



The PPII-to- α -helix transition of poly-L-lysine in methanol/water solvent mixtures accompanied by fibrillar self-aggregation: An influence of fluphenazine molecules

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ABSTRACT

Fourier-transform infrared, vibrational circular dichroism spectroscopy and transmission electron microscopy are used to follow the structural changes of pure and fluphenazine (FPh)-mixed poly-L-lysine (PLL) triggered by variations of the methanol to water ratio in solvent mixtures. FPh molecules are used as an effective psychotropic drug but with a strong Parkinson's-related side effect. To answer the question whether FPh molecules can modify the fibril development, the PLL polypeptide was used as a model of α -helix- and PPII-rich fibrils. It was stated that the presence of FPh molecules did not inhibit the creation of both types of PLL fibrils with clustering features. The methanol-poor aqueous solutions promote the formation of extended polyproline II (PPII) helices; however, the methanol-rich aqueous solutions induce the development of α -helices of both pure and FPh-mixed PLL. Unpredicted and interesting features of PLL fibrillogenesis are evidenced by the formation of uncommon fibrillar aggregates, which are developed in methanol/water solvents from PLL molecules rich in either α -helix or PPII structures. Possibility of PLL molecules to form β -sheet-, α -helix- and PPII-rich fibrils demonstrating that fibrillogenesis is a common phenomenon, and fibrillar aggregates can be based on all of the basic protein secondary structures.

1. Introduction

The structures of poly-L-lysine (PLL) in aqueous solutions have been extensively studied using different spectroscopic methods [1–3], which are highly informative techniques commonly used not only in studies of protein structures but also for many other biomolecules [4–7]. Results presented in the literature [1–3] revealed that the PLL peptide can adopt all of the most important secondary structures of proteins in various external environments. Hence, PLL is frequently used in studies as an excellent model of the structural properties of more complicated naturally occurring proteins. It was shown that PLL in an aqueous solution changes its secondary structure depending on the pH and temperature [1,8,9]. An acidic pH promotes the extended helix structures assigned, in the case of PLL, to polyproline II (PPII) structures [2,3,10] because of the similarity of their Fourier-transform infrared (FTIR) and vibrational circular dichroism (VCD) spectra to the spectra of extended left-handed helices of poly-L-prolines [11]. At alkaline pH, PLL adopts an α -helical conformation at low temperatures, whereas at the high-temperature range, PLL exists in an antiparallel β -sheet conformation [1,9,10]. Additionally, the heating of alkaline aqueous solutions of PLL induces its aggregation to form β -sheet fibrils, which

are structurally similar to amyloid fibrils present in pathological living cells [12,13]. The PLL fibrillogenesis is not based only on the formation of fibrillar aggregates with a common β -sheet-rich structure, but interestingly, the same peptide can also self-aggregate into α -helix-rich fibrils in alkaline aqueous solutions but at a low temperature [1]. Herein, we show that the α -helix-rich fibrils of long-chain PLL are characteristic of methanol/water solvents with not only one and unique methanol to water ratio but also these aggregates are present in a large range of methanol-rich aqueous solutions. An extension of the structural studies of long-chain PLL to methanol-poor aqueous solvents allowed for additional uncommon features of PLL fibrillogenesis to be revealed. The formation of PPII-rich fibrillar aggregates has also been demonstrated in a large range of methanol-poor aqueous solutions. The results presented in this paper confirm the high complexity of the process of fibrillogenesis and promote PLL peptides as excellent candidates to model different aspects of fibrillogenesis of naturally occurring amyloid peptides.

The PPII-to- α -helix transition of pure and fluphenazine (FPh)-mixed PLL in methanol/water solvent mixtures was studied using FTIR and VCD spectroscopy. The fibrillar self-aggregation of PPII and α -helix structures of pure and FPh-mixed PLL molecules was investigated using

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transmission electron microscopy (TEM). The secondary structures were defined on the basis of the amide I' band and the VCD signals in the range of amide I' vibrations. The influence of FPh molecules on the secondary structures and self-aggregation process of long-chain PLL has been determined to discuss the pro-fibrillar activities of FPh. FPh molecules have been recognized by the World Health Organization (WHO) as an indispensable drug for the treatment of psychotic disorders [14]. However, its clinical use is limited by a side effect manifested as, for example, drug-induced Parkinson's disease [15]. Parkinson's disease, as a neurodegenerative illness, is accompanied by the formation of amyloid fibrils present in the living cells affected by the disease. Hence, the mechanism of the Parkinson's-related side effects of FPh molecules can also be associated with the modifications of fibril development by FPh. An explanation of the molecular mechanism of the non-desired side effects of the FPh drug will facilitate findings of a new strategy for the chemical modification of the FPh structure to decrease the harmful effects.

2. Experimental methods

2.1. Materials

The long-chain poly-L-lysine hydrobromide (PLL, ~250 kDa), D₂O and deuterated methanol (CD₃OD) were purchased from Sigma Aldrich (Seelze, Germany) and used without further purification. Fluphenazine hydrochloride (FPh) was obtained from Jelfa S.A. (Jelenia Góra, Poland). The final concentration of PLL was 50 mg/mL and for FPh was 2.5 mg/mL in the methanol/water solvents. The methanol content in aqueous solutions was in the range of 40 to 95% (v/v). An appropriate amount of the stock solution of FPh in DMSO (163 mg/mL DMSO) was used to treat the previously prepared samples of pure PLL in the methanol/water solvents.

