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The Diastereomeric Assembly of Polylysine Is the Low-Volume Pathway for Preferential Formation of β-Sheet Aggregates

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Abstract: The interaction of left- and right-handed polylysine chains (poly(D-lysine) and poly(L-lysine)) results in a dramatic increase in the propensity to form aggregated β-sheet structure (and amyloid-like fibrils), which is reflected by an approximately 15 °C decrease of temperature of the α-helix-to-β-sheet transition. While a relative volume expansion of 13−19 mL·mol^{-1} accompanies the α-to-β-transition in a single enantiomer, this does not hold true for the mixture, which, along with substantially more negative heat capacity changes, points to a lower solvent-entropy cost of the transition as the possible thermodynamic driving force of the diastereomeric aggregation. The underlying solvational mechanism may be one of the decisive factors responsible for the spontaneous protein aggregation in vivo and, as such, may shed new light on the molecular basis of amyloid-associated diseases.

Introduction

The β-pleated sheet precipitates formed upon mixing of poly(L-lysine) (PLL) and poly(D-lysine) (PDL) solutions were first reported in a concise communication by Fuhrhop et al.[1] The relevance of this finding becomes clear only now, when protein aggregation, once associated with the banality of spoiled protein samples, has become a major puzzle in bioscience. The shift of interest toward protein aggregation is caused by the realization that this phenomenon lies behind several neurodegenerative diseases,[2] the BSE crisis,[3] and may have important implications for early stages of cancerogenesis (e.g., ref 4). The present understanding of its mechanisms offers little more than pointing to partial destabilization of a native protein as a prerequisite for its aggregation.[5]

There are several reasons why polylysine is an excellent, yet probably simplest, model for protein aggregation studies. First, it undergoes an α-helix-to-β-sheet transition, the hallmark of protein aggregation.[6] Second, it binds Congo Red, an amyloid-specific organic dye.[7] Third, it forms fibers with an amyloid-like morphology.[8] Finally, the sequenceless character of the polypeptide allows one to explore conveniently the recently voiced hypothesis of the aggregation as a common generic feature of proteins as polymers taking place whenever native protein tertiary contacts are overruled by "polymer-like" main-chain interactions.[8]

There is a wealth of studies on structural transitions in polylysine. At pH (pD) above 11.1, increasing temperature induces the α-to-β-transition and aggregation in PLL,[6] and the same is expected for its enantiomer. The process is reversible only at low concentrations. In acidic or neutral environments, neither helical nor extended structures of polylysine are energetically possible because of repulsive forces between positively charged side-chain residues. On the other hand, compensation of the polylysine's charges through interactions with a negatively charged polyglutamic acid allows both polypeptides to assemble into β-sheet even at pD = 7.[9] Several factors have been implicated to affect the α-to-β-transition in polylysine. Those promoting hydration of the polypeptide (e.g., high hydrostatic pressure[10]) were shown to favor the helical conformation, while the generally dehydrating lipidic bilayers[11] and anesthetics[12] have the opposite effect. The first to report the occurrence of the β-pleated sheet precipitates after mixing PLL and PDL solutions were Fuhrhop and co-workers.[1] Although there are studies addressing stereospecific interactions

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of PLL and PDL with sugars,[13,14] the phenomenon of stereospecific cooperative aggregation of polylysine's enatiomers has not been explored any further. The diastereomeric self-assembly of enantiomers is not unique to polylysine and has been observed for other compounds (e.g., refs 15 and 16). As we show here, for polylysine, this phenomenon is of quite intriguing nature, because as opposed to the interaction of polylysine with polyglutamic acid,[9] it is not driven by simple charge compensation, but an efficient chain/solvent packing in the 3D hydrogen-bonded polypeptide networks. We suggest that a similar explanation may apply to amyloidogenesis of proteins. The driving force of the diastereomeric aggregation appears to be increased water entropy gained by the packing mode of polylysine chains. The fact that the process occurs at a temperature at which single enantiomers form thermodynamically stable helical conformations proves that these structures remain, even at low temperature, in a dynamic equilibrium with aggregation-competent intermediate states.

While circular dichroism (CD) and Fourier transform infrared (FTIR) spectroscopy, as well as differential scanning calorimetry (DSC), are well-established techniques for monitoring structural and solvational transitions in proteins, pressure perturbation calorimetry (PPC) is an efficient method yielding precise data on the apparent volume expansion coefficient (α) of the protein as a function of temperature, which sensitively depends on perturbations in hydration caused by temperature- or denaturant-induced unfolding and aggregation.[17] Through integration of a PPC α vs T plot with temperature, a relative volume change accompanying the transition can be calculated. PPC α vs T plots largely reflect the kosmotropic (mostly hydrophobic) or chaotropic (polar and charged) character or amino acid side-chain residues interacting with the surrounding solvent. A more comprehensive description of the theory, methodology, and applications of PPC was given elsewhere.[17−19]

Materials and Methods

Samples. PLL and PDL of approximately 27 kD molecular weight were purchased from Sigma, USA. Typically, the samples were prepared by dissolving the polypeptide in either H_2O (most of the CD, DSC, and PPC measurements) or D_2O (FTIR) at 1 to 2 wt % (0.05 wt % for CD measurements) concentration. pD (pH) of the samples was adjusted to 11.6 with diluted NaOD (NaOH). To prevent spontaneous heat-induced aggregation, all sample handling was done in an ice-bath.

