

Do Parallel β -Helix Proteins Have a Unique Fourier Transform Infrared Spectrum?

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ABSTRACT Several polypeptides have been found to adopt an unusual domain structure known as the parallel β -helix. These domains are characterized by parallel β -strands, three of which form a single parallel β -helix coil, and lead to long, extended β -sheets. We have used ATR-FTIR (attenuated total reflectance-fourier transform infrared spectroscopy) to analyze the secondary structure of representative examples of this class of protein. Because the three-dimensional structures of parallel β -helix proteins are unique, we initiated this study to determine if there was a corresponding unique FTIR signal associated with the parallel β -helix conformation. Analysis of the amide I region, emanating from the carbonyl stretch vibration, reveals a strong absorbance band at 1638 cm^{-1} in each of the parallel β -helix proteins. This band is assigned to the parallel β -sheet structure. However, components at this frequency are also commonly observed for β -sheets in many classes of globular proteins. Thus we conclude that there is no unique infrared signature for parallel β -helix structure. Additional contributions in the 1638 cm^{-1} region, and at lower frequencies, were ascribed to hydrogen bonding between the coils in the loop/turn regions and amide side-chain interactions, respectively. A 13-residue peptide that forms fibrils and has been proposed to form β -helical structure was also examined, and its FTIR spectrum was compared to that of the parallel β -helix proteins.

INTRODUCTION

The rather unusual parallel β -helical fold was discovered when the crystallographic structure of pectate lyase C (PelC) was solved (Yoder et al., 1993). In this motif the polypeptide chain is coiled into a large, right-handed helix composed of three parallel β -sheets connected by three regions (variously called loops, turns, or bends) leading to three very large parallel β -sheets (Fig. 1, *B* and *E*). We will designate each “circuit” of the polypeptide chain in the β -helix a coil, rather than a turn, to prevent confusion with the “turns” (or loops) connecting the β -strands. Additional proteins have since been found to have a very similar fold, including other members of the pectate lyase superfamily (Heffron et al., 1998), the P22 tail-spike protein (TSP) from *Salmonella typhimurium* (Fig. 1, *C* and *F*) (Steinbacher et al., 1994), and *Bordetella pertussis* P.69 peractin (Emsley et al., 1996). In addition, a left-handed parallel β -helix is found in UDP-*N*-acetylglucosamine acyltransferase (LpxA) from *Escherichia coli* and its homologs (Fig. 1, *A* and *D*) (Raetz and Roderick, 1995). There are also reports of β -roll proteins with two β -strands per coil, as observed in alkaline protease (Baumann et al., 1993), or four β -strands per coil, as in rhamnolacturonase A (Petersen et al., 1997).

The interior of the parallel β -helices is predominantly hydrophobic, with stacks of aliphatic or aromatic residues lined up. Each coil consists of three β -strands with a distinct repetitive pattern of hydrophobic residue preferences at specific positions, corresponding to the side chains forming

the interior of the helix (Heffron et al., 1998; Raetz and Roderick, 1995). For the right-handed parallel β -helix, of nine such preferences, six are for valine, leucine, isoleucine, or alanine. The number of coils in the β -helix varies from 7 to 16 (Fig. 1).

The right-handed parallel β -helices have an elongated cross section, whereas the left-handed parallel β -helices have a triangular cross section (Fig. 1). The diameter of the β -helices ranges from 11 to 27 Å; this variation is observed even within the same molecule, e.g., TSP (Fig. 1 *F*). It is important to note that the parallel β -helix structure under discussion is quite different from the anti-parallel β -helix structure observed for gramicidin (Langs, 1988; Wallace and Ravikumar, 1988).

The extended β -sheet structure arises from hydrogen bonding between the β -strands in the corresponding strands in the coil above and below. Interestingly, in contrast to the other right-handed parallel β -helices, in the P22 TSP the hydrogen-bonding pattern extends into the connecting turns/bends to generate an almost uniform β -helix (Steinbacher et al., 1994). Monomeric parallel β -helical subunits in both the P22 TSP and LpxA are involved in forming a trimer in which adjacent β -helices form a β -sandwich, giving rise to a hydrophilic interior of the oligomerized protein (Steinbacher et al., 1994; Raetz and Roderick, 1995).

