Biophysical Chemistry 175–176 (2013) 47–53

Contents lists available at SciVerse ScienceDirect

Biophysical Chemistry

journal homepage: http://www.elsevier.com/locate/biophyschem

The structure of poly-L-lysine in different solvents

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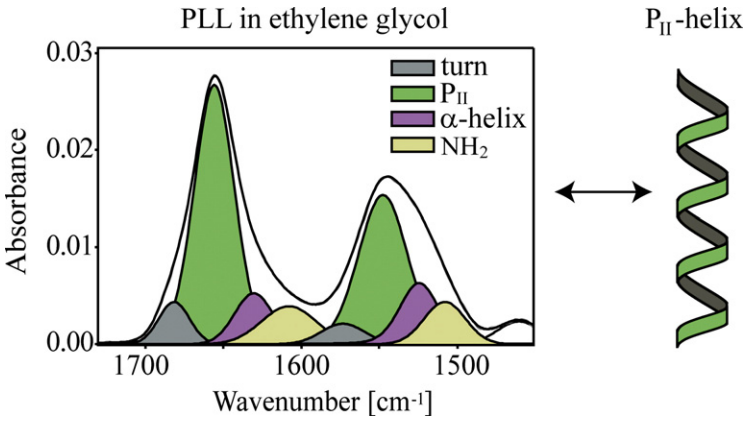
HIGHLIGHTS

- The structures of PLL was determined by analysis of amid I, II and III regions.
- High population of PII structure was found in PLL dissolved in non-aqueous medium.
- P_{II} is stabilized in ethylene glycol by H-bonds between solvent and peptide.
- TFE and DMSO stabilize α-helical structure of PLL.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 24 January 2013
Received in revised form 13 February 2013
Accepted 13 February 2013
Available online 22 February 2013

Keywords:
Poly-L-lysine
PII
Alpha-helix
Beta-conformation
ATR infrared spectroscopy
DFT calculation
Water
DMSO
TFE
Ethylene glycol

ABSTRACT

Understanding the factors that affect the conformational stability of the polypeptide main chain provides insight not only into the molecular basis of unfolded states but also into the earliest event that occurs during the protein folding. The presented study was concentrated on finding the conformational distributions of poly-L-lysine (PLL) by applying infrared spectroscopy. We assigned the amide bands for different conformations of PLL in water. At low pH values PLL mainly possesses the P_{II} and β structures while at higher pH values and low temperatures characteristic bands for the α-helical conformation are found. The increase in temperature induces the formation of β structures. The obtained assignment of the infrared bands for various conformations was used to determine the conformational populations of PLL in non-aqueous solvents. In TFE, PLL possesses an α-helix structure that is after heating partially transformed into the P_{II} conformation. DMSO enables a uniform α-helical conformation of PLL. A similar uniform conformation (P_{II}, 88%) was found for PLL dissolved in ethylene glycol, suggesting that the P_{II} structure is not limited to the presence of water molecules or charged side chains. The role of intermolecular interactions between the solvent molecules and PLL in stabilizing the P_{II} conformation is discussed.

1. Introduction

Understanding the mechanism of peptide folding at the molecular level is a subject currently under intensive study. A dynamic peptide

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http://dx.doi.org/10.1016/j.bpc.2013.02.004

structure with a large population of unstructured regions requires spectroscopic methods that provide detailed structural information probed at a very short timescale (picoseconds). Infrared spectroscopy is a powerful method in structural biology capable of monitoring structures with such short life times. However, to determine the various types of particular secondary structural elements, a uniform and detailed assignment of the spectral bands needs to be resolved first. We used FTIR spectroscopy to probe the distribution of various conformations of the homopolypeptide poly-L-lysine (PLL). PLL has positively charged side chain amino groups at a neutral and low pH resulting in electrostatic repulsion between them that allows only the extended conformation of polypeptides. The secondary structure of PLL has been characterized by numerous spectroscopic methods such as CD [1,2], IR [3], NMR [4–6], Raman optical activity [7–9], VCD [10] and most recently by UV resonance Raman spectroscopy [11–13]. By using UV resonance Raman spectroscopy (UVRS), Mikhonin et al. [14] demonstrated the conformation of PLL at pH 2 as an equilibrium between P_{II} and the extended β-strand conformations in the ratio 60:40, respectively. The P_{II} conformation, described by Tiffany and Krimm [1], is characterized by the lack of intramolecular hydrogen bonds. The factors that stabilize P_{II} conformation are not well established [15]. It was proposed that P_{II}-helical conformation arises from the combination of minimization of unfavorable intrachain steric interactions and favorable backbone solvation [16,17], even though the role of water remains debated. The resulting opened and rather flexible structure is becoming increasingly recognized as general structural characteristics of natively unfolded proteins [18,19] and as a candidate for the intermediate structure in amyloid fibril formation [13,20]. Moreover, it has been shown that the P_{II} structure is one of the most preferential conformations found in dipeptides in water [21,22].