Fourier Transform Infrared Spectroscopy. For acquisition of FTIR spectra, CaF_2 transmission windows and 0.05-mm Teflon spacers were used. The temperature in the cell was controlled through an external water-circuit connected to a PC-controlled thermostat. All FTIR 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 spectrum was subtracted. All data processing was performed with GRAMS software (ThermoNicolet, USA).

Circular Dichroism. The concentrations of samples of pure PLL and PDL were verified spectrophotometrically at 225 nm. The spectra were recorded on a JASCO J-715 spectropolarimeter equipped with a thermostated cylindrical cell (1-mm path length) from 260 to 190 nm under continuous nitrogen purge. Temperature was increased in a stepwise manner from 10 to 70 °C at a heat rate of 20 °C/h. The CD spectra were expressed as mean residue ellipticity (θ) in deg·cm^2·$dmol^{-1}$.

Atomic Force Microscopy. Fibrillar samples for AFM-imaging were obtained through a prolonged incubation (120 h) of mixed 1.5 wt % solutions of PLL and PDL in H_2O, pH = 11.6, at a temperature below the conformational transition of a single enantiomer (20 °C). Subsequently, the sample was applied onto freshly cleaved mica (muscovite mica from Plano GmbH, Wetzlar, Germany). After drying the samples in air (1−2 h), the data were acquired in tapping mode on a MultiMode AFM microscope equipped with a Nanoscope IIIa Controller from Digital Instruments (Santa Barbara, CA, USA). Commercial silicon cantilevers ("NCHR" from Nanosensors, Neuchatel, Switzerland) with 42 N/m stiffness and 300 kHz resonance frequency were used.

Differential Scanning Calorimetry. Differential scanning calorimetry measurements were carried out on a VP DSC calorimeter from MicroCal (Northampton, MA). The calorimeter's sample cell was filled with ca. 0.5 mL of solution, while the reference cell was filled with a matching buffer. In all measurements, a scan rate of 20 °C/h was applied.

Pressure Perturbation Calorimetry. Pressure perturbation calorimetry measurements were carried out on the VP DSC calorimeter equipped with MicroCal's PPC accessory. An extensive description of the technique is placed elsewhere.[17,19,20] Five bar gas (N_2) pressure jumps were applied to samples during all PPC cycles. Under the same experimental conditions, a set of reference sample−buffer, buffer−buffer, buffer−water, and water−water measurements was carried out each time.[19] In all measurements an effective 20 °C/h scan rate was applied. The value of partial specific volume of polylysine used for the volumetric calculations was 0.71 cm^3 g^{-1}.

Results

Figure 1A presents temperature-induced α-to-β-refolding and aggregation of PDL (top) and PLL (bottom) upon gradual heating from 10 to 70 °C at 20 °C/h scan rate, monitored by far-UV CD. The two minima (maxima for PDL) at 208 and 222 nm in the initial spectra are typical for α-helical structures. The positive values of ellipticity of PDL are expected for any left-handed polyamino acid. The data show that for a pure enantiomer the transition starts above 30 °C and is complete at 50 °C, reflected by the single ellipticity peak at 216 nm. The flat spectrum in the center corresponds to the equimolar racemic mixture of both enantiomers. Figure 1B shows how the conformational transition in polylysine affects the infrared amide I′ band. The characteristic splitting of the band into two components—minor peak ($[\tilde{\nu}(o,\pi)]$) at 1682 cm^{-1} and major peak ($[\tilde{\nu}(\pi,o)]$) at 1612 cm^{-1}—is typical for an antiparallel β-sheet.[6] At 20 °C, the interaction of the mixed basic solutions of PLL and PDL has enhanced a fast conformational α-to-β-transition and, on a time scale of several days, has led to the formation of amyloid-like fibrils, whose AFM images are shown in Figure 2. Judging by the shear thickness, the defect-rich fibrils represent apparently various stages of structural assembly: from protofibrils to mature amyloid-like forms. It should be stressed that, although the time scale of the incubation is similar to that

