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FTIR spectra of solid poly-L-lysine in the stretching NH mode range

M. Rozenberg *, G. Shoham

Department of Inorganic and Analytical Chemistry, The Hebrew University of Jerusalem, Jerusalem, Givat Ram 91904, Israel

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Abstract

Three bands at 3270 cm^{-1}, 3200 cm^{-1} and 3030 cm^{-1} are found in the IR stretching proton (ν_1) mode spectral range in spectra of solid poly-L-lysine (PLL). Strong quantitative changes of these bands are observed in samples dried from water solutions with different pH. The narrow band at 3270 cm^{-1}, which is strong in the spectrum of PLL precipitated from pH = 12 alkaline medium, is assigned to the ν_1 peptide proton mode of NH-CO (amide A) of the β-sheet structure type. The band at 3200 cm^{-1}, which is intensified in PLL precipitated from pH = 1 acidic medium, relates to the ν_1 peptide mode in the random coil structure. The band at 3030 cm^{-1}, whose peak intensity increases two-fold in going from alkaline to acidic medium, is assigned to the ν_1 modes of protonated NH_3^+ side chain groups. The frequencies of all bands were used for estimating H-bond energy relying on an empirical correlation between this property and the red shift of the ν_1 band. The enthalpy of the secondary structure transition from β-sheet to the random coil, which is observed in PLL at the change of pH from 11 to 1 amounts to 4.7 kJ mol^{-1}.

Keywords: Poly-L-lysine secondary structure; Hydrogen bonding; FTIR

1. Introduction

The IR band amide I–the almost pure carbonyl C=O stretching mode of the peptide group–is most commonly used for the analysis of the secondary structure of polypeptides [1–4]. The assignment of the amide I components to particular secondary structure relies upon correlations with band positions observed under specific experimental conditions. Despite the fact that FTIR bands in the amide I range directly correspond to secondary structure elements, deriving quantitative information with this approach meets with some difficulties because of the line broadening combined with strong overlapping of deconvoluted components [4,5].

The use of the ν_1 mode range for determining peptide secondary structure is limited [1–3], since stretching bands usually involve overlapping of all proton ν_1 modes of the NH and OH groups, forming a multitude of hydrogen bonds. For this reason, and in so far as peptide studies are concerned, as a rule, with water solutions, this IR range of spectra has been virtually removed from practical use. However, this band is extremely sensitive, in both position and intensity, to the changes in the hydrogen bonding structure [6,7], and consequently to H-bond energy and the type of conformation. The correlations of spectral, thermodynamic and structural parameters, which are well known in H-bonding spectroscopy [7,8] were recently extended to the area of solid amino acids and small peptides [9]. They may also prove useful in some cases for the study of hydrogen bonding in peptides with a large molecular weight, in particular when peptides are studied in solid state. The hydrogen bond interaction and its contribution into the energetics of protein structure and protein folding is being investigating intensively by theoretical and experimental methods (see, e.g., Advances in Protein Chemistry, vol. 72, 2005), which is possibly the best source of up-to-date information on this subject. Since, despite their popularity, theoretical and computational methods for description of hydrogen bonds in biomacromolecules are plagued by computational limitations, the application of empirical correlations based on model hydrogen bonded systems is fully justified.

A good subject for such investigation is poly-L-lysine (PLL), which often serves as a model system for more complex proteins, and which can be converted in the α-helix, β-sheet or random coil secondary structures by altering pH of the water solution [10]. In other words, by changing the pH, it is possible to induce the polypeptide to adopt any of the above-mentioned

* Corresponding author.
E-mail address: markroz@chem.ch.huji.ac.il (M. Rozenberg).

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three structures; alternatively, these transitions can be reached by heating [11,12], manipulating the level of hydration [13] or through the lyophilization process [14]. Extensive qualitative evidence correlates characteristic amide I absorption frequencies with the presence of α-helices (1650 cm^{-1}), β-sheets (1620 cm^{-1} and 1680 cm^{-1}) and random coils (1640–1650 cm^{-1}) [10]. This information facilitates the interpretation of PLL spectra in the ν_1 range.

