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A conformational α-helix to β-sheet transition accompanies racemic self-assembly of polylysine: an FT-IR spectroscopic study

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Abstract

The self-assembly of polylysine chains with opposite chiral senses is an intriguing phenomenon, suggesting that subtle hydrational effects may be a driving force of protein aggregation. We have used FT-IR spectroscopy to characterize the α-helix-to-β-sheet conformational transition that accompanies the aggregation of single and mixed enantiomers of polylysine. The preferential racemic self-assembly not only takes place at a lower temperature, but is also less prone to repulsive electrostatic interactions between lysine charged side chains, caused by decreasing pH (pD). While the process is generally irreversible, it yet appears to proceed in a stepwise manner through a sequence of thermodynamically, rather than kinetically controlled events involving gradual destabilization of α-helices. Interestingly, although the α/β-transition is in either case (single or mixed enantiomers) an endothermic process, it may also be induced by freezing of water, which leads to markedly more complete (and irreversible) aggregation of the mixed enantiomers. Relevance of these findings has been discussed in the context of protein aggregation studies.
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Keywords: Poly(L-lysine); Poly(D-lysine); Conformational transitions; Self-assembly; Aggregation; Amyloid; Beta-sheet

1. Introduction

The fascinating phenomenon of protein aggregation, which transforms native proteins into insoluble, biologically dysfunctional β-sheet-rich agglomerates, has started to draw attention since it was implicated in molecular aetiology of neurodegenerative diseases, such as Alzheimer, Parkinson, or the "prion"-associated Creutzfeldt-Jakob Disease [1,2]. Although significant research efforts have helped elucidate common scenarios of aggregation (such as partial destabilization occurring prior to the actual aggregation [1,2]), comprehensive mechanisms remain elusive. The observed common character of protein aggregation supports the hypothesis that the phenomenon may reflect a generic trait shared by all polymers, and persuades to seek further clues by studying generalized—sequenceless polypeptide models [3].

One of the most successfully studied model polypeptides is polylysine, which undergoes the α-helix-to-β-sheet transition—the hallmark of protein aggregation [4], binds Congo Red-an amyloid-specific dye [5], and forms fibrils with an amyloid-like outlook [3]. Since repulsive forces between positively charged lysine side chains would rule out any helical or extended structure, the charges must be either prevented by high pH, or compensated at neutral pH through interactions with a negatively charged polypeptide, e.g. polyglutamic acid [3]. Previous studies have shown that dehydrating media: lipid bilayers [6] and hydrophobic anesthetics [7] promote the extended conformation in PLL, while hydration (e.g. enhanced by high hydrostatic pressure) has the opposite effect [6,8]. Upon mixing of PLL and PDL, propensity to form β-sheet increases dramatically, yet the process remains endothermic [9,10]. The volumetric

Abbreviations: FT-IR, Fourier-Transform Infrared Spectroscopy; CD, Circular Dichroism; PLL, poly(L-lysine); PDL, poly(D-lysine).

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data obtained from Pressure Perturbation Calorimetry led us to conclude that the alternative stacking of the left- and right-handed chains enables a different packing mode of side residues, which reduces thermodynamically unfavorable low-density low-entropy water [10]. Given the virtually identical, as judged by FT-IR, β-sheet structure of single (chiral) and mixed (racemic) polylysine enantiomers, the driving thermodynamic forces of the process may have more to do with the hydration than the chains' configuration. The finding that changing hydration may render a polypeptide more prone to self-assembly has prompted our further spectroscopic inquiry into the mechanism of this transition. This is urgently needed to clarify the so far poorly understood role that protein interactions with water, co-solvents and co-solutes may play in triggering aggregation in vitro and in vivo.