In the present article, we not only determine the distributions of the conformations of PLL in water but also in several other solvents. The applied solvents preferentially stabilize one conformation by changing the preference of inter- and intramolecular hydrogen bond formation. Determination of the structure by the application of infrared spectroscopy is mainly based on analysis of the amide I region. This region in general contains bands that are strongly overlapped, especially in the case when the polypeptide establishes several distinct conformations. To ensure the reliable determination of the peptide (protein) structure, additional indicative bands sensitive to particular structures are needed. Thus, we introduced new structural parameters retrieved from the infrared spectra, which are able to distinguish between the α-helix and P_{II} conformations, as well as between the various types of β structures, such as β conformation, β-strands and β-sheets, by analyzing the amide I and amide II regions of peptide infrared spectra, as well as amide III.

2. Materials and methods

Poly-L-lysine (PLL) HCl was purchased from Sigma (MW_{MALLS} = 31185 Da) and used without further purification. The samples were freshly prepared in H_2O or D_2O (Cambridge Isotope Laboratories, Inc.) as a 4 wt.% solution. The pH was adjusted to pH 4 and pH 11.6 using appropriate amounts of HCl (DCl) and NaOH (NaOD). The solutions in DMSO (Sigma-Aldrich), DMSO-d6 (Cambridge Isotope Laboratories, Inc.), TFE (Sigma-Aldrich) and ethylene glycol (Sigma-Aldrich) were prepared as 4 wt.% concentrations.

2.1. Fourier transform infrared spectroscopy

The infrared spectra were measured using the PerkinElmer System 2000 and the Bruker Vertex 80 infrared spectrometers. Spectra were recorded in the range between 7000 cm^{-1} and 450 cm^{-1} in ATR mode. The spectra obtained from the ATR-infrared measurements were first recalculated to get the pure absorption spectra [23]. Before any spectral analysis, the spectrum of pure water was subtracted. The band overlapped regions were analyzed using the Grams band fitting procedure by optimizing the sum of the bands with the mixed Lorentzian and Gaussian shapes. The solution spectra of PLL were measured in a temperature range from 10 °C to 80 °C using a diamond ATR cell (Specac) equipped with a heated top plate. To reduce the strong bands due to the absorption of diamond, backgrounds were collected for each recorded temperature. The assignment of the structural sensitive bands in amide I, II, and III regions is based on previous results on blocked dipeptides [22] and vibrational spectra of proteins in solution with known structure [24–28].

2.2. Ab initio calculations of the pKa

The *ab initio* pKa calculations of propylamine in the different solvents were performed for the gas phase and in solution using Gaussian 09 [29]. The solvation energies have been calculated using the Polarisable Continuum Model (PCM) [30] at the B3LYP/6-31++G(d,p) [31–33] level.

3. Results and discussion

3.1. The assignment of the major spectral features in the FTIR spectra of the different conformers of PLL

The conformational sensitive regions in the infrared spectrum of polypeptides are known as amide I (1600 cm^{-1}–1690 cm^{-1}), amide II (1500 cm^{-1}–1580 cm^{-1}) and amide III (1220 cm^{-1}–1330 cm^{-1}). The amide I mode has a predominant contribution of backbone C═O stretching. Its frequency is sensitive to the conformation of the peptide backbone, particularly to various types of secondary structures. Since the backbone C═O group acts as a proton acceptor, the frequency of the amide I band possesses a great sensitivity to variations in the environment of the C═O groups. The establishing of hydrogen bonds and the extension of inter-residual vibrational coupling [12,34] may also influence the frequency and shape of amide I vibration. The amide II mode is less sensitive to the various secondary structural elements. However, its frequency reflects the participation of backbone NH groups in the various types of hydrogen bonds with proton acceptor groups from the protein or molecules from the protein environment. The amide III region is the most sensitive indicator for the peptide conformations. The bands from the amide III region, which chiefly result from N–H in-plane bending mixed with C–C and C–N stretching, C═O in-plane bending and C_α–H bending, possess information on the values of the ϕ and Ψ torsion angles from the peptide backbone [22].