The ν_1 absorption of PLL is expected to include contributions from the neutral amide NH group, side chain amine NH_2 groups, as well as from charged (protonated) side chain NH_3^+ groups. The relative weight of these contributions should depend, quantitatively, on the preceding history of the sample. All the above-mentioned groups absorb IR radiation in different spectral ranges, depending on whether they are free or bound with different H-bonds. The first two of these groups, which have been extensively studied in multiple peptide model systems [1,15–17], can generally be found at 3400–3100 cm^{-1}. The bands of NH_3^+ groups absorb in the range 3100–2500 cm^{-1}, as has been found for solid amino acids and short peptides [9,15]. The spectral bands which relate to both these extreme cases are over 300 cm^{-1} apart, which is far enough for quantitative differentiation. The groups in question may govern secondary structure by repulsive forces between positively charged lysine side chains [18]. The ν_1 band of the NH peptide group is expected to change its position and intensity only if the H-bond secondary structure of PLL changes in line with pH. Moreover, it is possible that the shape of these bands include a contribution of the Fermi resonant vibrational interaction in peptide and side chain amine groups, a fact that must undoubtedly be taken into account [1].

Out of a vast body of literature devoted to IR studies of PLL, the entire spectrum of PLL, including both ν_1 and amide I bands, is displayed only in one work, where conformational changes of PLL are studied as influenced by the drying rate [13]. Neither have IR spectra of side chain amine groups in the range of ν_1 mode (3μ) been discussed in the literature, although these, as has been already mentioned, are likely to influence changes in secondary structure. They are also interesting from a practical standpoint, since side chain effects can be an essential factor in forming amyloid structure [19] or in self-assembly process of ionic-complementary peptides into nanostructures [20].

This work is concerned with FTIR spectra of solid PLL prepared from water solutions with a wide range of pH. It was found that the intensities of bands assigned to both peptide amide and charged side chain amine groups are correlated and that their ratio reflects secondary structure changes.

2. Experimental

Samples (1–2 mg) of poly-L-lysine hydrobromide (PLL) with molecular weight (5–10)10^3, (30–70)10^3 ans (70–150)10^3 (two samples)–all from Sigma–were dissolved (2 mg/1–2 ml) in KBr/water (D_2O) solutions with pH of 10–12, 6–8 and ca. 1. The pH was adjusted by adding of sodium hydroxide or hydrochloric acid and measured with accuracy of 0.1 pH unit. The samples were lyophilized from water PLL solutions with the addition of 200 mg

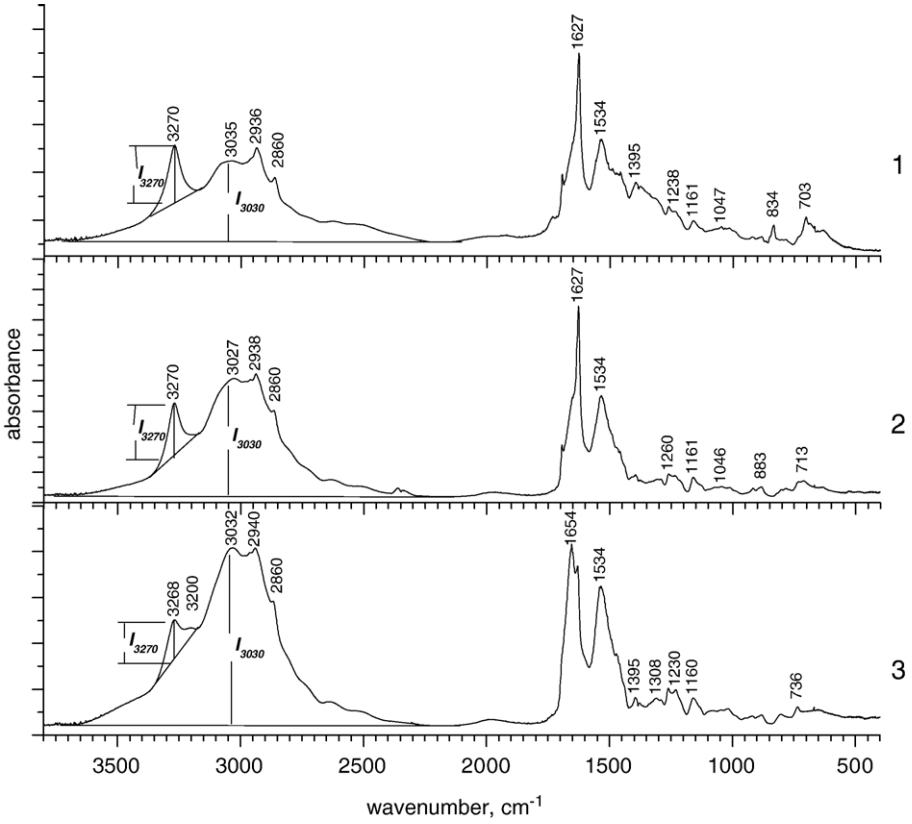

Fig. 1. Spectrum of poly-L-lysine (M.W. [(30–70)10^3]) in KBr pellet. Samples were lyophilized from water solution with different pH after addition of KBr: (1) pH ≅ 12, (2) pH ≅ 7, (3) pH ≅ 1. Vertical lines show absorbancies I_{3030} and I_{3270} used for calculations in Table 1.