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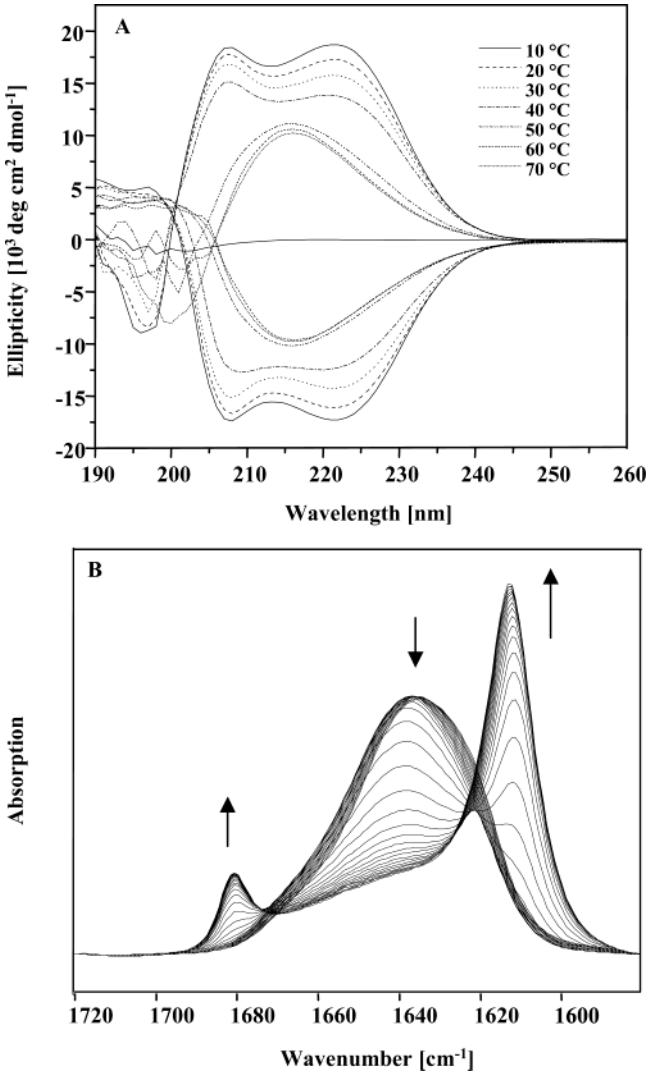

Figure 1. (A) α-to-*β*-transition in PDL, top, and PLL, bottom, upon gradual heating from 10 to 70 °C at 20 °C/h scan rate, monitored by far-UV CD. The flat spectrum in the center corresponds to the equimolar, racemic mixture of both the enantiomers, 0.05 wt % solutions in H_2O, pH = 11.6. (B) α-to-*β*-transition of PDL upon gradual heating from 10 to 60 °C at 20 °C/h scan rate, monitored by FTIR, 2 wt % solutions in D_2O, pD = 11.6. The arrows indicate directions of spectral changes with temperature.

warranting the fibrillization of a single enantiomer, PLL,[8] only mixed enantiomers would form the fibers at 20 °C, namely, below the temperature that triggers the conformational transition in a single enantiomer of polylysine. The inherent lack of optical activity in the equimolarly mixed PDL + PLL samples rules out application of CD for monitoring of conformational transitions in the sample. On the other hand, the α-to-*β*-transition affects the infrared spectra of polylysine essentially in the same manner, whether this occurs in the pure enantiomers or their mixture. Therefore, the infrared data may be used for drawing quantitative plots of the transition degree. This has been done accordingly in Figure 3A. The two curves plotted for the pure enantiomers are very similar. The minute differences between them are certain to reflect minor variations in molecular weight of both samples. The third curve (mixed enantiomers) reveals a dramatic decrease (by roughly 15 °C) of temperature of the α-to-*β*-transition. Apart from this change, and the process becoming irreversible, the FTIR data reveal one more difference. Namely, as shown in ensuing Figure 3B, the position of the

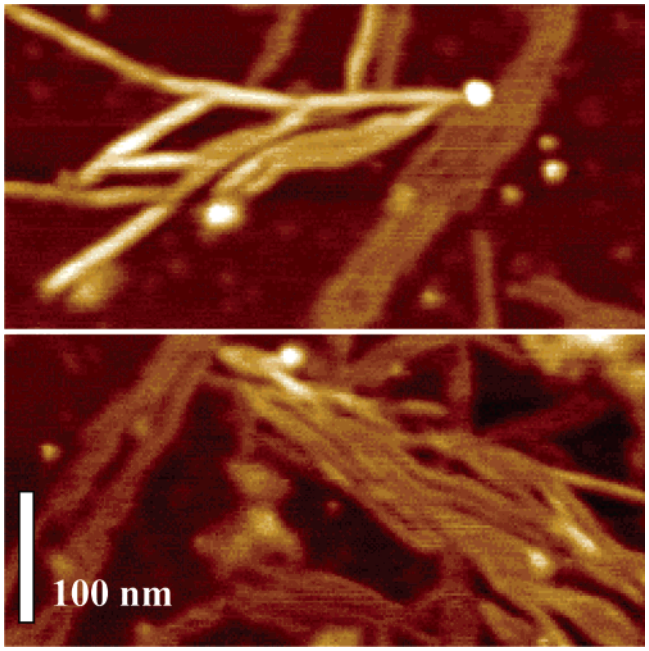

Figure 2. AFM images of fibrils obtained after 120 h incubation of an equimolar mixture of 1.5 wt % solution of PLL and PDL in water, pH = 11.6.