3.2. PLL in water (pH 11.6)

A high pH value (11.6) and low temperature stabilize the α-helical conformation of PLL in water. The band fitting algorithm resolves the amide I band on two band components at 1664 cm^{-1} and 1644 cm^{-1} respectively (Fig. 1). The strongest model band at lower wavenumbers is ascribed to the α-helix conformation. The band frequency of this amide I band is located close to the α-helix band found in the infrared spectrum published by Dzwolak et al. [26]. However, in the α-helical state, there is an additional band in the amide I region at 1664 cm^{-1}, the assignment of which is not conclusive. One of the possibilities, which should be proved by the presence of corresponding bands in the amide II and amide III regions, is that this amide I band belongs to turns [35]. The decomposition of the amide II band revealed two prevailing components located at 1550 cm^{-1} and 1572 cm^{-1}. These model bands are assigned to the α-helix conformation and the amide II counterpart of the 1664 cm^{-1} amide I band (Fig. 1). The remaining bands presented in Fig. 1 are due to the NH_2 symmetric and antisymmetric deformation of the lysine side chain located at 1602 cm^{-1} and 1532 cm^{-1} respectively. The remaining band in the amide I region located at 1628 cm^{-1} is attributed to the bending vibration of water molecules, which remains after the subtraction of the bulk water.

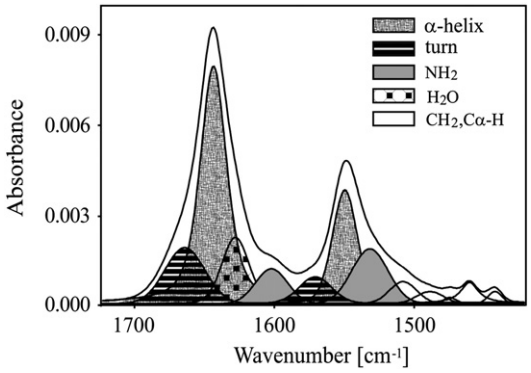

Fig. 1. The infrared spectrum of PLL at pH 11.6 and T = 10 °C with fitted model bands that represent: the α-helix (1644 cm^{-1}, 1550 cm^{-1}), the turn conformation (1664 cm^{-1}, 1572 cm^{-1}), the CH_2 and Cα–H vibrations (1508 cm^{-1}, 1489 cm^{-1}, 1474 cm^{-1}, 1461 cm^{-1}, 1443 cm^{-1}), the NH_2 groups of the lysine side chain (1602 cm^{-1}, 1532 cm^{-1}), and the water bending (1628 cm^{-1}).

The band structure of the extended amide III region is presented in Fig. 2. At the high frequency region, two sharp model bands are assigned to the CH_2 (1394 cm^{-1}) and C_α–H (1348 cm^{-1}) bending vibrations. The present assignment of the band at 1348 cm^{-1} is not unique. While Barron et al. [8] assigned this band as a component of the amide III region representing the vibration of hydrated α-helices, vibrational studies of short dipeptides showed that this band is already out of the amide III region [22]. It is predominately attributed to $C_\alpha H$ bending mixed with the amide III mode but has no potentials for the structural characterizations of peptides [36]. Therefore, the amide III region of the PLL is composed of two band components. The strongest band at 1295 cm^{-1} has been assigned to the α-helical conformation of the polypeptide. The band near 1300 cm^{-1} is generally found in the vibrational spectra of globular proteins composed mainly of α-helices [37–39]. Moreover, the component of the amide III vibration in the infrared spectra of dipeptides, which was assigned to an α conformation of the peptide backbone [22], was found at a similar position. The intensity of the second band in the amide III region located at 1257 cm^{-1} (Fig. 2) is much lower compared to the central band at 1295 cm^{-1}. Its appearance is correlated to the bands at 1664 cm^{-1} and 1572 cm^{-1} in the amide I and amide II regions respectively, which were assigned to turn structures.

The area of the component bands can be efficiently applied for a rough estimation of the populations of a particular conformation. This analysis revealed that the population of α-helix in PLL at 10 °C and at pH 11.6 is between 78% and 81% as obtained from the amide I or amide III region, respectively. These results are in accordance with results published by Jiji et al. [13].

At higher temperatures and pH 11.6, helical PLL gradually transforms into the β-sheet conformation. The infrared spectrum of β-sheet PLL

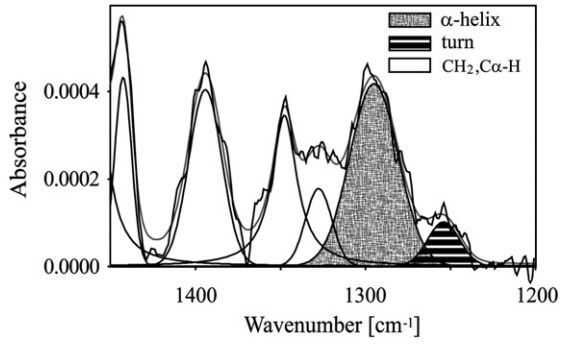

Fig. 2. The infrared spectrum in an amide III region of PLL at pH 11.6 and T = 10 °C with fitted model bands that represent: the α-helix (1295 cm^{-1}), the turn conformation (1257 cm^{-1}), and the CH_2 and C_α–H vibrations.