KBr in a home-made glass liquid nitrogen trap (10^{-3} mbar, T=77 K). Spectra were measured with Bruker Equinox 55 spectrometer in KBr pellets (at pressure ca. 10 T/cm^2), which had been pressed from powder immediately after drying from solution at resolution 2 cm^{-1}, and were processed with Bruker OPUS and Origin 7 software. All results provided and discussed below were obtained using the technique of sample preparation from KBr/water solutions. Comparison of spectra measured from the samples prepared by the standard method–mixing separately dried PLL sample with KBr powder and using commercial lyophilizer (10^{-2} mbar, T=150 K)–did not reveal substantial differences in band peak positions. This confirms previous studies, which do not find noticeable changes in dried proteins spectra at pellet preparation [21]. Similarly, no changes were observed when PLL samples were kept and treated at room or low (0–20 °C) temperatures. Spectra of samples prepared from water solutions by simple drying by air flow did not show essential differences as compared to the ones prepared by freeze drying.

The final drying of the PLL samples, which can contain substantial quantities of water after lyophilizing (even if prepared using commercial machine), was performed in a desiccator with P_2O_5 or with silica. The water content thereof was checked based on the presence of an absorbance band near 3450 cm^{-1} and in samples, whose spectra are shown in Fig. 1, was estimated as less than 3 wt.%. The bands in the range 3300–3000 cm^{-1} as well as amide I were insensitive to the presence of water.

It is noteworthy that some peculiar effects–the presence of broad background near 2500 cm^{-1} and in particular in the range below 1500 cm^{-1}–can be observed in spectra of PLL from solutions with pH=10 and pH=12. It must also be mentioned that, compared to standard methods, the quality of spectra were superior (the bands narrower) in our technique, i.e. precipitation of PLL from water solutions with KBr.

3. Results and discussion

3.1. Bands assignment

Spectra of PLL samples, dried from alkaline, neutral and acidic solutions, are shown in Fig. 1, panels 1, 2 and 3, respectively. Two features are prominent: (i) two or three overlapping bands resulting in very broad (350 cm^{-1}) absorbance in the range 3300–3000 cm^{-1} and (ii) a group of bands near 1600–1500 cm^{-1}. The assignment of the latter is well known: the band at 1620–1700 cm^{-1} relates to amide I (mainly CO group stretching mode); the band at 1534 cm^{-1} (amide II) is also well established: it relates to the in-plane deformational mode of the NH group. Both bands relate to the vibrational modes of the peptide group [1–4]. Deformational mode bands of charged side chain amine groups NH_3^+ also fall in the range at ca. 1500 cm^{-1}, as for example, for a zwitterion of alanine in solid state [22].

The narrow band near 3270 cm^{-1} with the band width of ca. 50 cm^{-1} can be safely assigned to the ν_1 proton mode band of the peptide group [1–3,23]. In PLL from D_2O solution, a new band with the bandwidth of ca. 30 cm^{-1} appears at 2413 cm^{-1}. The close to normal isotopic frequency ratio 1.357 confirms its assignment to the isotopic counterpart of the band at 3270 cm^{-1}.

The band at 3030 cm^{-1} should be assigned to the ν_1 band of the side chain NH_3^+ groups. Its isotopic counterpart is observed at ca. 2250 cm^{-1} in the D_2O solution with the isotopic frequency ratio of 1.35. It preserves its frequency in the spectra of all three samples prepared from acidic, neutral or alkaline media, but its intensity shows a clear dependence on pH. This dependence can be seen, for example, when the intensity of the band of the side chain NH_3^+ groups is compared with that of the amide I band at 1620–1650 cm^{-1}—the latter remains relatively constant, since the quantity of peptide groups does not change with pH (see Fig. 1). The 3030 cm^{-1} band intensity is maximal in spectra of PLL from the acidic media, where the majority of amine groups are protonated. As pH increases, the quantity of NH_3^+ species lessens and the 3030 cm^{-1} intensity decreases. The complex shape of this broad band clearly contains several components (see also the deconvoluted spectrum in Fig. 2), which can, in part, originate from the Fermi resonance with deformational modes of the NH_3^+ groups at 1510–1520 cm^{-1} ([9 and ref. therein). The assignment of this band to the NH_3^+ ν_1 mode is also supported by the presence of a band at 3000–3100 cm^{-1} in crystal spectra of simple peptides and amino acids [9], which form zwitterions with the NH_3^+ group in solid state.

