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Pressure titration of the monomeric variant of transthyretin

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Abstract Transthyretin (TTR) is an indispensable transporter protein of thyroxine and a retinol molecule in humans. TTR has a stable homo-tetrameric structure in its native state, while upon dissociation into monomers, it becomes aggregation-prone and can form an amyloid fibril. Although the amyloidogenic propensity of TTR has been known and investigated since the late 1990s, the structural information regarding TTR's amyloidogenic species is still elusive. Here, we employed high-pressure nuclear magnetic resonance (HP-NMR) approaches on the monomeric variant of TTR (TTR[F87M/L110M]; M-TTR) and observed that it experiences a two-step transition in response to the pressurized condition. Our study demonstrated that M-TTR in an ambient condition has heterogeneous structural features, which is likely related to the amyloidogenic propensity of TTR.

Keywords transthyretin, transthyretin amyloidosis, NMR spectroscopy, pressure titration

Introduction

Transthyretin amyloidosis (ATTR) is a pathogenic process caused by the aggregation of transthyretin (TTR).[1] TTR is abundantly present in human plasma and cerebrospinal fluid, working as an essential transporter protein for thyroxine (T_4) and retinol molecules. In its native state, this protein has a stable tetrameric quaternary structure, in which two hydrophobic binding pockets for T_4 are constructed.[2] However, it was shown that destabilization of its native tetrameric state and subsequent manifestation of monomeric species, which is facilitated by several factors, such as low pH, increased temperature, proteolysis, and genetic mutation, can induce the formation of amyloid fibrils.[3] Notably, a recent cryo-electron microscopic study indicated that TTR may experience significant structural rearrangement to form amyloid fibrils.[4,5] In addition, NMR-based structural characterization of TTR's monomeric variant (M-TTR) concluded that the monomeric state of TTR accommodates more increased mobility than the tetrameric state.[6]

To advance our understanding of the amyloidogenic state of TTR, we conducted NMR-based pressure titration experiments for M-TTR. HP-NMR is an appropriate approach to populate intermediate structural states by inducing a mildly denaturing condition.[7] Moreover, the pressurized condition was previously used to investigate aggregation mechanisms of α-synuclein and TTR.[8] In our analysis, we observed that M-TTR stabilizes more homogeneous structural states at 500 bar than those at ambient pressure, while further pressurization induces the global unfolding of the protein.

Experimental procedures

The uniformly ^{15}N-labeled ([U-^{15}N]) M-TTR protein

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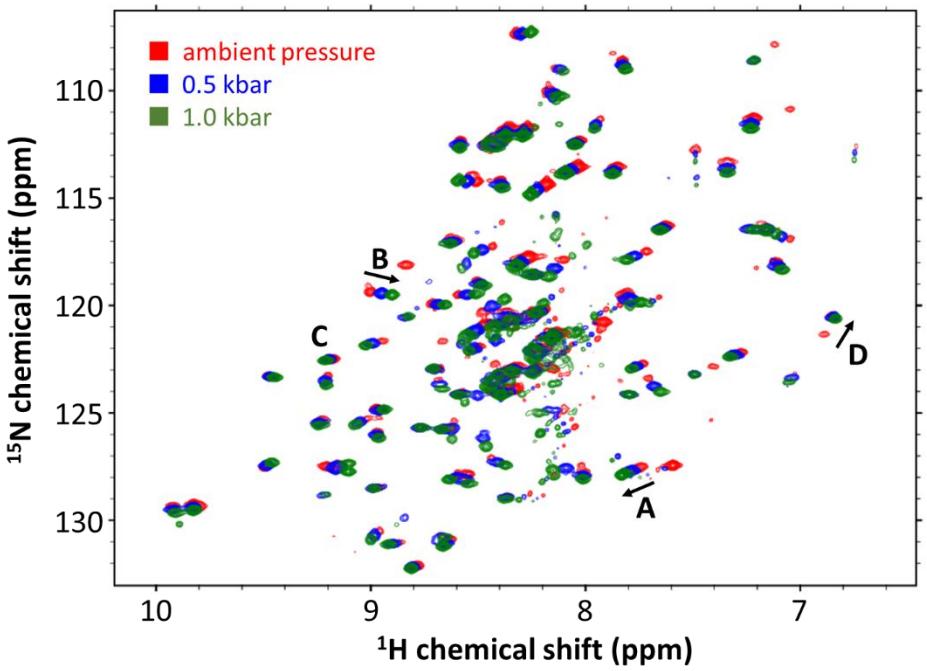