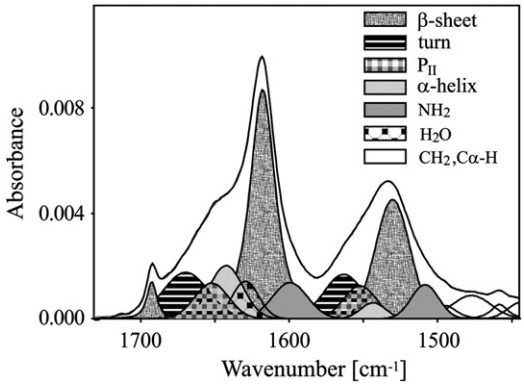

Fig. 3. The infrared spectrum of PLL at pH 11.6 and T = 80 °C with fitted model bands that represent: the β-sheet (1692 cm^{-1}, 1618 cm^{-1}, 1530 cm^{-1}), the turn conformation (1669 cm^{-1}, 1563 cm^{-1}), the P_{II} conformation (1652 cm^{-1}, 1543 cm^{-1}), the α-helix (1642 cm^{-1}, 1553 cm^{-1}), CH_2 and Cα–H vibrations (1495 cm^{-1}, 1477 cm^{-1}, 1458 cm^{-1}, 1444 cm^{-1}), the NH_2 groups of the lysine side chain (1600 cm^{-1}, 1508 cm^{-1}), the water bending (1629 cm^{-1}), and the CH_2 and C_α–H vibrations.

(Fig. 3) shows two characteristic amide I bands, weaker at 1692 cm^{-1} and stronger at 1618 cm^{-1} that are typical for the (aggregated) antiparallel β-sheet (Table 1).

In the decomposed amide III region presented in Fig. 4, three components of different β conformations can be observed; the first component is located at 1270 cm^{-1}, which is assigned solely to amino acids in the β conformation that are not involved in the secondary structure [22], the second is located at 1243 cm^{-1} and is assigned to the β-strands and the third is located at 1222 cm^{-1} and is assigned to the strong hydrogen bonded β-sheets of aggregated PLL. The frequency of the second component is close to the value of the amide III band found in the infrared spectra of β structured proteins [7], while the amide III components near 1220 cm^{-1} have already been found in the spectra of aggregated proteins [7,25,40,41]. The bands representing the β-sheet conformation populates 61% and 58% of all the conformations obtained from the amide I and III regions respectively. The structure of the amide I, amide II and amide III bands revealed that besides the prevailing β-sheets, the final state of the fibrillation of PLL also contains some amino acids in the turn, α-helical and P_{II} conformations.

3.3. PLL in water (pH 4)

The poly-L-lysine side chains at pH 4 are fully ionized, so electrostatic repulsion prevents the formation of the α-helical conformation. The characteristic infrared spectrum of PLL in a water solution at 10 °C and a low pH shows broadened and asymmetrical amide I and amide II bands (Fig. 5). Similar to that at a higher pH, the fitting algorithm is used to determine the intrinsic components of the amide bands as shown in Fig. 5. The band analysis shows that the major

Table 1
The assignment of the model bands from the amide I, II and III region, retrieved with the band fitting algorithm from the corresponding spectral regions of PLL in water.

Conformation	Frequency (cm^{-1})		
	Amide I	Amide II	Amide III
Turn	1664–1674	1563–1572	1257–1260
P_{II}	1648–1654	1543–1546	1308–1311[a]
α	1643–1645	1550–1552	1290–1295[a]
β-Conformation	/	/	1270–1280[a]
β-Strand	1625–1630[b]	/	1240–1243
β-Sheet	1630–1640[c]	/	1230
Aggregated β-sheets	1692, 1618	1530	1219–1222

[a] Obtained from Grdadolnik et al. [22].
[b] Obtained from Huang et al. [27].
[c] Obtained from Baumruk et al. [28].