2. Materials and methods

2.1. Samples

PLL and PDL of approx. 27 kD molecular weight were purchased from Sigma, USA. The "light" PLL had molecular weight of approx. 20 kD. Preparation of samples began with dissolving the polypeptide in D_2O at 1.5 wt.% concentration, followed by pD-adjustment. In order to prevent spontaneous aggregation, all sample handling was done in an ice-bath. In the freezing experiment small amount of H_2O (<0.5 wt.%) was present to enable using the –OH stretching band as a probe of the liquid-to-ice transition.

2.2. Fourier-transform infrared spectroscopy (FT-IR)

For acquisition of FT-IR spectra, CaF_2 transmission windows and 0.025 mm Teflon spacers were used. The temperature in the cell was controlled through an external water-circuit connected to a PC-controlled thermostat. All FT-IR spectra were collected on a Nicolet Nexus FT-IR spectrometer equipped with a liquid nitrogen-cooled MTC detector. For each spectrum 256 interferograms of 2 cm^{-1} resolution were co-added. The sample chamber was continuously purged with CO_2-free, dry air. From each sample's spectrum a corresponding D_2O and water-vapor spectra were subtracted. The semi-quantitative plots in Figs. 2, 3 and 4C, which approximates progress of the conformational transitions, were calculated as $(I_\alpha - I)/(I_\alpha - I_\beta)\times 100$, where "$I_\alpha$" is spectral intensity at 1612 cm^{-1} corresponding to samples with highest α-helical content (initial conditions), "I_β" is the intensity value at 1612 cm^{-1} of completely aggregated polylysine, likewise "I" is a transient intensity of a spectrum at a given temperature. All data processing was performed with GRAMS software (ThermoNicolet, USA).

3. Results and discussion

Since CD spectroscopy is inapplicable to samples lacking optical activity, which is the case of equimolar mixtures of PLL and PDL, FT-IR spectroscopy can be employed instead to follow the conformational changes accompanying the aggregation. Fig. 1A shows the changing infrared amide I' band of racemic polylysine (PLL+PDL, 1:1) upon the temperature-induced α/β-conformational transition. The band assigned to α-helical structures splits gradually into two components—minor ([$\tilde{\nu}(,\pi)$]) at 1682 cm^{-1} and major ([$\tilde{\nu}(\pi,)$]) at 1612 cm^{-1}, which are indicative of an antiparallel β-sheet [8]. We have proposed earlier that the antiparallel orientation of the β-strands may be a consequence of the pre-transitional antiparallel alignment of dipolar α-helices, which is energetically favored [11]. It should be stressed, though, that neither the fresh "α-helical",