Figure 1. The ^{1}H-^{15}N HSQC NMR spectra of [U-^{15}N]-M-TTR obtained under differently pressurized conditions. Three spectra are overlaid in the figure: red, ambient pressure; blue, 0.5 kbar; green, 1 kbar. Note that some signals (e.g., the signal marked as A) showed downfield shifts in their ^{1}H chemical shift, while there were other signals (e.g., the signal marked as B) exhibiting upfield shifts during pressure titration. On the other hand, there were other signals with minimal shifts (e.g., the signal marked as C), or a set of signals (e.g., the signal marked as D) showing a stepwise movement.

sample was prepared with the protocol described elsewhere.[6,9] The NMR experiments was conducted with a 700 MHz NMR spectrometer (Bruker) equipped with a cryogenic HCN probe. The pressurized condition (0.5 and 1 kbar) was made with a commercial pressure instrument (Daedalus Innovations LLC, PA), at which 2D ^{1}H-^{15}N HSQC spectra were collected. The ^{1}H-^{15}N signal assignment information for M-TTR at 500 bar was obtained from the BMRB data (accession number 25986),[6] and the signal assignment results of wild-type TTR were referred as well.[10] Subsequent assignments for signals obtained at ambient pressure and 1 kbar were inferred from the signal shifts during pressure titration, some of which were confirmed with 3D HNCA experiments. Signal perturbation by pressure application was plotted, either by subtraction of the ^{1}H or ^{15}N chemical shift of the signals at ambient pressure from those of the signals at 0.5 or 1 kbar (for $\Delta\delta_H$ or $\Delta\delta_N$, respectively), or according to the following equation: $\Delta\delta_{NH}=[(\Delta\delta_N/5)^2+(\Delta\delta_H)^2]^{1/2}$. Topspin 3 (Bruker) was used for data collection and processing, and POKY software package was used for data analysis.[11]

Results and discussion

To monitor the structural perturbation of M-TTR in a pressurized condition, we collected 2D ^{1}H-^{15}N HSQC spectra of [U-^{15}N]-M-TTR at three different pressures: ambient pressure, 0.5 kbar, and 1 kbar (Fig. 1). As expected, many M-TTR signals exhibited significant perturbations, indicating overall structural changes upon pressurization. However, it was also notable that different perturbation patterns were observed during pressure titration. For example, some signals showed consistent increase (downfield shift) in their ^{1}H chemical shift (Fig. 1A and Fig. 2), which mostly corresponds to the disordered region of M-TTR (such as N-terminal and C-terminal tails). On the other hand, some signals, such as the residues M13, V20, N27, E42, A45, E51, K70, and V93, exhibited consistent