major spectral component [$\tilde{\nu}(\pi,o)$] in the racemic *β*-sheet spectra is blue-shifted by roughly 2 cm^{-1}. This apparently holds true at high temperature, i.e., the once-formed racemic *β*-sheet remains stable even under conditions that could favor competitive L-only and D-only *β*-sheet aggregates. In the case of hydrogen-bonded structures, like this one, a blue-shift of the amide I′ band equals a weakening of the interchain hydrogen bonding.

The fact that for the pure enantiomers the transition is reversible and, under the experimental conditions, thermodynamically controlled has become a good starting point to use the FTIR data presented in Figure 3A to calculate corresponding van't Hoff's ΔH values, ΔH_{vH} (Figure 3C and Table 1). Because of different molecular weight distributions of the samples, the values of ΔH_{vH} and ΔS_{vH}, the corresponding entropy change, must be judged cautiously. Moreover, the values calculated for the racemic samples would not bear any thermodynamic meaning because of the irreversibility and clearly biphasic character (Figure 3) of the transition. The implied endothermic character of the α-to-*β*-transition has been confirmed by DSC scans shown in Figure 4A. The peaks' positions (T_m) correlate roughly with the midpoints of the transition estimated through FTIR, although the separation between the transitions for single and mixed enantiomers becomes 20 °C. The previously expressed caution as to the thermodynamic validity of ΔH and ΔS values applies to the data obtained through DSC (Table 2). Nevertheless, in either case the transition appears to proceed with marked positive ΔH_{calcd} and $\Delta S_{calcd} = \Delta H_{calcd}/T_m$ values. These values agree roughly with the van't Hoff's ΔH_{vH} values calculated from either the FTIR data (Table 1) or DSC data (Table 2), proving a marked degree of cooperativity of the transition. The relative decrease in heat capacity (ΔC_p) deepens when the transition leads to the diastereomeric *β*-sheet.

DSC and CD have also been used to address the issue of preferential stoichiometry of the PDL + PLL assembly. Although for a system of self-assembling enantiomers only the

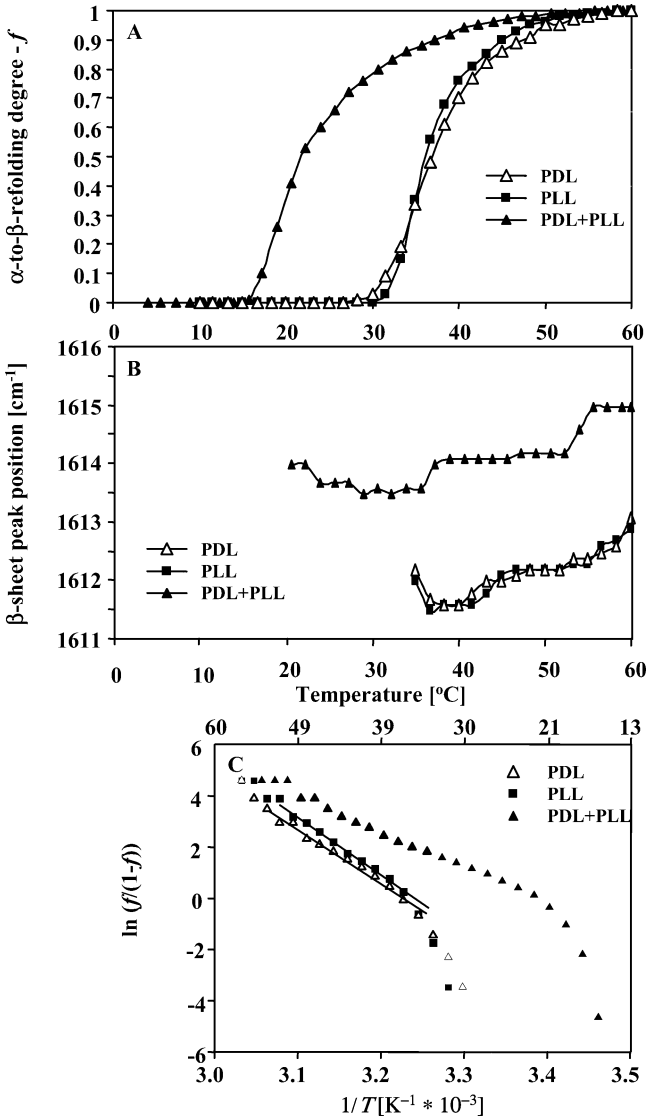

Figure 3. (A) α-to-β-transition in the chiral and racemic polylysine samples monitored by FTIR spectroscopy, 2 wt % solutions in D_2O, pD = 11.6, scan rate 20 °C/h. The degree of transition is approximated by the relative intensity of the β-sheet content at 1612 cm^{-1}. (B) Spectral position of the β-sheet [$\tilde{\nu}(\pi,o)$] component of the amide I′ band. (C) The corresponding van't Hoff plots; *f*, mole fraction of β-sheet structures.