The narrow bands at ca. 2863 cm^{-1} and ca. 2936 cm^{-1}, which do not show isotopic displacement in D_2O, should be assigned to CH_2 stretching modes.

With the lowering of pH, the 3030 cm^{-1} band intensity increases drastically, while the band at 3270 cm^{-1} weakens (Fig. 1, panels 1 and 3, compare, for example, with the band amide II intensity at 1534 cm^{-1}). This correlation is also illustrated by the deconvolution of the spectra near 3000 cm^{-1}, which is shown in Fig. 2 (for the sake of simplicity, the narrow CH_2 bands are not included). Along with the decrease of the component at ca. 3270 cm^{-1}, a new weak band component appears at ca. 3200 cm^{-1}, at pH change from 12 to 7 and further to 1. At the same time, the structure of the broad NH_3^+ band remains without noticeable change. This new component at 3200 cm^{-1} is clearly seen in the original spectrum of PLL at pH=1 (Fig. 1, panel 3) and can be assigned with certainty to the amide A band of the changed peptide group. An alternative assignment, namely to the amide I overtone, is highly improbable, since the doubled amide I frequency (which is ca. 1654×2=3308 cm^{-1}) is too far from its peak position at 3200 cm^{-1}. It follows from the above that the new band indicates a change of the peptide group state in acidic media as compared to neutral or alkaline media, which is also reflected by the position of the amide I band. The latter band, which is quite narrow, has its peak position at 1627 cm^{-1} in PLL prepared from alkaline and neutral media (Fig. 1, panels 1 and 2) and at 1654 cm^{-1} in spectra of PLL from acidic media (Fig. 1, panel 3). The peak at ca. 1630 cm^{-1} in panel 3 can be assigned to the remnants of PLL molecules with a multitude of uncharged side chain amine groups; it correlates with a decreased intensity of the peak at 3270 cm^{-1} in the spectrum of the same sample.

It can thus be concluded that PLL precipitated from water solutions with different pH undergoes changes which are