There are also some specific side-chain interactions in these parallel β -helices. The pectate lyases and LpxA and its homologs exhibit an asparagine stack (termed an asparagine ladder) characterized by Asn side chains forming H-bonds with adjacent asparagine side chains or, sometimes, main-chain amides. In addition, in pectate lyase a serine stack forms H-bonds between the side chain and main chain. Parallel stacking of aromatic rings is also observed in both

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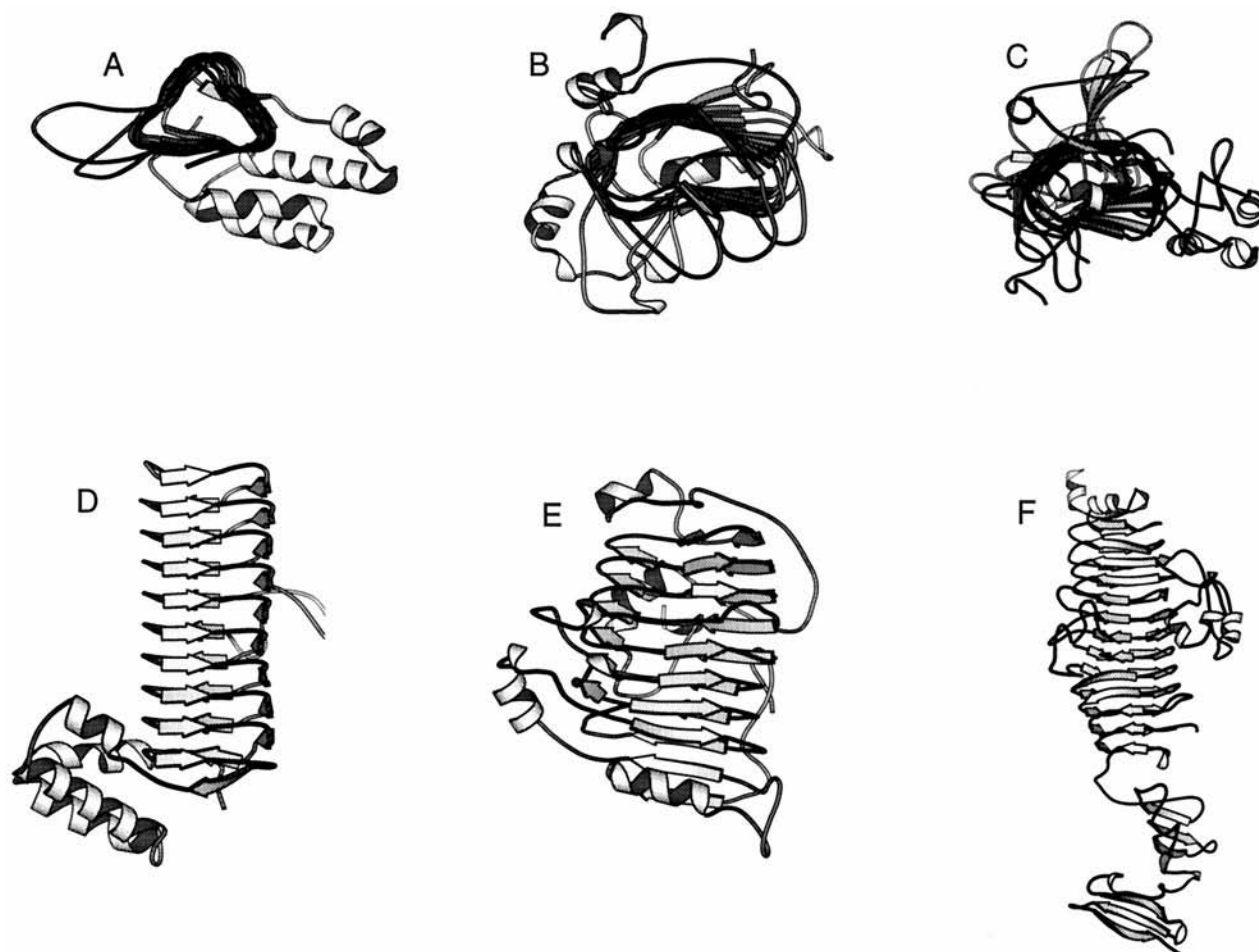


FIGURE 1 The native conformations of the proteins examined, based on their x-ray crystallographic structures, using Molscript (Kraulis, 1991). Top views (A–C) and side views (D–F) of LpxA, pectate lyase C, and P22 tailspike protein, respectively.

the interior and exterior of these proteins. LpxA also has small polar residues (Cys, Thr, Asn) that form H-bonds to either side chains or main-chain amides, in adjacent coils, stabilizing the tight turns at each “corner.”

