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Research paper

Alpha-helix to beta-sheet transition in long-chain poly-L-lysine: Formation of alpha-helical fibrils by poly-L-lysine

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ABSTRACT

The temperature-induced α-helix to β-sheet transition in long-chain poly-L-lysine (PLL), accompanied by the *gauche*-to-*trans* isomerization of CH_2 groups in the hydrocarbon side chains of Lys amino acid residues, and formation of β-sheet as well as α-helix fibrillar aggregates of PLL have been studied using Fourier-transform infrared (FT-IR) and vibrational circular dichroism (VCD) spectroscopy, and transmission electron microscopy (TEM). In a low-temperature alkaline water solution or in a methanol-rich water mixture, the secondary structure of PLL is represented by α-helical conformations with unordered and *gauche*-rich hydrocarbon side chains. Under these conditions, PLL molecules aggregate into α-helical fibrils. PLLs dominated by extended antiparallel β-sheet structures with highly ordered *trans*-rich hydrocarbon side chains are formed in a high-temperature range at alkaline pD and aggregate into fibrillar, protofibrillar, and spherical forms. Presented data support the idea that fibrillar aggregation is a varied phenomenon possible in repetitive structural elements with not only a β-sheet-rich conformation, but also an α-helical-rich conformation.

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1. Introduction

The structure of proteins is related to their biological activity. Hence, a determination of the protein structure at different levels of organization is still one of the most important tasks in many research projects. However, the high complexity of a natural protein structure makes it difficult to study. An alternative approach is to use a polypeptide model system. Poly-L-lysine (PLL) is an excellent candidate system for modeling properties of protein structures because PLL can easily adopt all of the most important secondary structures of proteins after a relatively simple manipulation [1,2]. PLL undergoes an α-helix to β-sheet transition, which is a fundamental process for amyloid formation [3–5]. Moreover, the β-sheet structures of PLL aggregate into fibrils, which have an amyloid-like appearance in transmission electron microscopy [6,7]. Amyloid refers to insoluble protein aggregates that are responsible for amyloid diseases, such as Parkinson's, Alzheimer's, and Huntington's [8]. However, the self-assembly of amyloid fibrils offers a promising application in nanotechnology, especially in electronics, photonics, biosensing as well as in enzyme immobilization and biocompatible materials [9,10]. In this context, structural studies of PLL are often related by many researchers to the mechanism of fibrillogenesis and to the application of fibril structures in nanotechnology.

Additionally, PLL itself has been reported to exhibit important and interesting biological effects, including a high efficiency of dissolving preformed βA fibrils in vitro [11], antibacterial activity [12], and PLL can form biodegradable nanoparticles suitable for gene delivery [13]. All of these activities are related to the structure of PLL.

Although numerous research groups have been working on determining a structural transition in PLL modulated by changes in pH, temperature and solvent composition [1,2,4,5,14], these same structural aspects are still to be explained. A combination of Fourier transform infrared (FT-IR) with vibrational circular dichroism (VCD) spectroscopy, and transmission electron microscopy (TEM) are used to study, in detail, a thermal structural transition in long-chain PLL in an alkaline aqueous solution. We observe that a transition of the α-helix to the antiparallel β-sheets of long-chain PLL is accompanied by the disappearance of two additional structural components, which are assigned to disordered forms with turns and to end fragments of the α-helix. To the best of our knowledge, this is the first report showing that the α-to-β transition of PLL is triggered by changes in the ratio of the *gauche* to *trans* conformation in the hydrocarbon side chains of Lys and by alterations in morphology of the protein aggregates. Long (fibril-like) and spherical aggregates are imaged using TEM. Surprisingly, fibrillar

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aggregates, particularly promoted by methanol, are present for PLL rich in α-helices.

2. Materials and methods

Poly-L-lysine hydrobromide of approximately 250 kDa molecular weight, D_2O and NaOD were purchased from Sigma. The polypeptide was dissolved in D_2O (40 mg PLL/1 ml D_2O), and then pD was adjusted to 11.8 with concentrated NaOD using a model FiveEasy pH-meter (Mettler Toledo) with a correction of 0.4 for the pD value. To prevent room temperature-induced formation of β-sheets, the protein samples were prepared in an ice bath.

2.1. FT-IR and VCD measurements

The FT-IR and VCD spectra were recorded on a Nicolet iS-50 spectrometer (Thermo Scientific) extended for VCD measurements and equipped with an MCT liquid nitrogen cooled detector. The external heating system was an F25 Julabo water bath (Julabo, Labrotechnic, GMbH). The FT-IR and VCD spectra of PLL were collected in a heating cycle from 10 to 60 °C, at intervals of 5 and 2.5 °C around the temperature of the α-helix to β-sheet transition. At each temperature, the time for equilibration was 1 h. A CaF_2 cell with a 56-μm Teflon spacer was used. 128 scans were collected for each FT-IR spectrum with a resolution of 2 cm^{-1}, and for each VCD spectrum, 15 000 scans of 8 cm^{-1} resolution were co-added. The FT-IR and VCD spectra of both pure water and water solution of peptide were recorded under the same conditions, then the water spectrum was subtracted from the peptide spectrum. The spectral pretreatment was performed using version 8 of the GRAMS/32 AI software (Galactic Industries Corporation, Thermo Scientific, Poland).