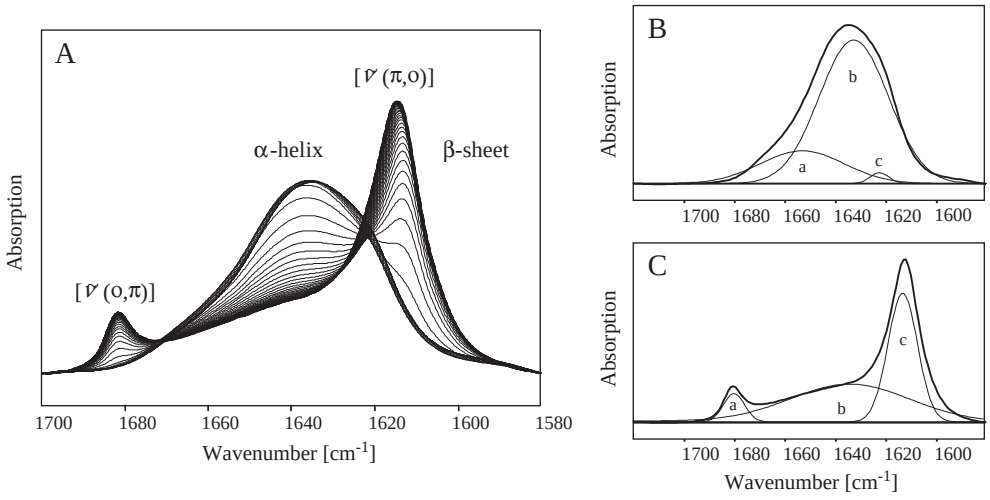

Fig. 1. Changes in the infrared amide I' band reflect the α-helix-to-β-sheet conformational transition in the equimolar mixture of PDL and PLL induced by gradual heating from 5 to 60 °C at 20 °C/h scan rate (A). The splitting band corresponds to the intermolecular antiparallel β-sheet. Peak fitting with Gaussian functions of the first (B) and the final (C) spectra of the racemic polylysine. The ratios of the areas marked as *a*, *b*, and *c* are 14:49:1 (B) and 1:8:6 (C).

nor the fully aggregated "β-sheet-rich" polylysine samples are conformationally homogenous. Fig. 1 shows results of peak fitting with Gaussian functions of the first (1B) and the final (1C) spectra from Fig 1A, i.e. corresponding to the "pure" conformers of the polymer. The relative areas of the components marked as *a*, *b*, and *c* become 14:49:1 (1B) and 1:8:6 (1C). The spectral position of the α-helical component centered on 1632 cm^{-1}, is quite untypical and red-shifted compared to helices in globular proteins (ca. 1655 cm^{-1} in deuterated forms). While this well-known aspect of spectral properties of polylysine is attributed to the presence of highly hydrated [12] and distorted helices [11], the exact peak position has been further shown to depend on the polylysine's chains length [11,12]. In context of quantification of the progressing transition, this, and the inherent to polymer samples broad molecular weight distribution may result in ambiguity of certain computational approaches (such as peak-fitting-a conventional method of analyzing FT-IR spectra of globular proteins) wherein resolved spectral components are specifically assigned to structural motifs. The herein employed manner of handling quantification of the transition (by plotting $(I_\alpha - I)/(I_\alpha - I_\beta)$ versus the temperature, as described in Materials and methods) has been successfully employed in studies on polylysine [10,11], yet, the obtained curves only approximate the actual α-helix-to-β-sheet conversion. Throughout the transition, the total polypeptide concentration is constant thus partial spectral intensities of different secondary conformations (α-helix, β-sheet, "random coil"/polyproline II-conformation) are linearly inter-dependent. Therefore differences in molar absorption coefficients of these secondary structure components ([12]) do not evoke systematic deviations of the transition curves computed according to the formula.

Efficiency and rate of the transition are expected to depend on a fraction of charged lysine side-chains and therefore of pD, which is confirmed by the data in Fig. 2. The racemic β-sheet is already formed at temperature roughly 15 °C lower. The sigmoidal curves computed for single PLL (Fig. 2A) and PDL (Fig. 2B) reveal a highly cooperative transition. That is clearly not the case of the racemate (Fig. 2C), when the process acquires a multiphasic character. This may also be interpreted as existence of subpopulations of helices of varying temperature-stability and propensity to form the racemic β-sheet structure [10,11].

Formation of any ordered, especially aggregated structure in polylysine is hampered by decreasing pH (pD), namely when lysine side chains' amine groups become protonated, and because of electrostatic repulsion, polypeptide chains are kept apart in solution in the "random coil" (polyproline II conformation) state. Fig. 2 shows that decreasing pD shifts the α/β-transition to a higher temperature range and renders it less complete. The minor differences between PLL and PDL visible at pD 11 and 10.7 should be attributed to different molecular weight distributions—a typical aspect of the lot-to-lot variability in polymerized amino acids samples. One pronounced difference between the pD-dependencies of the single and mixed PLL and PDL concerns the initial decrease of pD from 11.6 to 11.3, which does affect aggregation of single enatiomers, but virtually has no impact on the racemic mixture (Fig. 2A and B versus Fig. 2C). This suggests that the self-assembly of the left- and right-handed chains is unlikely to involve any direct interactions between the charge-bearing amine's groups (such as hydrogen bonding), and that the high stability of the racemic structure is capable of overriding a minor destabilization caused by the increasing presence of uncompensated charges. Both these facts are easy to reconcile with our earlier claim as to the relative gain in solvent entropy as the thermodynamic factor contributing to the preferential aggregation of mixed PLL and PDL [10]. A changing mode of packing of the left- and right-handed polylysine chains may reduce thermodynamically unfavorable void volumes and/or structured water around the side-chains. It seems that through essentially the same mecha-