Molecular modeling suggests that alternating hydrophobic and hydrophilic residues can minimize the contact of hydrophobic side chains with water by adopting a β -helical conformation (Downing 1995). A 13-residue peptide, KLKLELELELG (KLEG), was designed by Downing and co-workers to maximize the opportunity for salt bridges between ionized side chains; this peptide has been demonstrated by electron microscopy to form fibrils (Lazo and Downing, 1997). KLEG is thought to form a β -helical structure based on its circular dichroism spectrum (Lazo and Downing, 1997). The positions of the leucine residues in KLEG correspond to the favored positions for Leu in the consensus sequence for right-handed parallel β -helices (Heffron et al., 1998), lending support to its adoption of such a conformation. In fact, parallel β -helix proteins have a unique sequence profile, and a search of the database for

homologs identified 19 families, of which 39% were carbohydrate-binding proteins and 50% were proteins containing leucine-rich repeats (Heffron et al., 1998).

In this report we have used thin-film attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) to analyze the amide I region of three representative parallel β -helical proteins and the peptide KLEG to determine their secondary structure. The β -helical proteins used in this study were pectate lyase C (Yoder et al., 1993) and P22 TSP (Steinbacher et al., 1994), both of which form right-handed β -helices, and LpxA, which forms a left-handed β -helix (Raetz and Roderick, 1995). This study was initiated 1) to determine whether there were unique peaks in the amide I region for the parallel β -helical proteins that would distinguish them from other types of β -sheet structure, and 2) to look for any similarities between the spectra of β -helical structures and amyloid fibril β -structures, because it has been suggested (Lazo and Downing, 1998) that amyloid fibrils, which are known to have a very high β -sheet content, may also contain antiparallel β -helices,

although a recent report suggests that the Alzheimer's A β peptide has parallel β -strands (Benzinger et al., 1998).

Hydrated thin-film ATR-FTIR was chosen over transmission FTIR because of its technical superiority. For example, the ATR mode has a much higher signal-to-noise ratio, data acquisition is much faster and easier, the samples can be in H₂O rather than D₂O (D₂O may affect protein conformation in some cases), and analysis of the spectra is facilitated by the absence of liquid water. Thin-film ATR-FTIR spectra of proteins are comparable to those obtained by transmission FTIR, and the secondary structure analysis by both methods gives equivalent results (Goormaghtigh et al., 1990, 1999; Oberg and Fink, 1998). Proteins in the thin films are fully hydrated (de Jongh et al., 1996) and hence would be assumed to exist in their native conformation. The thickness of the films is such that only a very small fraction, the monolayer in contact with the internal reflection element (IRE) surface, undergoes conformational perturbation due to interactions with the surface.

MATERIALS AND METHODS

KLEG, LpxA, PelC, and P22 TSPs were generous gifts from Profs. Don Downing, Steven L. Roderick, Frances Jurnak, and Jonathan King, respectively.

ATR-FTIR measurements

All of the protein solutions were prepared by dissolving either the lyophilized powders in 10 mM sodium phosphate buffer (pH 7.5) or by diluting concentrated stock solutions to give a final concentration of 1–2 mg/ml. Aliquots of 50 μ l of the protein solution were loaded onto the germanium IRE (surface area \sim 7 cm²), and a hydrated thin film was formed by using a stream of nitrogen gas to rapidly evaporate most of the solvent. We have previously shown that this technique allows analysis of the spectrum of the native conformation of proteins (Oberg and Fink, 1998). The IRE was then placed in an out-of-compartment attenuated total reflectance accessory (SPECAC), and FTIR spectra were collected using a Nicolet 800SX instrument, with a liquid nitrogen-cooled MCT detector (Oberg and Fink, 1998). The background spectrum was collected using the same IRE before the sample measurements. Water vapor spectra were collected by reducing the dry-air purge with the clean IRE.