2.2. PCA calculations

PCA calculations were performed separately on both amid I′ and $\nu_{as,s}CH_2$ vibrations regions. Prior to performing PCA, the spectrum of solvent was subtracted from the spectrum of PLL, then a baseline correction was applied with a linear function, and finally a normalization to a constant total area in the analyzed regions and a mean center process were used. Pretreatments processes and PCA were curry out using version 8.0 of the GRAMS/32 AI software (Galactic Industries Corporation, Thermo Scientific) and Matlab R2009a software (The MathWorks Inc., Natick, Massachusetts) with the PLS Toolbox (Eigenvector Research, ICN, Wenatchee.).

2.3. Transition electron microscopy (TEM)

A FEI Tecnai G2 20 X-TWIN transmission electron microscope (Thermo Fisher Scientific) operating at accelerating voltages of 200 kV was used. A 5 μL droplet of a twice diluted solution of PLL was placed on a 400 mesh carbon coated microscope grid. After 1 min, excess fluid was removed. A 2% solution of uranyl acetate dissolved in distilled water was used to negatively stain the PLL samples for 50 s.

3. Results

3.1. Temperature-dependent FT-IR studies of long-chain PLL at pD 11.8

The temperature-induced transition of α-helix to β-sheet antiparallel structures in the 250-kDa heavy PLL was studied using FT-IR spectroscopy. Evolution of a secondary structure of PLL was monitored by the spectral changes in the amide I′ band. The *trans*/*gauche* isomerization in the hydrocarbon chain of a Lys amino acid side chain was tracked by the spectral alterations in a region of antisymmetric and symmetric stretching vibrations of CH_2 groups ($\nu_{as,s}CH_2$).

3.1.1. Amide I′ vibration region of PLL

FT-IR spectra of PLL at pD 11.8 as a function of temperature are presented in Fig. 1. To avoid a strong overlapping of the amide I band of PLL with the δOH band of water molecules, PLL was studied in a D_2O solution. In the low-temperature range, a broad amide I′ band was observed with a maximum centered at 1638 cm^{-1}. A deconvolution procedure showed that this band was composed of three subbands, see Fig. 1B. The main subband with a maximum at 1638 cm^{-1} was derived from an α-helix conformation. The assignment of this band (amide $I'_{(\alpha\text{-helix})}$) was based on the FT-IR

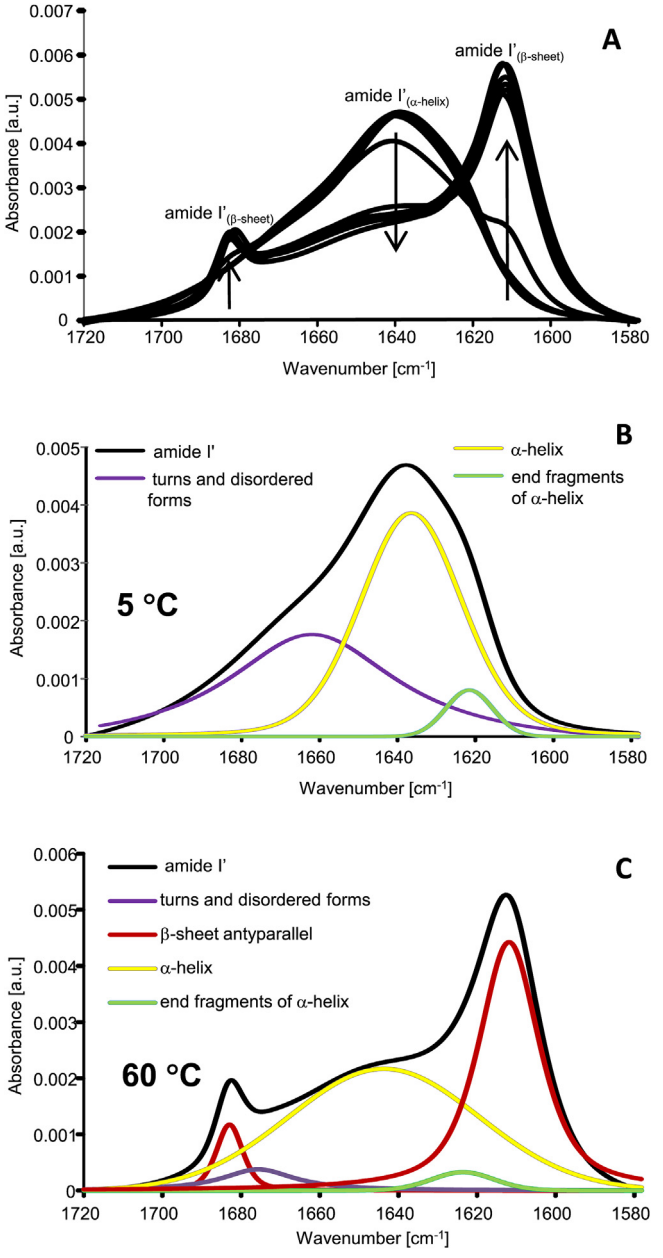

Fig. 1. (A) Evolution of amide I′ bands of long-chain PLL in the water solution of at pD 11.8 as a function of increasing temperature in a range of 5–60 °C. Peak fitting with Gaussian functions of amide I′ bands of PLL at 5 °C (B) and 60 °C (C).

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