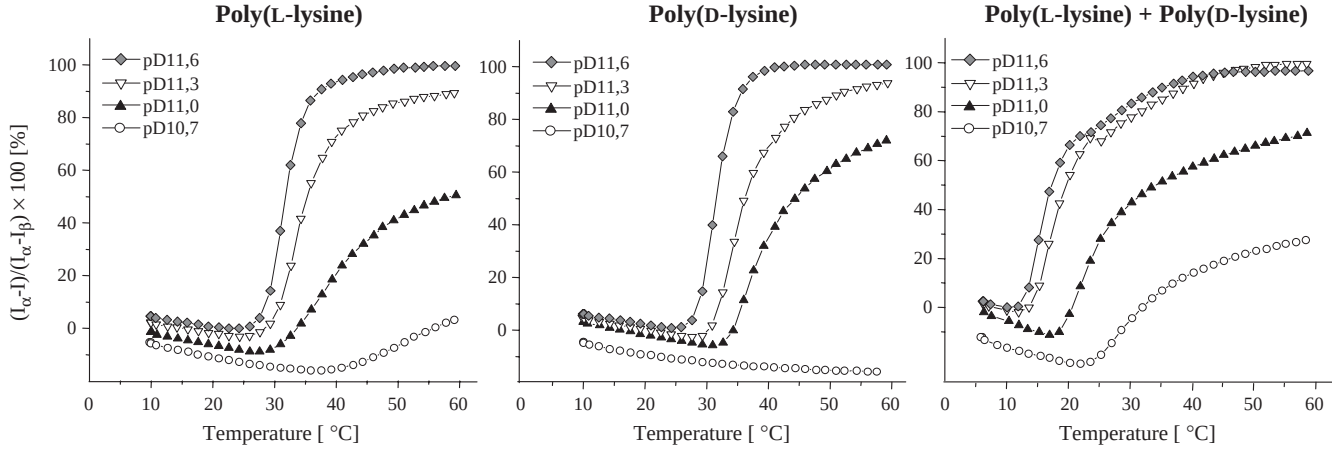

Fig. 2. pD-effect on the α-helix-to-β-sheet conformational transition in PLL, PDL and the racemic mixture of PLL and PDL. The degree of transition is approximated a relative intensity of the β-sheet-assigned [$\tilde{\nu}(\pi,o)$] component of the amide *I′* band at 1612 cm^{-1}.

nism small hydrophobic molecules, such as halothane [7], may trigger formation of β-sheet aggregates in the chiral polylysines upon partitioning into polypeptide hydration layers and through sharing the entropic cost of solvating water [11].

The profoundly irreversible character of the PLL–PDL self-assembly first communicated by Fuhrhop [9], and the partly reversible transition in single enantiomers were compared at different heating rates. The data shown in Fig. 3 reveals that the increasing heating rate does not retard appreciably the transition as a function of temperature, as is expected for any kinetically controlled process. On the contrary, in each case, the shape of the sigmoidal plots is unaffected by the increasing heating rate. The plots showing the reversibility of the transition (empty symbols) overlap quite exactly (the cooling rate in each set of measurements is the same as the corresponding heating rate). These observations hold true for single PLL, single PDL, and their mixture.