Table 1. ΔH_{vH} and ΔS_{vH} Values Corresponding to the α-to-β-Transition in Polylysine Calculated from the FTIR Data According to the van't Hoff's Equation and the Data Shown in Figures 3A,C[a]

sample	range of temp considered (°C)	ΔH_{vH} (kJ mol^{-1})	ΔS_{vH} (kJ K^{-1} mol^{-1})
PLL	33−37	458 (±32)	1.48 (±0.12)
PDL	30−37	345 (±17)	1.10 (±0.05)

[a] Error bars given in parenthesis reflect only deviations derived from the linear regression calculations.

1:1 stoichiometry is plausible, as any other would, in the end, violate the symmetry of interactions law, a question remains whether the diastereomeric β-sheet is capable of incorporating an excess of one enantiomer. Figure 4B shows that at a relative 50% excess of PLL, the endothermic peak becomes markedly asymmetric. A 100% excess of PLL renders the DSC curve composed of two peaks: one as for an equimolar mixture of PLL + PDL, while the other is typical for a single enantiomer.

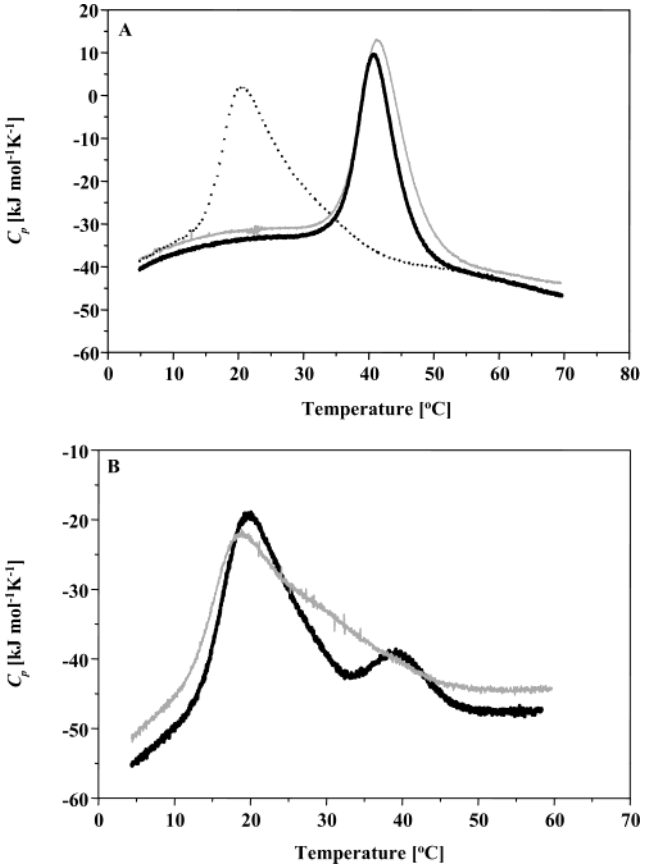

Figure 4. (A) DSC scans of PLL (black solid line) and PDL (thick gray line) and their equimolar mixture (black dashed line). (B) DSC scans of PDL + PLL mixture at the molar ratios 1:2 (black solid line) and 2:3 (thick gray line). The polypeptides were dissolved in H_2O at 2 wt % concentration, pH = 11.6, and the scan rate was 20 °C/h.

Table 2. ΔH_{calcd} and ΔS_{calcd} Values Corresponding to the α-to-β-Transition in Polylysine Calculated According to the DSC Curves and the Data Shown in Figure 4A[a]

sample	T_m (°C)	ΔH_{vH}[b]	ΔH_{calcd}[b]	ΔS_{calcd}[c]	ΔC_p[c]
PLL	41 (±1)	452 (±7)	306 (±7)	0.97 (±0.02)	−4.1 (±0.2)
PDL	40 (±1)	383 (±7)	389 (±7)	1.24 (±0.02)	−5.4 (±0.2)
PLL + PDL	21 (±2)	≥233	≥342	1.16 (±0.02)	−8.1 (±0.8)

[a] Error bars are given in parenthesis. For the mixture, the data are of limited value because of the irreversible nature of the transition. [b] In kJ mol^{-1}. [c] In kJ K^{-1} mol^{-1}.