Data analysis

The spectra were analyzed using GRAMS software (Galactic Corp.). All of the sample and water vapor spectra were Fourier transformed with medium Norton Beer apodization and converted to absorbance, using the IRE spectrum as a background. The water vapor component was then subtracted from each of the sample spectra. We confirmed that contributions from residual water vapor are not responsible for the observed bands by comparisons with slightly over- and undersubtracted water vapor. The region between 1700 and 1800 cm⁻¹ (where no protein bands are present) was found to be featureless in the second-derivative spectra, indicating satisfactory removal of water vapor components (Oberg and Fink, 1995). As further confirmation, the band positions of water vapor were compared to the observed bands. Second-derivative and Fourier self-deconvolution were used to identify components of the raw spectrum in the amide I region between 1700 and 1600 cm⁻¹, and those peaks were then fitted to the raw spectrum, using the curve-fit routine, by slowly increasing the width of

each peak during the fit, starting with a peak width of 15 and a maximum width of 28 cm⁻¹. The area under each peak was then used to calculate the percentage of each component as represented in the tables and finally used to analyze the percentage of secondary structure components. The calculated side-chain contributions, based on the amino acid composition, were subtracted from the amide I region (Venyaminov and Kalnin, 1990) before the curve fitting using SAFAIR software (developed by Dr. Keith Oberg). In these analyses it was assumed that the extinction coefficient for each type of secondary structure is the same.

The amide I region of the infrared spectrum, 1600–1700 cm⁻¹, predominantly reflects the C=O stretching mode of the amide carbonyl. β -Sheet structure is manifested in the amide I region of the IR spectrum by major bands in the 1623–1643 cm⁻¹ region; furthermore, small absorbance bands in the 1685–1695 cm⁻¹ range are also often observed from β -sheets. Most commonly, β -sheet conformations in globular proteins are evident as bands in the vicinity of 1625, 1632, and 1637 cm⁻¹. Second-derivative FTIR spectra are useful for revealing the major component bands.

RESULTS

Examination of the second-derivative spectra of the amide I region of the FTIR spectra of the β -helical proteins in this study (in H₂O) reveals a dominant band at 1638 cm⁻¹ in each case (Fig. 2), reflecting the high β -structure content. The spectra of PelC and TSP also have a much smaller peak in the vicinity of 1692 cm⁻¹, which may also reflect β -structure, and the PelC spectrum has a second significant band due to β -sheet at 1626 cm⁻¹. After resolution enhancement, curve fitting was done (Fig. 3) to allow quantitative analysis of the FTIR spectra. Secondary structure assignments were based on standard amide I band assignments (Byler and Susi, 1986; Jackson and Mantsch, 1995; Dong et al., 1990; Lee et al., 1990). The results are shown in Table 1. Interestingly, whereas the TSP has just the one major band, at 1638 cm⁻¹, corresponding to 56% β -structure, pectate lyase C and LpxA have a smaller 1638 cm⁻¹ band but additional low-frequency β -bands. All three proteins have 13–18% turn, based on bands in the 1675–1680

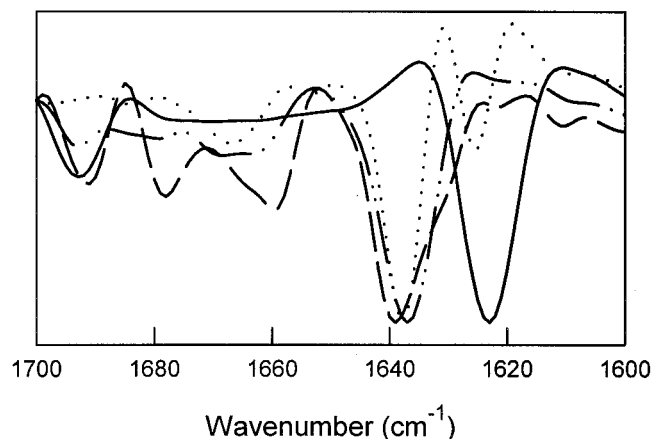


FIGURE 2 FTIR spectra of the β -helix proteins and the peptide KLEG. Second-derivative spectra of the amide I region, showing the major β -structure components in the vicinity of 1638 cm⁻¹ for the proteins and 1623 cm⁻¹ for KLEG. \cdots , PelC; $---$, LpxA; $- \cdot -$, TSP; $—$, KLEG.