Attempts to elucidate mechanisms of the racemic self-assembly must address the fact that the transition begins at a temperature, at which each single enantiomer is in the stable α-helical conformation. This proves that, at least above 15 °C, the still helical structures of PLL and PDL become aggregation-prone. Given that in this temperature range no changes in FT-IR, nor CD spectra of single enantiomers are visible [10], it is likely that the intermediate state is not completely unfolded (no visible broadening of the amide I′ band above 15 °C), but rather reflects o more loose (and hydration-permissive) 3D arrangement of helices:

$$\alpha^N \leftrightarrow \alpha^*$$

An extra free energy gain from the increased solvent entropy in the racemic mixture may be responsible for the capability of the "intermediates" (α*) to form racemic, but not chiral β-sheet, which would require a more profound destabilization at a higher temperature. Therefore, the increasing temperature seems to be the factor controlling–initially–partial, and then, more pronounced destabilization of helices, which are prerequisites for aggregation of mixed, or single enantiomers, respectively. This parallels the well-known fact that aggregation of biological proteins also requires a degree of destabilization of the native structure [2]. It has been suggested recently that intermediate stages of the α-to-β-transition in polylysine may be populated by distinguishable conformations of the lysine side chains [13]. We have shown earlier that independently from the inherent molecular weight distribution in commercially available poly amino acid samples, there is distribution of helical stretches of different thermodynamic stability within a single polypeptide chain [11]. Temperature-induced destabilization of such helical stretches may be fast, reversible and thermodynamically controlled, but the following events of association of freed chains into large multi-stranded β-sheet structure acquire a more irreversible character. That a degree of protein destabilization required prior to its aggregation may depend on accompanying solvent entropy gains, is an interesting suggestion in light of the ongoing debate on critical amount of structural disruption needed to trigger protein amyloidogenesis (e.g. [14]). In other words, it may as well depend on the environment and protein hydration, whether partially destabilized intermediate states become aggregation-competent.

At this point, it is very tempting to discuss the rather perplexing data on freezing effect on helical polylysine. Fig. 4 shows how, upon abrupt freezing of water (reflected by narrowing of –OH stretching band around 3300 cm^{-1}) the infrared amide *I′* band undergoes the spectral changes indicative of the α/β-transition and aggregation. Although traces of H_2O were used-along with a temperature sensor-to probe the liquid/solid state of a sample (Fig. 4A), it still contained more than 97 wt.% of D_2O (melting point 3.81 °C, under 1 bar), which explains why the freezing occurrs above 0 °C. The semi-quantitative plots in Fig. 4C show that the process can be triggered for either single or mixed enantiomers, but only in the latter case is almost quantitative

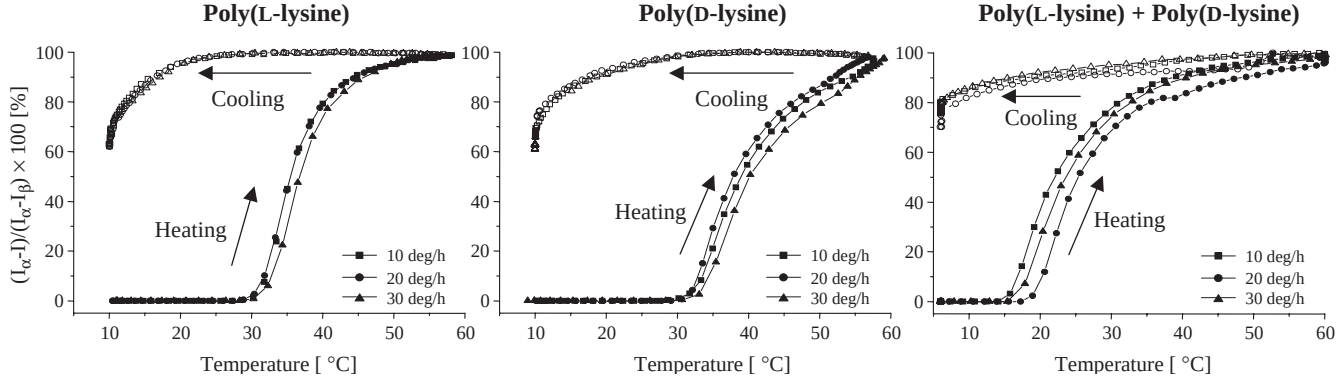

Fig. 3. Influence of heating rate on the conformational transition in polylysine at pD 11.6, and its reversibility. The filled symbols correspond to increasing temperature, while the empty ones reflect the gradual cooling.