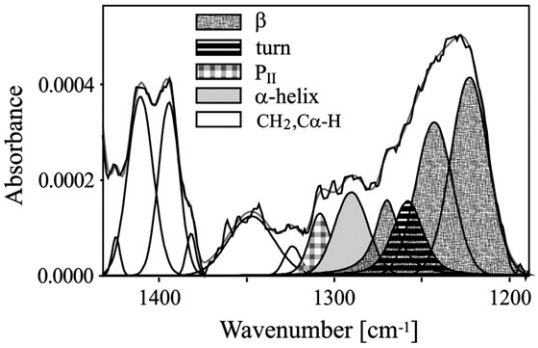

Fig. 4. The infrared spectrum in an amide III region of PLL at pH 11.6 and T = 80 °C with fitted model bands that represent: β conformations (β conformation at 1270 cm^{-1}, β-strand at 1243 cm^{-1}, hydrogen bonded β-sheet at 1222 cm^{-1}), the turn conformation (1258 cm^{-1}), the P_{II} conformation (1308 cm^{-1}), the α-helix (1290 cm^{-1}), and the CH_2 and C_α – H vibrations.

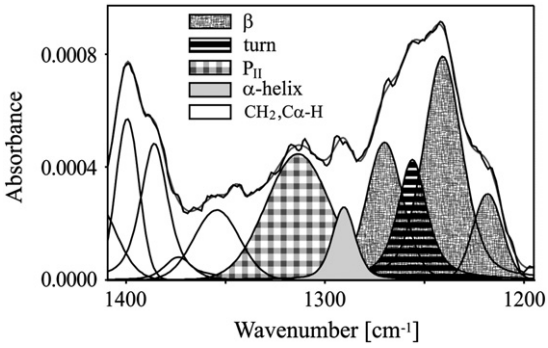

Fig. 6. The amide III region of the infrared spectrum of PLL, pH 4, at 20 °C fitted with model bands that represent: β conformations (β conformations at 1272 cm^{-1}, β-strands at 1243 cm^{-1}, aggregated β-sheet at 1219 cm^{-1}), the turn conformation (1257 cm^{-1}), the P_{II} conformation (1311 cm^{-1}), the α-helix (1291 cm^{-1}), and the CH_2 and C_α – H vibrations.

population of PLL at pH 4 is the P_{II} conformation (45%) followed by the β-structures. The amide I and II band components attributed to P_{II} are located at 1649 cm^{-1} and 1547 cm^{-1} respectively. The corresponding bands for β structures can be found at 1618 cm^{-1} and 1522 cm^{-1}, while small bands at 1644 cm^{-1} and 1671 cm^{-1} were assigned to the α-helix and turn structures. The amide II counterpart of the later structure is located at 1570 cm^{-1}. The bands at 1594 cm^{-1} and 1506 cm^{-1} are assigned to the NH_3^+ stretching of the lysine side chains. The assignment of those bands was checked with deuterium substitution of the solvent (data not shown).

Similar structural elements of PLL conformation in water at a low pH was found by decomposing the amide III region. The extended amide III region of the FTIR spectrum of PLL in water at pH 4 shows at least four well-defined bands with the frequencies 1311 cm^{-1} (P_{II}), 1291 cm^{-1} (α-helix), 1272 cm^{-1} (β conformation), 1257 cm^{-1} (turn), 1243 cm^{-1} (extended β-strand) and 1219 cm^{-1} (aggregated β-sheet). However, it needs to be emphasized that the decomposition of the amide III region presented in Fig. 6 yielded slightly different distributions of the presented conformers. By comparison of the integral intensity, the ratio of the P_{II} conformation falls to 25%. The reason for this inconsistency is not known. The comparison of the results obtained from the analysis of amide I and amide III regions reveals that the decomposition of the latter region retrieves more complete information about the populations of different conformers of PLL. The band components of the amide III region are more resolved already in the original spectrum. Consequently, the application of the band fitting algorithm is therefore more reliable. This is the reason why we are able to determine some conformations connected to the β-structure, in which the characteristic patterns are strongly overlapped in the amide I region with water and NH_3^+ bending.

The temperature dependence of the PLL conformation at pH 4 is not as extensive as at pH 11.6. It culminates in a small upshift of the amide I band that corresponds to a small downshift of the amide II band. The frequency of the amide II band is the most sensitive for the formation of hydrogen bonds and consequently to hydration [42]. Therefore the change in hydration of PLL causes a downshift of this band by 16 cm^{-1}. This temperature dependence derives from the hydrogen bonds of water molecules to amide carbonyl and N – H groups [42–44]. With a higher temperature, the water–amide hydrogen bond strengths decrease, which consequently form stronger carbonyl bond resonance and weaker C(O) – N bonding resonance of the peptide bond. Comparison of the band intensities of the bands corresponding to the P_{II} conformation reveals that heating PLL at a low pH from 20 °C to 80 °C decreases the population of P_{II} by roughly 10%.