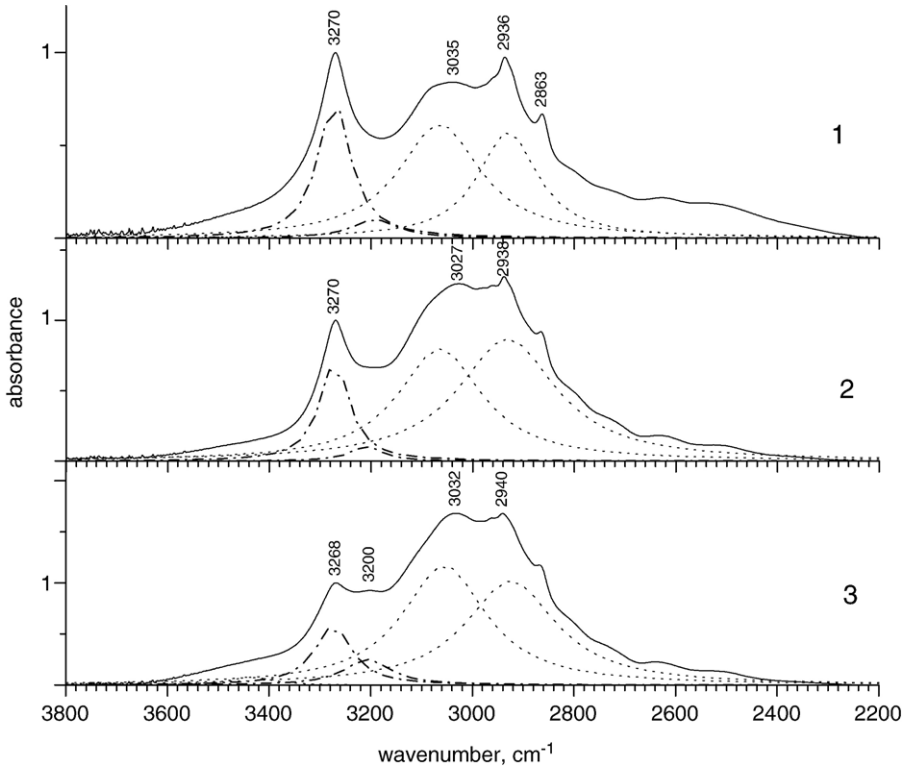

Fig. 2. Approximate deconvolution of spectra of poly-L-lysine (M.W. [(30–70)10^3]) in KBr pellets. Samples were lyophilized from water solution with different pH after addition of KBr: (1) pH ≅ 12, (2) pH ≅ 7, (3) pH ≅ 1. All spectra were normalized relative to optical density at 3270 cm^{-1}.

reflected not only on the amide I band but also, as expected, on the ν_1 band. An especially pronounced change is evident from the intensity of the side chain NH_3^+ group band.

It is important to take into account that the position of the amide I band in all samples does not depend on the presence of water.

In summary, we claim that the band at 3030 cm^{-1} is a marker band for protonated side chain NH_3^+ groups in solid PLL, and as such can be used to estimate their relative content. Observed narrowing of the main peak at ca. 3030 cm^{-1} (from 280 cm^{-1} at pH = 12 to 190 cm^{-1} at pH = 1) (Fig. 1, panel 3) indicates the more ordered structure of solid PLL prepared from acidic media as compared to those prepared from alkaline media.

3.2. Quantitative estimation

Table 1 presents the results of the quantitative estimation of the ratio of NH_3^+/NH_2 groups in PLL from different pH. Such estimation of protonated chain groups relative to the number of peptide groups is possible because peptide group content remains constant irrespective of the secondary structure type of PLL. The estimation was made by the base line method, which is the simplest available. Fig. 1 shows the way of calculating I_{3030}/I_{3270} (I—absorbance measured from the base line). On the basis that PLL from acid (pH = 1) medium contains maximal quantity of ionized NH_3^+ groups, possibly even 100 %, it can be concluded that, at the high pH value of 12, approximately 30% of amine groups remain protonated. Statistical evaluation, which is presented in Table 1, shows that this ratio, for PLL prepared from all media, has the standard deviation from the mean value lower than 15–20% (with 95% probability).

In normalized spectra of PLL (Fig. 2), the integral intensity of the 3270 cm^{-1} component from pH = 12 does not deviate substantially from the sum of the 3270 and 3200 cm^{-1} components in PLL from a neutral solution. In the spectrum of PLL from acidic media, the sum of intensities of the 3270 and

Table 1
Absorbance ratio I_{3030}/I_{3270} of the bands at 3270 cm^{-1} and 3030 cm^{-1} in spectra of poly-L-lysine from water solutions with different pH in KBr pellets and films

N	Poly-L-lysine M.W. 10^{-3}	I_{3030}/I_{3270}		
		pH = 1	pH = 7	pH = 12
1	5–10	4.3	2.2	1.6
2		5	2.8	1.8
3		6.7	1.9	1.7
4	30–70	4.7	2.4	1.5
5		6.0	3.2	–
6			2.1	
7	70–150 (I)	–	2.3[a]; 2.00	1.6[a]; 0.9
8	70–150 (II)	5.3[a]	2.7[a]; 2.7	1.8[a]; 1.1
9	30–70 (films)	5.5[a] (pH = 0.3)	2.7[a]	1.8[a]
		4.45[a] (pH = 1.1)	2.4[a]	1.4[a]
		2.90[b] (pH = 1.3)		
Average ± σ		5.3 ± 0.7	2.5 ± 0.2	1.5 ± 0.3
(NH_3^+/NH_2) ratio		1	0.45	0.3

I_{3030} and I_{3270} were calculated from the baselines drawn between (3700–2200 cm^{-1}) and (3330–3180 cm^{-1}) points of spectra, respectively (see Fig. 1). σ—standard deviation for 95% probability.

[a] Samples with water contamination.

[b] Not used in statistical treatment.

3200 cm^{-1} bands (Fig. 2, panel 3) grows by approximately 25% on account of the increase of the molar intensity of the red shifted band at 3200 cm^{-1} [7].