Figure 5 illustrates analogous experiments with 50 and 100% excesses of the left-handed polylysine monitored by CD. Should a small fraction of the excess enantiomer be trapped in the (L−D) β-sheet, this would act as a conformational label permitting a look into the secondary structure of the otherwise racemic, thus invisible in CD, aggregate. Despite the approximately 20-fold concentration decrease, as compared to the DSC experiment, the course of conformational changes in Figure 5 corresponds closely to the changes seen in Figure 4B. Namely, the excessive PLL undergoes the transition either at a temperature typical for single enantiomer (Figure 5A: the large 100% excess partially "rejected" by the (L−D) β-sheet) or at the much lower temperature of the diastereomeric transition (Figure 5B: the 50% excess incorporated in the (L−D) β-sheet).

The PPC data are shown in Figure 6. The negative slopes of the α vs *T* plots are expected for any (monomer or polymer) chaotropic amino acid, such as PLL, its optical isomer PDL, or

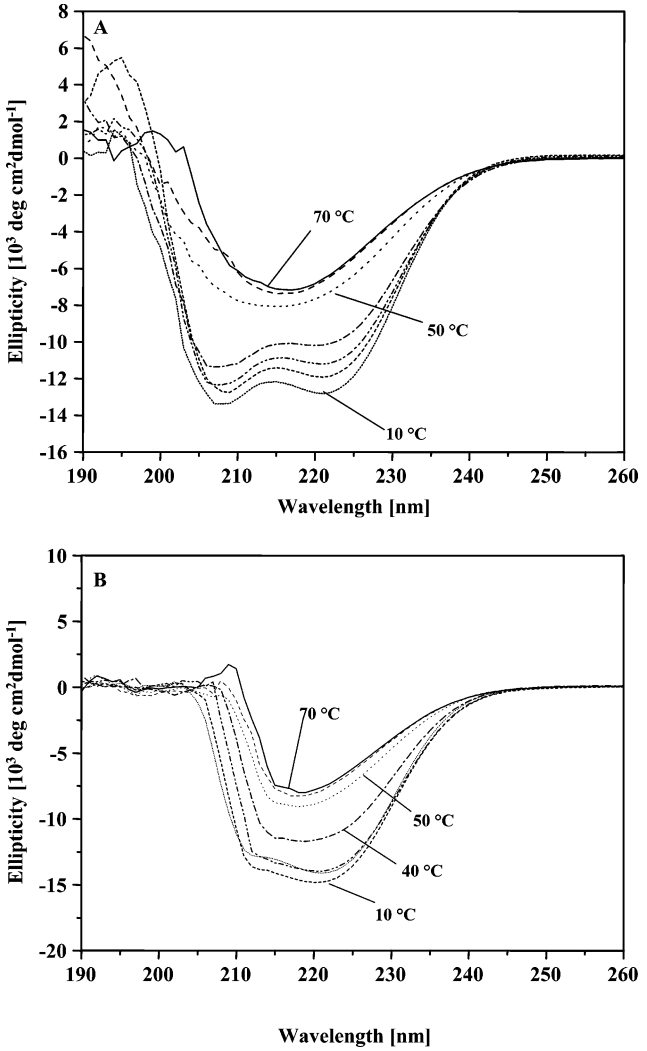

Figure 5. (A) Far-UV CD spectra of 1:2. (B) 2:3 PDL + PLL mixtures at different temperatures. The effective concentration of an optically active excess of PLL is 0.05 wt %, pH = 11.6.

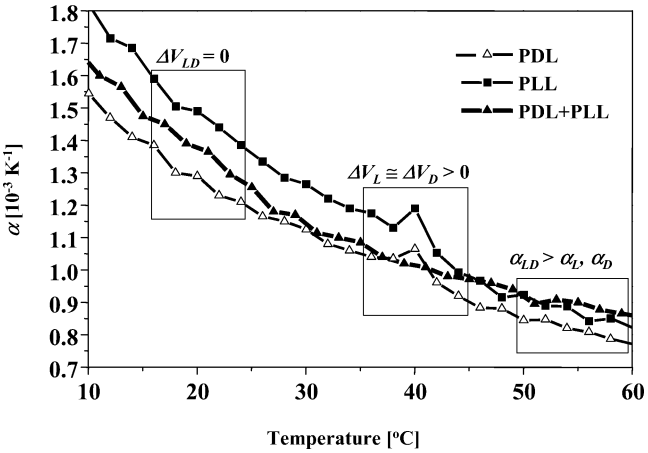

Figure 6. PPC plots of PLL, PDL, and their equimolar mixture. The polypeptide was dissolved at 2 wt % concentration, pH = 11.6, and the effective scan rate was 20 °C/h.

their mixture. The minute differences between PLL and PDL PPC curves are again likely to reflect different molecular weight distributions of the samples. Thus, as expected, in a parallel PPC experiment on pure monomers, D-lysine and L-lysine (where no molecular weight distribution problem exists),