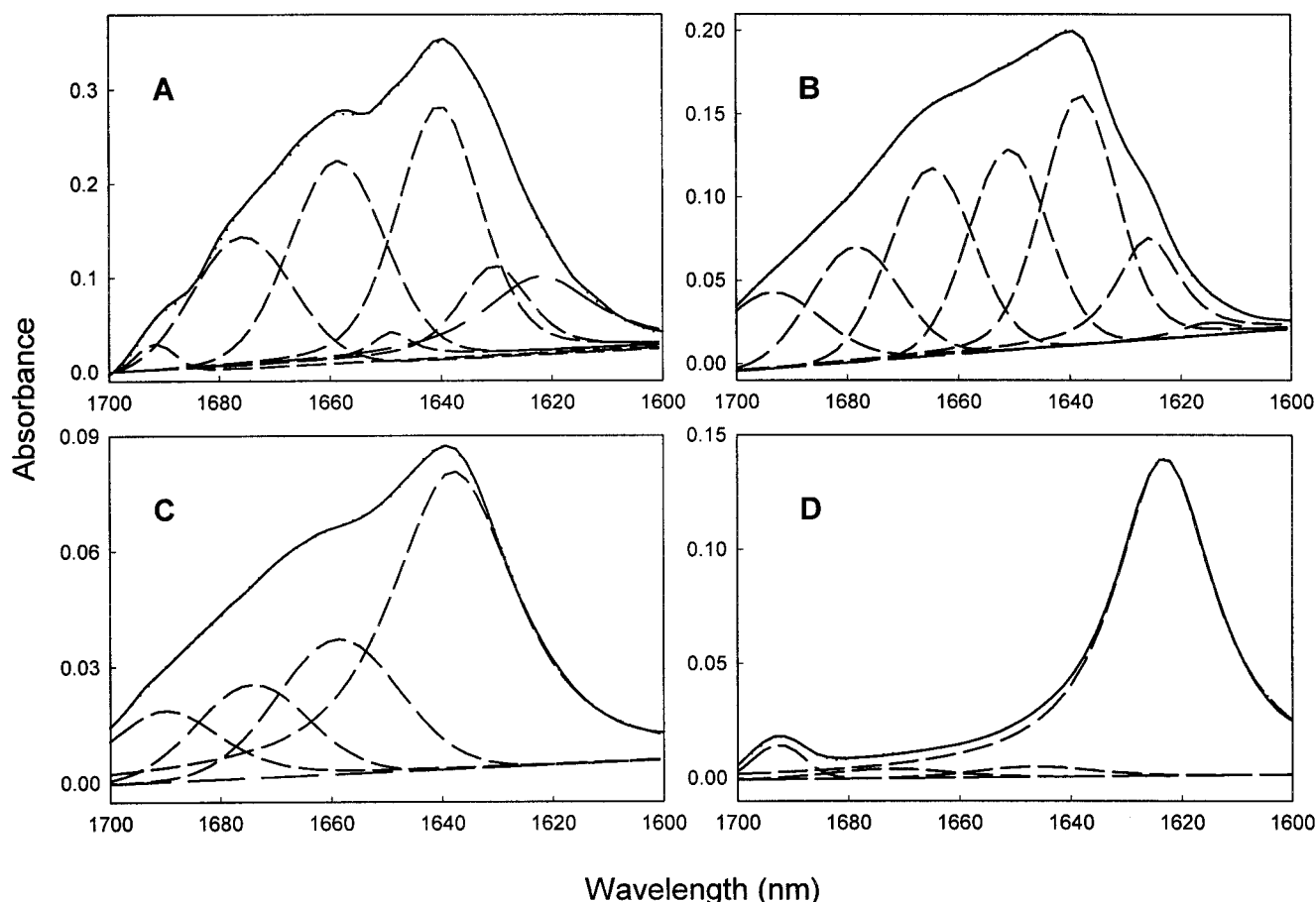


FIGURE 3 The spectra for the amide I region are shown by solid lines for LpxA (A), pectate lyase (B), P22 tail-spike protein (C), and the peptide KLEG (D). The dotted lines in each panel are the sums of the individual curve-fit components (dashed lines) (see text).

cm^{-1} region, and 20–27% loop components, corresponding to bands in the 1660–1666 cm^{-1} region. Only PelC has a distinct helical component of 21% at a band position of 1652 cm^{-1} . The band in the vicinity of 1660 cm^{-1} in LpxA and TSP may also contain some α -helix contributions.