3.3. Connection with the secondary structure

The secondary structure of the samples under investigation can be determined by using any more or less reliable method. Thus, the peak position of the amide I band, as measured in our PLL samples, can be used for this purpose. PLL from neutral media, which, in solution, exists mainly as unordered polypeptide, at freeze drying converts into a highly ordered β-sheet [14]. Similarly, lyophilization from the pH 11.2 solution, where PLL adopts an α-helical conformation, induces transition to a β-sheet [14]. The observed peak position of the amide I band at 1627 cm^{-1} (together with the very weak narrow band at 1690 cm^{-1}) indicates the formation of the β-sheet structure, a fact which is confirmed in extensive literature [4]. From our technique, in which solutions with high pH were treated for a long time at room temperature [24], it follows with all probability that the spectra of PLL samples from alkaline (pH = 10–12) media relate to the β-sheet structure. One can reasonably conclude that this structure can be characterized by the bands at 3270 cm^{-1} and 1627 cm^{-1} for amide A and amide I modes, respectively. The quantity of ionized amine groups in this case is minimal, averaging about 30%.

A definitely different structure is forming in PLL from acidic media; its characteristic band is at ca. 3200 cm^{-1}. The amide I band, concurrently observed at 1654 cm^{-1}, indicates either a random coil or an α-helical structure [4]. It is impossible to differentiate with certainty between these two structures relying on the literature on the amide I band in IR spectra. However, in so far as this new structure is formed primarily in PLL in the maximally charged state, it can be related to the random coil [25], which can be characterized by the ν_1 (amide A) and amide I modes bands at 3200 cm^{-1} and 1654 cm^{-1}, respectively.

It can thus be unequivocally concluded from our experiment that PLL from acidic solutions, whose side-chain structure is ionized to the greatest degree, contains a minimal quantity of the β-sheet structure, and, conversely, PLL from an alkaline solution, which includes approximately 30% of ionized side-chain groups, contains a maximal quantity of the β-sheet structure. It follows that the secondary structure changes drastically depending on the degree of ionization of PLL [25].

In summary, two extreme ratio values at pH 1 and pH 12 reflect quantitatively the presence in the sample of either a random coil or a β-sheet structure, respectively.

Authors [13] observed the formation of β-sheet structure with the bands at ca. 1625 and 1690 cm^{-1} in PLL, prepared from neutral media at air drying, when the drying time exceeded 6 min. When the drying time was shorter (ca. 2 min), the spectrum of this "rapidly" dried PLL prepared from a neutral solution was happened to be very similar to that of freeze dried PLL from a solution with pH = 1 (Fig. 1, panel 1) with the band amide I at 1654 cm^{-1}. This indicates in agreement with [13], that random coil structure is forming at fast drying from neutral medium, possibly due to higher water content in samples of [13], as was suggested by our Referee; indeed the higher water content is seen from the spectrum shown in [13] when compared with Fig. 1. It is noteworthy that a full spectrum of PLL, one that includes the ν_1 (amide A) band, has so far been found only once in the literature [13].

3.4. Estimation of H-bond strength

For systems exhibiting the frequency red shift ($\Delta\nu_1$) of the ν_1 stretching vibration of the donor group from 100 to 850 cm^{-1} due to H-bond formation, the empirical relation $(\Delta H)^2 = 1.92(\Delta\nu_1 - 40)$ was found between $\Delta\nu_1$, on the one hand, and the square of the H-bonding enthalpy (ΔH, kJ mol^{-1}), on the other [7]. The red shift $\Delta\nu_1$ equals $\nu_1^H - \nu_1^0$, where ν_1^H and ν_1^0 are peak positions (cm^{-1}) in a bound state and in a free from H-bonding state, respectively. Though it is impossible to check the applicability of this correlation to PLL using independent thermodynamic data, an approximate estimation of ΔH can be undertaken. The accuracy of the ΔH value depends on the accepted ν_1^H and ν_1^0 peak positions. The value of the ν_1^0 of 3473 cm^{-1} is accepted as the mean value of (i) free NH band positions at 3495 cm^{-1} of free *N*-methylacetamide in N_2 matrix [26]; (ii) 3454 cm^{-1}, which was measured for isolated "linear" non-hydrogen bonded dipeptide Ac-Val-Phe-OMe (Val—valine, Phe—phenylalanine) in the gas phase [27]; and (iii) 3470 cm^{-1} of acetamide in diluted solution [17]. The scatter in ν_1^0 values defines the ΔH error in the limits of ±1.0 kJ mol^{-1}.