2.2. FTIR, VCD and TEM measurements

The sampling techniques as well as the parameters of instruments and the equipment have been described in detail elsewhere [1]. All samples were measured at a room temperature. The FTIR and VCD spectra of the methanol/water solvents were respectively subtracted from the FTIR and VCD spectra of pure or FPh-mixed PLL in a solvent with the same chemical composition. The principal component analyses (PCAs) were performed on the amide I' vibrations region of the FTIR spectra of pure and FPh-mixed PLL recorded in solvents with a different methanol to water ratio. The details of the PCA procedures, including the manipulation of FTIR data needed before PCA calculations, have been described elsewhere [1].

3. Results

3.1. The PPII-to- α -helix transition of PLL in methanol/water solvent mixtures

The influence of the changes in the methanol to water content ratio of the solvent mixtures on the secondary structure of the long-chain PLL was studied at room temperature using FTIR and VCD spectroscopy. Fig. 1 presents the evolution of the amide I' vibrations band and the VCD spectra in the range of amide I' vibrations of PLL dissolved in water (D₂O) mixed with an increasing amount of deuterated methanol (from 40 to 95% (v/v)). In methanol-rich solvent mixtures, the long-chain PLL under study adopted an α -helical conformation, the presence of which was explicitly proved by both FTIR and VCD spectra, characteristic of the α -helix structure, which has already been presented in the literature [1–3]. An increase in the intensity of the amide I' (α -helix) band, with a maximum centered at 1642 cm⁻¹, together with a simultaneous increase in the intensity of the VCD spectrum, represented by three peaks at 1660, 1649, and 1638 cm⁻¹ with a sign pattern of “–

+ –”, were triggered by an increase in the number of α -helix conformations in the structure of PLL dissolved in methanol-rich solvents, see Fig. 1. However, a decrease in the methanol concentration in the mixture with water was accompanied by a high-frequency shift of the maximum of the amide I' band to 1655 cm⁻¹, see Fig. 1A. This amide I' band change was also accompanied by distinct alterations in the spectral parameters of the VCD signal as a result of the solvent-dependent structural variations in the PLL under study. In methanol-poor solvent mixtures, the VCD spectrum was represented by two bands: the positive band with a maximum at 1668 cm⁻¹ and the negative band at 1639 cm⁻¹, see Fig. 1B. These two VCD bands with opposite signs are characteristic of extended left-handed helices, which have been observed in many different proteins and peptides, and are termed as the PPII structure [10,11,16].

The methanol-induced transition of PPII to α -helix structures of the long-chain PLL under study was further (deeply) characterized using PCA calculations in an analysis of the PLL spectra in the range of the amide I' band, which were measured as a function of the increasing methanol content in the water/methanol solvent mixtures. The obtained PCA model emerged the main process, assigned to the first principal component (PC1), and driven by the changes in the methanol to water content ratio of the solvent mixtures, see Fig. 2. The character of the evolution of the dominant solvent-dependent structural variations of PLL was depicted in Fig. 2A by the sigmoidal plot of the scores on PC1 as a function of increasing methanol content. In the 70 to 85% range of the methanol concentration, the secondary structure of PLL was drastically changed. The nature of the structural reorientations during the PC1 process was described by the plot of the loadings on PC1 as a function of the wavenumbers (see Fig. 2B), which shows how the signals are related to each other during the PC1 process. For the methanol-poor solvents, the biggest changes in the amide I' band took place in the lower-frequency range with a maximum at 1665 cm⁻¹ on the positive scale of the loadings on PC1, which is characteristic of PPII structures. However, in the methanol-rich solvents, two negative maxima of the loadings were centered at 1641 and 1627 cm⁻¹, see Fig. 2B. This high-frequency loading band was attributed to changes in the population of the α -helical conformation of PLL, and the low-frequency band originated from the corresponding alterations in the end fragments of the α -helix. It has already been reported that the amide I' band with a maximum at 1627–1620 cm⁻¹ is also present in a structure of PLL during the temperature-induced α -to- β transition of PLL in an alkaline pure aqueous solution [1,9,10]. The methanol-induced PPII-to- α -helix transition of PLL comprised almost all (98.5%) of the variations in the total absorption present in the amide I' region of PLL, and the rest of the absorption changes were attributed to the noise in the spectra.

3.2. Fibrillar self-aggregation of PPII and α -helix structures of PLL

The effect of the variations in methanol concentration in the range of 40 to 95% (v/v) on the self-aggregation process of PLL molecules with PPII or α -helical secondary structures was studied using the TEM technique, see Fig. 3. α -Helix-rich PLL molecules, induced by methanol, self-assembled into long fibrillar aggregates with a substructure represented by laterally aggregated thread-like subunits, see Fig. 3A and B. A decrease in the methanol content did not inhibit the formation of such fibrillar aggregates, unusually formed by α -helical PLL molecules. It is interesting that PLL dominated by PPII structures, whose formation was promoted by a decrease in the methanol concentration and started from a methanol concentration of 70% (see Fig. 2A), were also self-associated into long fibrillar aggregates, see Fig. 3C and D. Even without the presence of methanol molecules, that is, in a pure water solvent, PLL molecules with the PPII structure still self-aggregated into fibrillar forms, see Fig. 3E and F. The PPII-rich fibrillar aggregates were structurally very similar to α -helix-rich fibrillar aggregates of the long-chain PLL. Both types of fibrils had the same substructure, described as

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