Table 3. Volumetric Effects upon the α-to-β-Transition in Polylysine Calculated According to the PPC Plots and the Data Shown in Figure 6[a]

sample	T_m (°C)	ΔV (mL mol^{-1})
PLL	40 (±2)	19 (±1)
PDL	40 (±2)	13 (±1)
PLL + PDL		∼0

[a] The value of molecular weight of polylysine taken for the DV calculations was 27 200 g·mol^{-1}. The error bars are given in parenthesis.

identical α vs T plots were obtained (data not shown). An important and well-reproducible difference between any of the enantiomers and the mixture consists of the presence of a local peak around the transition temperature only for the pure compounds. Such a feature in a PPC plot signifies a volume expansion of the system. The volume expansion upon the transition for PLL and PDL is estimated to be 19 and 13 mL·mol^{-1}, respectively (Table 3). Given the average molecular weight of the samples (27 kD) and the partial specific molar volume of polylysine (0.71 cm^{3}g^{-1}), these values translate into relative volume changes of 0.1 and 0.07%, respectively. The data show no measurable volumetric effect when the diastereomeric β-sheet is formed, however.

Discussion

Irrespective of the stereochemical composition, the FTIR spectra of polylysine in β-sheet conformation are virtually identical, which implies that the antiparallel fold is maintained upon the diastereomeric self-assembly. The transition in the racemate begins at a temperature at which each of the single enantiomers remains in the stable α-helical conformation (Figure 3A). This proves that, at least above 15 °C, the helical structures of PLL and PDL must be in a dynamic equilibrium with an aggregation-prone intermediate (I): $\alpha \overset{K}{\leftrightarrow} I$. 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.[21] It appears that the transition can be triggered off either reversibly by a temperature-induced change in the equilibrium constant, K, or irreversibly through converting of the aggregation intermediates, I, into the diastereomeric β-sheet until the whole helical content of both the enantiomers has undergone the reaction. In the time scale of all the FTIR, CD, DSC, and PPC measurements carried out in this study, only early β-sheet aggregates are likely to occur, which, only when given enough time for further structural rearrangement, transform into fibrillar structures, such as shown in Figure 2. The fibrils appear to have more defects than those composed of a single enantiomer observed by Fandrich and Dobson.[8] One may speculate that the pronounced irreversibility of the diastereomeric aggregation has some contribution here, as this, on the molecular scale, would be likely to reduce the chains' capability of a topological rearrangement of a once-formed β-strand. The minor nonideality of correlation between the FTIR and DSC data (vide the corresponding intertransition 15 and 20 °C separations), as well as the visible asymmetry of the DSC peaks (Figure 4A) and the sigmoidal plots (Figure 3A), can be explained in terms of slightly different molecular weight distributions in the samples. Namely, these are likely conse-

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quences of subpopulations of chains interacting with different mutual affinity and facing various activation energy barriers on the transition pathway. The 2 cm^{-1} blue-shift reported for the diastereomeric β-sheet (Figure 3B) indicates weaker interchain hydrogen bonds, which are likely to bring about larger separations between the polypeptide chains as compared to the chiral β-sheet. Interestingly, such a "horizontal" expansion of the diastereomeric β-sheet must be compensated profusely by a perpendicular (or "vertical") compression of the multistranded β-sheet layers,[21] since the PPC data show an overall volume decrease (Figure 6, Table 3). The volume expansion observed during the α-to-β-transition in a single enantiomer is a sum of contributions from low-density low-entropy water around the partly hydrophobic, thus water-structuring lysine side chains, and to some extent changing void volumes. We might speculate and mention that the low-density water around hydrophobic residues (if mainly hydrophobic!) consists of more pentagonal ring structures, as described earlier.[22,23]We believe that the latter contribution does not dominate, because the polypeptide, unlike folded proteins, has a flexible structure, permissive to a rapid solvent exchange and penetration. Therefore, the observed small volume expansion most likely stems from the larger amount of structured water trapped between β-sheet strands compared to the helical state. In light of the study by McColl et al., the volume expansion could be related directly to the transient conformational state of the side chains.[21] Under closer scrutiny, the PPC plot in Figure 6 reveals another interesting difference between the structural transitions in mixed and pure enantiomers of polylysine. As has been stated in the Results section, the positive, yet quickly decreasing α vs T dependencies reflect the chaotropic character of lysine and are expected to be identical for both enantiomers. The minor differences seen in the figure in the low-temperature range (Figure 6) may again be attributed to the different distributions of molecular weights in the samples. As expected for a mixture of equal amounts of PLL and PDL, the initial values of α are intermediate with respect to the ingredient enantiomers. However, at temperatures above 45 °C, the apparent thermal expansion coefficient corresponding to the diastereomeric β-sheet is slightly larger than that for the chiral β-sheet aggregates. This effect cannot be explained by anything other than a stronger binding (and hindered release) of water molecules in the diastereomeric β-sheet.