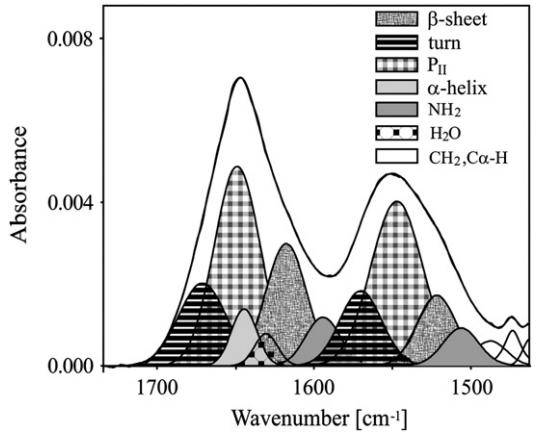

Fig. 5. The infrared spectrum of PLL at pH 4 and T = 25 °C with fitted model bands that represent: the β-sheet (1618 cm^{-1}, 1522 cm^{-1}), the turn conformation (1671 cm^{-1}, 1570 cm^{-1}), the P_{II} conformation (1649 cm^{-1}, 1547 cm^{-1}), the α-helix (1644 cm^{-1}), the NH_3^+ groups of lysine side chain (1594 cm^{-1}, 1506 cm^{-1}), the water bending band (1630 cm^{-1}), and the CH_2 and C_α – H vibrations.

3.4. pKa calculation in different solvents

The experimental values of pKa for PLL in TFE and ethylene glycol are not known. The protonation state of the side chain amino group of PLL in different solvents was determined indirectly by comparison with the calculated pKa values of model molecule propylamine using the DFT approach. Propylamine was used as a model molecule for the side chain of lysine possessing a similar pKa value in water as PLL. Solvation free energy was calculated using the polarized continuum model (PCM) with the B3LYP/6-31++G(d,p) method and calculated using the equation [45]:

$$\begin{aligned}\Delta\Delta G = {} & \Delta G_{GP}(\mathrm{PropylNH_3^+}) - \Delta G_{GP}(\mathrm{PropylNH_2}) + \Delta G_{solvent}(\mathrm{PropylNH_3^+}) \\ & - \Delta G_{solvent}(\mathrm{PropylNH_2}) + \Delta G_{GP}(\mathrm{solvent - H^+}) - \Delta G_{GP}(\mathrm{solvent}) \\ & + \Delta G_{solvent}(\mathrm{solvent - H^+}) - \Delta G_{solvent}(\mathrm{solvent})\end{aligned} \quad (1)$$

The obtained ΔΔG values were then used to calculate the pKa values, which were compared to experimentally determined pKa values for propylamine in different solvents (Table 2).

A very good agreement with the experimental values was obtained for water and DMSO, suggesting a rather good match between the calculated pKa value for ethylene glycol and TFE. The low values of the calculated pKa of propylamine in ethylene glycol and TFE suggest the deprotonation of amino groups from side chains in both solvents.

Table 2
pKa values for the propylamine model molecule in different solvents obtained *ab initio* from Eq. (1) and experimental values.

Solvent	pKa calculated	pKa experimental
Water	10.56	10.6[a]
TFE	4.54	/
DMSO	9.76	10.4[b]
Ethylene glycol	1.82	/

[a] Obtained from Perrin et al. [46].
[b] Obtained from Makowska et al. [47].

3.5. PLL in different solvents

To confirm the presented assignment and to elucidate the origin of the bands of individual conformation, we extended the conformational studies to cover various solvents that are able to promote particular types of conformations. We used the common α-helix promoting solvent TFE to stabilize the α-helix conformation of PLL [48,49]. PLL was titrated with different ratios of water:TFE at pH 4. With an increasing TFE concentration from 0% to 80% (v:v), the spectra of PLL remain unaffected. An additional increase of the TFE concentration induces a shift of the amide I 3band maximum from 1649 cm^{-1} to 1652 cm^{-1}, indicative of P_{II}/α-helix transformation, *i.e.* the mainly P_{II} conformation of PLL in water converts to the α-helical conformation in 100% TFE. The calculated amount of α-helix conformation in 100% TFE is 84%. It should be mentioned that the amide I band characterizing an α-helix of PLL is blue-shifted in TFE due to the low dielectric constant of TFE compared to water. Heating PLL in 100% TFE from 10 °C to 70 °C results in a decrease of the α-helix conformation by 13%, which runs parallel with the formation of the P_{II} conformation at 1659 cm^{-1} (Fig. 7). These results are consistent with the data published by Xiong and Asher [50], where polyalanine peptide in 50% TFE, which possesses mainly helical conformation, melts 9.1% of its α-helices into P_{II} after heating the peptide from 25 to 40 °C.