The positions of both observed peaks at 3270 cm^{-1} and 3200 cm^{-1}, which present the most intensive components of the Fermi doublet[1] [1], do not provide the required positions of ν_1^H bands; the latter must be taken as a centre of gravity of the whole structured band [7,17,28]. The second component is usually observed in the spectra of self associated amides at ca. 3100 cm^{-1} and belongs to the overtone (2δ) of amide II in-plane deformational (δ) mode; its intensity does not exceed 20% relatively the main component. As it was shown on the example of the ν_1 (NH) band of ε-caprolactam in different solvents [28], when the main maximum of the NH band is near 3260 cm^{-1} (similar to our case), the centre of gravity (ν_1^H) is approximately 10 cm^{-1} lower than the position of the main maximum. With increasing of the H-bond energy the ν_1^H band displaces to the lower frequencies closer to the position of the 2δ mode, the resonance strengthens and the intensity of the 2δ component increases, and as a result, the center of gravity ν_1^H of the band differs essentially from the peak position of the most intensive component. When the main maximum is near 3200 cm^{-1}, the position of the ν_1^H is ca. 40 cm^{-1} lower than the main maximum. In both cases, these corrections were taken into account and the values of the ν_1^H frequencies of the NH bands of 3260 cm^{-1} ($\Delta\nu_1 = 213$ cm^{-1}) and 3160 cm^{-1} ($\Delta\nu_1 = 313$ cm^{-1}) were accepted in the β-sheet and coil structures, respectively, for the estimation of the H-bond energies.

It transpires that the H-bond energy in the β-sheet based on position of the 3260 cm^{-1} band amounts to 18.2 kJ mol^{-1}. In PLL from acidic media, the ν_1^H band at 3160 cm^{-1} is red shifted more by ca. 100 cm^{-1}. This means that the energy of the amide

[1] The second component is not seen in our spectra because of overlapping with the broad absorbance at 3030 cm^{-1}.

H-bond increases up to 22.9 kJ mol^{-1} in the coil structure and the enthalpy of the transition β-sheet—coil amounts to ca. 4.7 kJ mol^{-1}. It should be noted that an empirical spectroscopic estimation based on the red shift reflects only changes in the H-bond structure while calorimetric measurements include other interactions as well. It is difficult to compare these results to the relevant findings in the literature, since, although secondary structure transitions have been extensively studied, the focus has always been on the α-helix to coil transition, whose enthalpy was estimated ca. 2.5 kJ mol^{-1} in peptides with polar side chains [29].

The red shift of the ν_1 band at ca. 3030 cm^{-1}, which is assigned to the NH_3^+ group, is about 350 cm^{-1} (estimated relative to ν_1^0=3380 cm^{-1} as calculated in [30] for free alanine neutral form). This means that side chain protonated amine groups form H-bonds, presumably for the most part with water, with the empirically estimated energy of ca. 24.0 kJ mol^{-1} per one proton. It should be noted that, in small peptides, the H-bond energy of different groups was found to be in the limits 16–30 kJ mol^{-1} [9].

The estimated H-bond energy in β-sheet and coil structures (18.2 and 22.9 kJ mol^{-1}, respectively) are close to a direct measure (by atomic force microscopy) of H-bond energy (20.2 kJ mol^{-1}) as a force stretching α-helix to a linear chain, albeit in the slightly different peptide $cysteine_3$–$lysine_{30}$–cysteine [31] prepared at pH 11.

4. Conclusion

A new parameter, namely, the intensity ratio of the ν_1 bands of charged side chain amine NH_3^+ and amide NH bands, is suggested for the characterization of the secondary structure of PLL. When the concentration of charged side chain NH_3^+ groups in PLL is low, this intensity ratio is minimal and PLL is in the β-sheet structural form. When, on the other hand, all (or the majority of) side chain NH_3^+ groups are charged, this ratio turns out to be four times bigger, and the amide A band shows conversion into a different structure, which, however, cannot be differentiated with certainty on the basis of the amide I band. The ν_1 amide NH band at 3270 cm^{-1} was conclusively assigned for the β-sheet structural form, and its frequency was used for estimating H-bond energy relying on the well established empirical correlations between spectral and thermodynamic properties of H-bonding [7]. The newly discovered ν_1 amide NH band at 3200 cm^{-1} indicates a change in the secondary structure of PLL, which, as has been argued above, assumes a coil structural form. The enthalpy of the secondary structure transition, which is observed in PLL at the change of pH from 11 to 1 amounts to 4.7 kJ mol^{-1}.