The diastereomeric self-assembly is apparently a more effective way of packing the chains, also in terms of the overall reduction of hydrating water. A number of hydrophobic compounds have been shown to promote extended structures in PLL.[12] These predominantly small and geometrically flexible molecules, such as halothane, are fit to partition into the hydrational layers of PLL and, possibly through sharing common low-density water, reduce the entropic costs of the formation of the extended structure. Although no explicit volumetric data of this effect were put forward, high-pressure FTIR spectroscopy showed a marked stabilization of the polylysine's β-sheet structure against pressure,[11,12] i.e., the volume increase accompanying the α-to-β-transition in polylysine is apparently reduced in the presence of hydrophobic molecules. It seems that in the case of the diastereomeric self-assembly, the particular geometry of the L−D side chains' packing enables effective sharing and reduction of the thermodynamically unfavorable component of polypeptide hydration: the highly structured water. The radically more negative values of the heat capacity change, ΔC_p, upon the formation of the diastereomeric β-sheet support this claim (Table 2). Namely, the data imply that the decrease in the number of water-polypeptide contacts is more substantial when the diastereomeric β-sheet is formed. In other words, the racemic β-sheet aggregate imbibes less water than the single enantiomer β-sheet does.[18] This is in excellent accordance with the 2D-FTIR study by Muller et al., who showed that a red-shift in the amide I′ band of PLL would not only signify strong hydrogen bonding, but strong hydration of the polypeptide backbone as well.[24] In light of this finding, the blue-shift reported here suggests a reduction in the amount of water participating in the hydration of diastereomeric β-sheet. On the other hand, the corresponding retardation of the decrease of α observed above 45 °C in PPC (Figure 6) suggests that the diastereomeric β-sheet traps water more tightly.

The data presented in Figures 4A and 5 show that the diastereomeric self-assembly has, as expected, a 1:1 preferential ratio, but at the same time, the process permits some nonideality, and a small excess of one enantiomer may be trapped in the stoichiometric L−D β-sheet, subsequently following the temperature-induced transition in the manner typical for the "host" structure. This flexibility reminds us of the cooperative character of the α-to-β-transition in PLL itself.[25] The work reported that PLL samples of a broad molecular weight distribution undergo the structural transition at an intermediate temperature compared to the ingredient chains.

The preferential self-assembling interactions of peptides of opposed chirality are neither unique to polylysine[15,16] nor generally expected.[26] The existence of extended flat multistranded β-sheets in pure PLL has been recently implicated.[21] We propose that a similar structure is maintained in the diastereometric β-sheet, though it becomes more horizontally (along the interchain hydrogen bonding) expanded and vertically (perpendicular to the chains) collapsed.

The juxtaposed spectroscopic, calorimetric, and volumetric data convey a picture of the drastic changes in the polypeptide's preferences for the β-sheet fold and the simultaneous aggregation stemming from fine coupling of its own structure and the solvent-structuring properties. The dramatically increased propensity to aggregate is accompanied by a marked decrease in water−polypeptide contacts and the restrained flexibility of the solvating water molecules. Apparently, similarly constrained water molecules (compared to the protein native state) are a distinguished feature of amyloids, which when formed are accompanied by negative heat capacity changes (e.g., refs 19 and 27) and reveal unmatched resistance to heat- (e.g., ref 28) and pressure-denaturation (e.g., ref 29), both of which are enhanced by the presence of flexible water molecules. A number of studies implicated a profound connection between hydrophobic and hydrophilic hydration of a polypeptide and its

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propensity to aggregate (e.g., refs 30 and 31). It thus appears that polypeptide hydration may be one of the key aspects of the protein aggregation phenomenon. A very recent study addressing this problem in a case study on transthyretin (the protein recognized to form in vivo amyloids in onsets of familial polyneuropathies) proved that the disease-associated point mutations simply induce dramatic changes in protein hydration, and the same effect can be evoked by a physical process of pressure-treatment.[32]

In conclusion, we have shown that a change in hydration of polylysine that occurs upon interaction and fine packing of the polypeptide's chains of opposed chirality causes a marked reduction in low-density water solvating the chains' backbone and leads to the spontaneous and irreversible conformational transition. Though the two enantiomers have marginal (under the experimental conditions) propensity to form intermolecular β-sheets, this becomes overruled through their solvent-mediated interactions. In vivo, a similar role may be played by proteins' amino acid side-chain residues, which, while sharing low-density water, may lead the protein, through the aggregation, to a free-energy minimum that is inaccessible to the conformation of a single molecule.

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Note Added after ASAP. In the version posted 3/4/04, poly(D-lysine) and poly(L-lysine) were reversed in the first line of the Abstract, and there was a typographical error in the seventh line of the first paragraph of the Results section. The version posted 3/8/04 and the print version are correct.

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