The ATR-FTIR data for KLEG show a major peak at 1623 cm^{-1} (Figs. 2 and 3 and Table 2), indicative of

β -structure with very strong hydrogen bonds, which constitutes 91% of its secondary structure. We attribute this band to the presence of intermolecular β -structure. A minor peak at position 1692 cm^{-1} is also associated with β -structure. Minor bands, at 1673 cm^{-1} (2.5%) and 1646 cm^{-1} (2.7%), may be due, respectively, to turn and extended chain in an unusual local environment. This kind of “pure” all- β struc-

TABLE 1 Secondary structure analysis of the amide I peaks in three naturally occurring parallel β -helical proteins, LpxA, pectate lyase C, and P22 tail-spike protein

LpxA		PelC		P22 tail-spike protein		Protein structure assignment
Peak position	% peak	Peak position	% peak	Peak position	% peak	
1692	1	1694	10	1690	11	β or turn
1676	18	1678	13	1674	13	Turn
1659	26	1665	20	1659	20	Loop/turn/bend or α -Helix
1649	2	1651	21			Disordered/ α -helix
1640	31	1638	26	1638	56	β -Sheet (parallel)
1630	9					β -Sheet
1622	13	1626	10			β -Sheet

The analysis is based on curve-fitting following band deconvolution (see Materials and Methods). The errors are $\pm 1 \text{ cm}^{-1}$ for the band positions and $\pm 5\%$ for the peak areas.

TABLE 2 Secondary structural analysis of the amide I peaks for the peptide KLEG

Peak position (cm ⁻¹)	Percentage peak	Protein structural component
1692	3.8	β or turn
1673	2.5	Turn
1646	2.7	Unknown
1623	91.0	β -Sheet

The errors are ± 1 cm⁻¹ for the band positions and $\pm 5\%$ for the peak areas.

ture ($\geq 95\%$) is quite unique and is not observed with proteins, as even all- β -sheet proteins also have substantial unordered and turn components. Moreover, the structure of KLEG was unchanged regardless of the conditions. The solid peptide, was analyzed using attenuated total reflectance (ATR) FTIR or KBr pellets, and suspensions in water or up to 100 mM NaCl (as hydrated thin films for ATR); all gave identical spectra.

DISCUSSION

As expected, the FTIR spectra of the polypeptides studied are dominated by their β -components. For the three parallel β -helix proteins the main component in each case is located at 1638 ± 2 cm⁻¹. That the major secondary structural component in all three proteins is the parallel β -sheet suggests that the amide I band position for parallel β -sheet may be at this frequency, i.e., 1638 cm⁻¹. This is a position that has previously been attributed to parallel β -sheet conformation (Bandeckar and Krimm, 1988; Susi and Byler, 1987); however, it is very important to note that parallel β -sheet may also be observed at various other frequencies in the 1643–1623 cm⁻¹ region and, furthermore, that antiparallel β -sheet may also be observed at 1638 cm⁻¹ (see discussion below). In contrast, the spectrum of the peptide KLEG showed the main component at 1623 cm⁻¹, reflecting intermolecular β -sheet structure (Jackson and Mantsch, 1995). The spectra of all three proteins showed the presence of substantial loop structure with bands around 1660 cm⁻¹, consistent with the crystallographic structures, which show relatively long loops connecting the β -strands. A notable difference between the spectra of the parallel β -helix proteins and that of the peptide KLEG is that the three proteins show significant amounts of non- β -sheet secondary structure, whereas the peptide is essentially “pure” β -sheet.