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References

[1] H. Susi, in: S.N. Timasheff, G.D. Fasman (Eds.), Structure and Stability of Biological Macromolecules, Marcel Dekker, Inc., New York, 1969, pp. 576–663, (Ch. 7).
[2] S. Krimm, J. Bandekar, Adv. Protein Chem. 38 (1986) 181–364.
[3] J. Bandekar, Biochim. Biophys. Acta 1120 (1992) 123–143.
[4] P.I. Haris, in: B.R. Singh (Ed.), Infrared analysis of peptides and proteins, ACS Symposium Series, 2000, pp. 54–95.
[5] W.K. Surewicz, H.H. Mantsch, D. Chapman, Biochemistry 32 (1993) 389–394.
[6] P. Schuster, G. Zundel, C. Sandorfy (Eds.), The Hydrogen Bond, North-Holland, New York, 1976.
[7] A.V. Iogansen, Spectrochim. Acta 55A (1999) 1585–1612.
[8] M. Rozenberg, A. Loewencshuss, Y. Marcus, Phys. Chem. Chem. Phys. 2 (2000) 2699–2702.
[9] M. Rozenberg, G. Shoham, I. Reva, R. Fausto, Phys. Chem. Chem. Phys. 7 (2005) 2376–2383.
[10] H. Susi, S.N. Timasheff, L. Stevense, J. Biol. Chem. 242 (1967) 5460–5466.
[11] B. Davidson, G.D. Fasman, Biochemistry 6 (1967) 1616–1629.
[12] M. Jackson, P.I. Harris, D. Chapman, Biochim. Biophys. Acta 998 (1989) 75–79.
[13] W.F. Wolkers, M.G. van Kilsdonk, F.A. Hoekstra, Biochim. Biophys. Acta 1425 (1998) 127–136.
[14] S.J. Prestrelski, N. Tedeschi, T. Arakawa, J.F. Carpenter, Biophys. J. 65 (1993) 661–6711.
[15] L.J. Bellamy, The Infrared Spectra of Complex Molecules, John Wiley & Sons, London, 1957.
[16] S. Suzuki, Y. Iwashita, T. Shimanouchi, M. Tsuboi, Biopolymers 4 (1966) 337–350 (-10).
[17] A.V. Iogansen, G.A. Kurkchi, L.A. Dementjeva, J. Mol. Struct. 35 (1976) 101–114.
[18] W. Dzwolak, V. Smirnovas, Biophys. Chemist. 115 (2005) 49–54.
[19] M. Fändrich, C.M. Dobson, The EMBO J. 21 (2002) 5682–5690.
[20] P. Chen, Colloids Surf., A Physicochem. Eng. Asp. 261 (2005) 3–24.
[21] J.D. Meyer, M.C. Manning, J.F. Carpenter, J. Pharm. Sci. 93 (2004) 496–506.
[22] M. Rozenberg, G. Shoham, I. Reva, R. Fausto, Spectrochim. Acta, Part A: Mol. Biomol. Spectrosc. 59 (2003) 3253–3266.
[23] M.H. Baron, C. Deloze, C. Toniolo, G.D. Fasman, Biopolymers 18 (1979) 411–424.
[24] S.C. Yasui, T.A. Keiderling, JACS 108 (1986) 5576–5581.
[25] J. Applequist, P. Doty, in: M. Stahmann (Ed.), Polyamino Acids, Polypeptides, and Proteins, University of Wisconsin, Madison, 1962, p. 161.
[26] S. Ataka, H. Takeuchi, M. Tasumi, J. Mol. Struct. 113 (1984) 147–160.
[27] C. Unterberg, A. Gerlach, T. Schrader, M. Gerhards, J. Chem. Phys. 118 (2003) 8296–8300.
[28] L.A. Dementjeva, A.V. Iogansen, G.A. Kurkchi, Opt. Spectrosc., (USSR), Engl. Trans. 29 (1970) 461–465.
[29] G.I. Makhatadze, Adv. Protein Chem. 72 (2005) 199–220.
[30] S.G. Stepanian, I.D. Reva, E.D. Radchenko, L. Adamowicz, J. Phys. Chem., A 102 (1998) 4623.
[31] M.A. Lantz, S.P. Jarvis, H. Tokumoto, T. Martynski, T. Kusumi, C. Nakamura, J. Miyake, Chem. Phys. Lett. 315 (1999) 61–68.