectric constant of an organic solvent tends to increase the electrostatic forces in proteins and other polyamino acids. Water apparently favors the right-handed helical form by reducing the backbone-side chain electrostatic interactions that would otherwise stabilize the left-handed α-helix.

The presence of the ω-helix conformation in the monolayer films at room temperature is most surprising since this fourfold helix is usually obtained by heating solid films of PBLA to at least 100°C. However, Goodman et al. (1963) reported the presence of amide I bands close to 1675 cm^{-1} attributed to the ω-helix conformation in the infrared measurements of chloroform solutions of poly(γ-methyl-L-aspartate). According to this author, the ω-helix may also exist at room temperature in the solution state. The change of helical sense from the right-handed to the left-handed α-form, according to Malcolm (1970), does not necessarily involve a net rotation of the whole molecule about its axis. Intramolecular hydrogen bonds may open, the molecule refolding in the opposite sense by rotation about the bonds to the α-carbon atoms, and unfolded regions propagating along the molecule, the driving force for this helix transition being the higher stability of the left-handed form. When both a change of helix sense and pitch occur, such as the transformation from an α-helix to the ω-helix, the same process may be involved with the propagation of unfolded regions along the molecular chain.

CONCLUSIONS

On the basis of the results from spreading experiments and infrared measurements, it was found that films of poly(β-benzyl-L-aspartate) could exist in different structural forms at the air-liquid interface. Structural characterization was achieved directly at the air-liquid interface using external reflectance infrared spectroscopy. The conformations at the air-liquid interface were found to be independent both of the spreading solvent (helical-promoting or random coil-promoting solvent) and film compression. The right-handed α-helical structure formed spontaneously on pure water substrates, whereas the left-handed α-form was more stable on isopropanol containing water subphases. The frequencies of the characteristic amide bands for the two helical forms were used to show that the monolayer spread from chloroform solution undergoes a transition from the right-handed to the left-handed α-helix when altering the polarity of the liquid substrate. In addition, the left-handed ω-helix, whose formation usually requires high temperature conditions, was stabilized at ambient temperature. Finally, the change in pH of the aqueous substrate led to the gradual hydrolysis of the polypeptide chains and complete denaturation at ~pH 12.

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