The major factors contributing to the frequency of the amide I carbonyl stretching IR vibrational mode are the nature of the hydrogen bond (the stronger and shorter the H-bond, the lower the frequency) and the transition-dipole coupling. It is the latter factor that makes it very difficult to make unambiguous secondary structure assignments in either the amide I or III regions, because the specific environment around a given amide bond may cause significant shifts in the vibrational frequency (Bandeckar and Krimm,

1988). It is generally accepted that IR cannot distinguish between parallel and antiparallel β -sheet structures; for example, a comparison of the spectra of proteins with parallel and antiparallel β -strands showed similar components in the 1639, 1634, and 1624 cm⁻¹ region (Susi and Byler, 1987; Krimm and Bandeckar, 1986; Bandeckar and Krimm, 1988).

Although the three parallel β -helix proteins show substantial amounts of β -sheet structure at 1638 cm⁻¹ (26–56%), this characteristic is insufficient to provide a *unique* signature indicative of a parallel β -helix protein. In fact, the FTIR spectra of the three parallel β -helix proteins are effectively indistinguishable from those of many globular proteins rich in β -structure. The fact that the major β -sheet IR band in parallel β -helix proteins overlaps that of “normal” β -sheets indicates that the hydrogen bonding patterns in β -helical proteins are very similar to those in the β -sheet structures found in typical globular proteins. It is important to note that antiparallel β -sheets can also give bands at 1638 cm⁻¹; for example, the antiparallel β -helix in gramicidin has an IR band at 1638 cm⁻¹ (Naik and Krimm, 1986).

The x-ray crystallographic structures of the three parallel β -helix proteins reveal that the fraction of secondary structure in the β -sheet conformation ranges from 22% to 37%. However, the quantitative analysis of the deconvoluted amide I spectra suggests much higher contributions of β -structure when they are determined by IR (Table 3). What is the source of this apparent discrepancy? We believe it is due to the increased hydrogen bonding in the β -helical proteins, due to two types of interaction, namely those involving hydrogen bonds among side chains, forming asparagine ladders, and between side chain and main chain, observed in LpxA and PelC (Raetz and Roderick, 1995; Yoder et al., 1993), and to β -sheet-like H-bonding in the turns/loops connecting the β -strands in the coils observed in TSP (Steinbacher et al., 1994).

For the TSP many main-chain H-bonds between coils are observed in the turn (loop) regions, leading to a continuous or uniform β -sheet; i.e., the H-bonding pattern of a β -sheet is continued through the turn/loop connecting the parallel β -helix segments (Steinbacher et al., 1994). These residues would not be assigned to β -sheet secondary structure in the crystallographic structure, because their dihedral angles are

TABLE 3 Comparison of β -sheet composition (%) from x-ray crystallography and FTIR

	X-ray	1638 cm ⁻¹	1624–1630 cm ⁻¹	~1692 cm ⁻¹
LpxA	27	31	23	1
PelC	29	26	10	10
TSP	37	56	0	11

The three regions of the amide I spectral region in which β -structure is observed are shown. For LpxA and PelC the data suggest that the contributions at frequencies other than 1638 cm⁻¹ may be due to the non- β -strand regions of the β -helix. In the case of the tail-spike protein these contributions, especially the turns/loops, are also found at 1638 cm⁻¹.

different, but would be classified as β -structure in the analysis of the amide I region of the FTIR spectrum, because the position of the corresponding amide C=O stretch frequency would be very similar to that of the amide residues in the β -helix regions.

In contrast to the P22 TSP, in PelC the main chains in the connecting loops/turns do not form H-bonds with the main-chain atoms of the coils above and below; instead there are some unusual short H-bonds between side chains and side or main chains. Many of these involve the asparagine ladders on the interior of the helix, or in some cases the exterior of the β -helix. Because both LpxA and PelC form asparagine ladders as well as participate in other stacked side-chain interactions, which might be expected to result in lower frequencies of the amide I frequencies, these H-bonds could be the source of the low-frequency (1622–1630 cm^{-1}) bands observed with these proteins, accounting for 10–20% of the observed amide interactions. In light of these considerations, with reference to Table 3, for LpxA there is good agreement between the amount of β -sheet observed at 1638 cm^{-1} and that determined from the crystal structure; thus the 23% additional β -structure observed by FTIR at lower frequencies can be ascribed to the side-chain interactions, such as the asparagine ladders, and possibly additional H-bonding in the loop/turn regions. This would also hold true for pectate lyase C. For the TSP the situation is a bit more complex. In this case, we attribute the extra 20% β -sheet structure corresponding to the 1638 cm^{-1} band to the hydrogen bonding between the coils in the loop/turn regions. Interestingly, the predicted amount of loop-like structure by the PHD secondary structure prediction algorithm corresponds reasonably well to the additional β -structure observed by FTIR over that seen by crystallography, namely 27% for LpxA, 32% for PelC, and 27.5% for TSP.