We measured PLL in DMSO as a solvent with only hydrogen bond acceptor abilities. At low temperatures, PLL in DMSO exhibits an even higher α-helical population than in TFE, *i.e.* a solvent with hydrogen bond donor abilities. The spectrum of PLL in DMSO is characterized by a sharp amide I band at 1650 cm^{-1}, an amide II band at 1548 cm^{-1} (Fig. 8) and an amide III band at 1295 cm^{-1}. The amide III region is only applicable when the DMSO solvent is substituted with DMSO-d_6. The amide I band is upshifted due to the low dielectric constant of DMSO. Contrary to the data that DMSO disrupts or weakens the intramolecular hydrogen bonds [51], our results indicate that DMSO strongly stabilizes the α-helix conformation, reaching 97% of the population in PLL. It was shown that polar amino acid side chains have dipole–dipole interactions with the oxygen atom of DMSO and form hydrogen bonds, whereas apolar side chain alkyl groups become solvated by DMSO

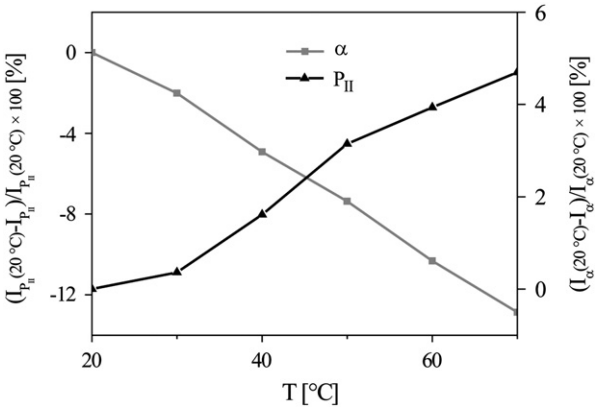

Fig. 7. The melting of the α-helix and the formation of the P_{II}-helix with increasing temperature relative to the α-helix and P_{II} content of PLL in TFE at 20 °C.

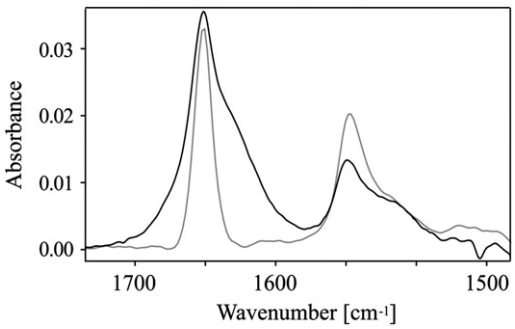

Fig. 8. Spectra of PLL in 100% TFE (black curve) and in DMSO (gray curve) at 20 °C.

through the formation of a hydrophobic pocket [52]. The dual solvation properties of DMSO cause it to be a good membrane-mimicking solvent [52].

Ethylene glycol was the next solvent where the structure of PLL was probed. As a solvent it has many applications especially as a cryogenic liquid. Calculation of the pKa values showed that the ε-amino group of the lysine side chain in ethylene glycol has a significant potential to be deprotonated. The reduced electrostatic repulsions of the side chains of PLL in ethylene glycol enable the stabilization of P_{II} conformation compared to water, where repulsions between ionized ε-amino groups promotes extended β-strand conformation [14]. PLL in ethylene glycol populates 88% of the P_{II} conformation, as can be seen in Fig. 9. The bands characteristic of the P_{II} conformation are located at 1656 cm^{-1} in the amide I region, 1546 cm^{-1} in the amide II region, and 1311 cm^{-1} in the amide III region. Due to the low dielectric constant of ethylene glycol, the amide I band characterizing the P_{II} conformation of PLL is blue-shifted compared to the amide I position in water. From the amide III region in Fig. 9, a band is observed at 1238 cm^{-1} that corresponds to the β-strand structure. After heating PLL in ethylene glycol from 10 to 65 °C, 10% of the P_{II} conformation melts as observed from the difference spectra.

3.6. Stabilization of the P_{II} conformation

Vibrational studies of the Lys dipeptide showed that the amino acid lysine possesses a strong propensity to P_{II} conformation. A recent study revealed that Lys in a short dipeptide populates 55% of P_{II} conformation [22]. This high propensity of lysine to adopt the P_{II} conformation was explained by the strong backbone electrostatic interactions and by the screening of those interactions with water molecules, which is influenced by the side chain [21,22]. The P_{II} conformation is supposed to be stabilized by water hydrogen bonding to the backbone amide and carbonyl group [53]. However, Drozdov et al. found no evidence for a role of water bridges in stabilizing P_{II} [17]. Contrary, Nerenberg and Head-Gordon found that the P_{II} conformation is optimizing the packing of water molecules in the hydration shell of the peptide [54].