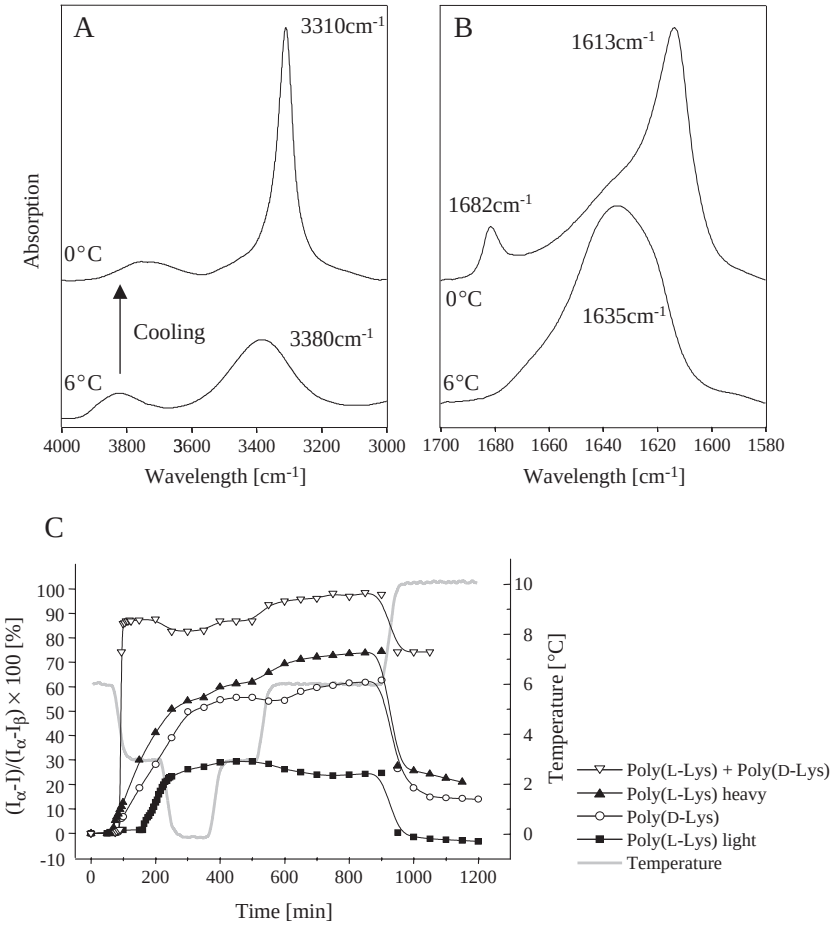

Fig. 4. Cooling and freezing of the PLL+PDL sample, reflected by pronounced spectral shift and narrowing of the –OH stretching band (A) induces the α-helix-to-β-sheet conformational transition in the polypeptide (B). To a lesser degree, the freeze-induced and partly reversible aggregation is also the case of single enatiomers of polylysine (C). The thick grey line denotes cooling/heating profile.

and irreversible. While there is no doubt, that concentration-effect caused by water crystallization and removal (as ice) would favor any aggregation process [15], it still does not explain how freezing of water would trigger a profoundly endothermic process. While the positive enthalpic effects of aggregation of single and mixed polylysine enantiomers are comparable [10], the extents of the freeze-induced transitions (at identical concentrations) are not. It could be speculated though, that because the thermodynamically disfavored low-entropy, low-density water is proposed to form around hydrophobic side-chains (e.g. [16]), then upon freezing, its formation comes at reduced expense, since at low temperature such forms are abundant in bulk water, as well.