Circular dichroism investigations of PelC, and its close relative PelE, revealed a shape for the far-UV spectra similar to that of other all- β proteins, but with a much larger amplitude for the trough at 216 nm and maximum at 195 nm (Sieber et al., 1995). It was on the basis of a similar CD spectrum for the peptide KLEG that its conformation was assigned as parallel β -helix, based on the spectrum calculated for 100% β -helix (Lazo and Downing, 1997). However, it is known that far-UV CD may not be the best technique for deciphering the differences between different kinds of β -structures (Venjaminov et al., 1991; Manning, 1989; Johnson, 1990), because quantitative analysis based on changes in the intensity and shifts in peak positions is not yet very clearly understood. In fact, calculations have shown that the intensity of the circular dichroism spectrum of a β -sheet is sensitive to the nature of the side chains involved in the β -sheet formation (Manning et al., 1988).

The spectrum of the peptide KLEG revealed a β band at a frequency (1623 cm^{-1}) significantly lower than that observed for the β -helix proteins, a position potentially more consistent with the presence of stronger hydrogen bonding,

and one that has been attributed to intermolecular β -strand interactions in aggregated proteins, such as thermally denatured gels (Dong et al., 1995), inclusion bodies (Fink et al., 1999), and amyloid fibrils (Bauer et al., 1994; Caughey et al., 1991).

The other unusual feature of the KLEG spectrum is the absence of components corresponding to both turns (1675–1680 cm^{-1}) and loops (1660–1665 cm^{-1}) that were observed to make significant contributions to the spectra of the β -helical proteins (see Tables 1 and 2). In fact, the spectrum of KLEG is unique, suggesting that the peptide is solely in the extended conformation, with no turns, loops, or disordered structure. This is in contrast to typical “all- β -proteins,” which actually have very significant amounts of non- β -sheet secondary structure due to the chain topology. Interestingly, the spectrum of KLEG is very similar to that obtained for the β -strand component extracted from a reference set of protein IR spectra (Cabiaux et al., 1997) by principal component analysis (PCA).

Given that KLEG exists in an extended conformation, without turns or loops, the simplest model for the aggregated peptide chains is one in which the strands are arranged in an antiparallel fashion, involving dimers with complementary electrostatic interactions between the side chains, and hydrophobic interactions between the dimer subunits.

Lazo and Downing (1997) have proposed that because the far-UV CD spectrum of KLEG exactly matches that of PelC, KLEG and, by extension, other amyloid fibrils are likely to have β -helical structure (in the sense of the β -helix proteins investigated in this study). However, the far-UV CD spectra of some amyloid fibrils are very similar to those of a typical globular β -sheet protein (Cascio et al., 1989). Peptides with leucine-rich repeats form fibrils and have several properties similar to those of β -helices; however, fiber diffraction studies show that they are not composed of β -helix structures (Symmons et al., 1997). In studies to be reported elsewhere, we have examined the FTIR spectra of amyloid fibrils from several proteins and peptides (Khurana and Fink, unpublished results). These show the major β -sheet band at a variety of different frequencies, ranging from 1638 to 1622 cm^{-1} . Thus there is certainly no strong evidence, from the secondary structure analysis by FTIR, for β -helix structure in amyloid fibrils.

In summary, we conclude that parallel β -helix proteins do not have a uniquely distinctive IR spectrum, although they are characterized by a major amide I component at 1638 cm^{-1} corresponding to parallel β -sheet structure. In fact, the spectra are similar to those of other globular proteins of high β -sheet content, such as the immunoglobulin fold. We also conclude that the associated state of the peptide KLEG has a structure distinct from that of the parallel β -helix proteins.

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