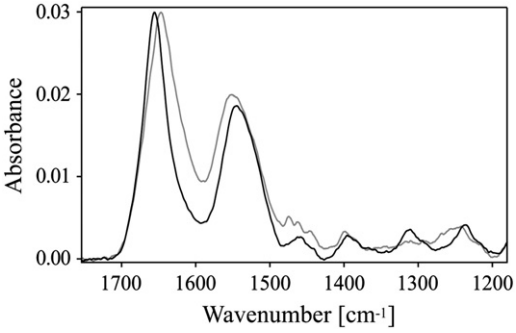

Fig. 9. Spectra of PLL in ethylene glycol (black curve) and in water at pH 4 (gray curve).

The water molecules reduce the attractive electrostatic interactions between the peptide atoms, leaving the steric interactions as the main force for determining conformational preferences [15]. P_{II} minimizes these interactions and thus becomes the most prominent conformation. However, in this study the P_{II} conformation was determined in a non-aqueous solvent for the first time. Moreover, Law and Daggett [55] found no correlation between the dielectric constant of the solvent and the P_{II} structure. Thus we suggest that the stabilization of the P_{II} structure in ethylene glycol occurs due to the deprotonated side chain of the lysine leading to a more sterically optimized structure of the P_{II}-helix. The question of "Why there is no formation of the P_{II}-helix in TFE and DMSO, where PLL also has a deprotonated ε-amine" definitely needs an answer. Ethylene glycol has, like a water molecule, both proton donor and proton acceptor groups that are able to form intermolecular hydrogen bonds with the NH and CO group of the peptide backbone and thus hinder the formation of the intramolecular hydrogen bonds characteristic of α-helices.

4. Conclusions

We used infrared spectroscopy to characterize the spectral bands of individual conformational states of PLL stabilized by different solvents. PLL changes its conformation depending on the solvent, pH value and temperature. In water at a low pH, it is populated predominately by the P_{II} conformation. The increase of the pH value stabilizes the α-helical structure, which transforms into the aggregated β-sheet structure after heating the peptide (Fig. 10).

The conformation of PLL is radically changed by the addition of TFE, ethylene glycol or DMSO. The analysis of the amide I, II and III bands revealed that TFE and DMSO induced the α-helix conformation, while ethylene glycol, like water, induced the P_{II} conformation. Thus, the P_{II} conformation is not connected only to water as a solvent and the hydration of peptide bonds. Ethylene glycol even more efficiently stabilizes the P_{II}-helix. We suppose that this stabilization has similar roots as predicted for water, *i.e.* the main forces that stabilize the P_{II} conformation are steric interactions of the uncharged side chains of PLL and the establishment of hydrogen bonds between the CO and NH groups from the peptide backbone and the solvent's proton donor and acceptor groups.

The conformational analysis of PLL in various solvents showed that the decomposition of the amide III region provides a more detailed distribution of the peptide conformations. Less extensive overlapping of the characteristic bands that belong to a particular conformation improve the accuracy of the structural determination. This is especially true for two typical helical secondary structural elements, *i.e.* P_{II} and α. Both conformations have well separated bands in the amide III region, the frequencies of which are less sensitive to the type of solvent compared to the corresponding amide I bands.

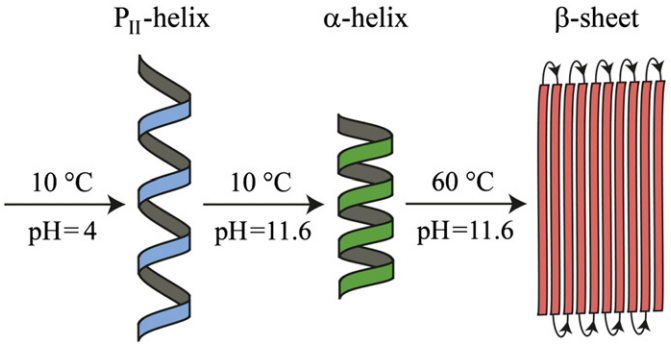

Fig. 10. Schematic representation of conformational changes of PLL induced by changing a pH value and temperature.

Acknowledgements

This work was supported by a grant from the Ministry of Education, Science, Culture and Sport of the Republic of Slovenia. We thank Franc Avbelj and Rok Borštnar for stimulating discussions.

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