In conclusion, processes that closely mimic aggregation and freeze-denaturation of proteins may be evoked in a sequenceless, charge-depleted and lacking any tertiary structure polymerized amino acid. This suggests that thermodynamic effects that determine conformational fate of a protein may stem from fundamental properties of polypeptide backbone and its hydration, rather than particular properties of amino-acid sequences.

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References

[1] C.M. Dobson, Protein folding and misfolding, Nature 426 (2003) 884–890.
[2] G.S. Jackson, A.R. Clarke, Mammalian prion proteins, Curr. Opin. Struct. Biol. 10 (2000) 69–74.
[3] M. Fandrich, C.M. Dobson, The behaviour of polyamino acids reveals an inverse side chain effect in amyloid structure formation, EMBO J. 21 (2002) 5682–5690.
[4] A.L. Fink, Protein aggregation: folding aggregates, inclusion bodies and amyloid, Fold. Des. 3 (1998) R9–R23.
[5] W.E. Klunk, J.W. Pettegrew, D.J. Abraham, Quantitative evaluation of congo red binding to amyloid-like proteins with a beta-pleated sheet conformation, J. Histochem. Cytochem. 37 (1989) 1273–1281.
[6] D. Carrier, H.H. Mantsch, P.T.T. Wong, Protective effect of lipidic surfaces against pressure-induced conformational changes of poly(L-lysine), Biochemistry 29 (1990) 254–258.
[7] J.S. Chiou, T. Tatara, S. Sawamura, Y. Kaminoh, H. Kamaya, A. Shibata, I. Ueda, The α-helix to β-sheet transition in poly(L-lysine):

effects on anesthetics and high pressure, Biochim. Biophys. Acta 1119 (1992) 211–217.
[8] D. Carrier, H.H. Mantsch, P.T.T. Wong, Pressure-induced reversible changes in secondary structure of poly(L-lysine): an IR spectroscopic study, Biopolymers 29 (1990) 837–844.
[9] J.H. Fuhrhop, M. Krull, G. Büldt, Precipitates with β-pleated sheet structure by mixing aqueous solutions of helical poly(D-lysine) and poly (L-lysine), Angew. Chem., Int. Ed. Engl. 26 (1987) 699–700.
[10] W. Dzwolak, R. Ravindra, C. Nicolini, R. Jansen, R. Winter, The diastereomeric assembly of polylysine is the low-volume pathway for preferential formation of β-sheet aggregates, J. Am. Chem. Soc. 126 (2004) 3762–3768.
[11] W. Dzwolak, T. Muraki, M. Kato, Y. Taniguchi, Chain-length dependence of α-helix to β-sheet transition in polylysine: model of protein aggregation studied by temperature-tuned FT-IR spectroscopy, Biopolymers 73 (2004) 463–469.
[12] M. Jackson, P.I. Haris, D. Chapman, Conformational transitions in poly(L-lysine): studies using Fourier transform infrared spectroscopy, Biochim. Biophys. Acta 998 (1989) 75–79.
[13] I.H. McColl, E.W. Blanch, A.C. Gill, A.G.O. Rhie, M.A. Ritchie, L. Hecht, K. Nielsen, L.D. Barron, A new perspective on beta-sheet structures using vibrational Raman optical activity: from poly(L-lysine) to the prion protein, J. Am. Chem. Soc. 125 (2003) 10019–10026.
[14] M. Fändrich, V. Forge, K. Buder, M. Kittler, C.M. Dobson, S. Diekmann, Myoglobin forms amyloid fibrils by association of unfolded polypeptide segments, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 15463–15468.
[15] F. Franks, Protein destabilization at low temperatures, Adv. Protein Chem. 46 (1995) 105–139.
[16] M.F. Chaplin, A proposal for the structuring of water, Biophys. Chem. 83 (2000) 211–221.