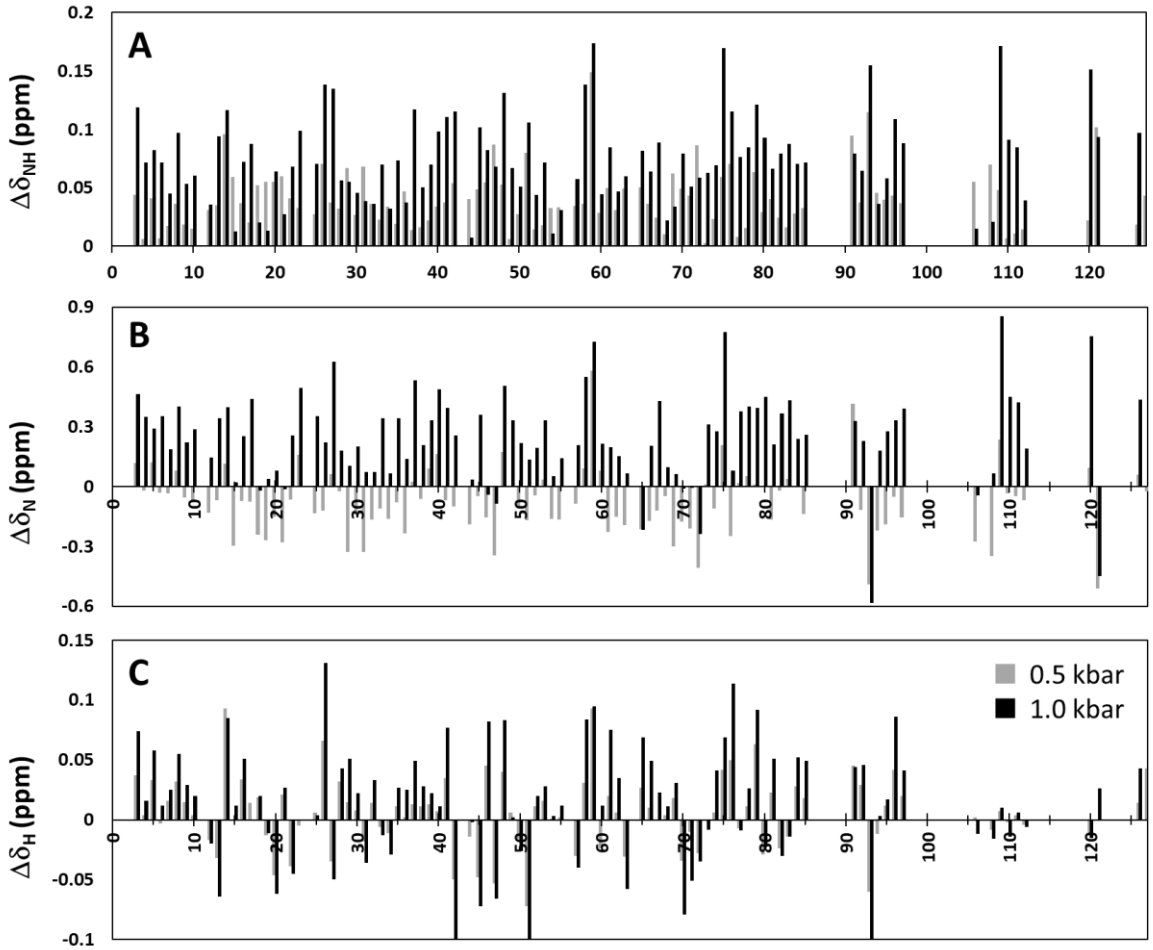

Figure 2. The NMR signal perturbations of M-TTR upon changing the pressure from ambient to 0.5 or 1 kbar. The ^{1}H-^{15}N signal perturbations were shown as a combined value ($\Delta\delta_{NH}$; A), or as a subtracted value ($\Delta\delta_N$ and $\Delta\delta_H$; B and C, respectively). See 'Experimental Procedures' for details.

decrease (upfield shift) in their ^{1}H chemical shift (Fig. 1B and Fig. 2). There were also some other signals, such as L12, V32, R34, F44, L55, I68, and V94, showed much less perturbations (less than the value obtained from the average minus one standard deviation) during pressure titrations (Fig. 1C and Fig. 2). Finally, there were also a set of signals, which showed considerable perturbations at 0.5 kbar whereas the increase to 1 kbar caused only minimal additional signal perturbations (Fig. 1D and Fig. 2; e.g., the signals for K36, G47, E63, V71, A91, and V121) or even reverse shifts closer to its original signals at ambient pressure (e.g., the signals for K15, D18, A19, R21, H31, Y69, E72, T106, and A108). Moreover, it is notable that the ^{1}H-^{15}N HSQC spectrum at 1 kbar showed the significant number of signals at the central region of the spectrum (8~8.4 ppm in the ^{1}H chemical shift axis; Fig. 1), indicating manifestation of the unfolded state of M-TTR at this condition. Indeed, further increase of pressure over 1 kbar resulted in signal increase at the central region of the spectrum with concomitant decrease of other well-dispersed signals (data not shown).

These results indicate that M-TTR experiences two-step structural transition by pressure. The first transition is evidenced by the residues whose signals changed significantly only at the first pressure change from ambient condition to 0.5 kbar; in these residues,

subsequent pressure increase from 0.5 to 1 kbar caused minimal or inconsistent shifts with the first pressure changes. Notably, previous NMR-based structural elucidation of M-TTR was also done under the pressure of 0.5 kbar, suggesting the presence of other structural states of M-TTR at ambient condition.[6] This is also consistent with several distinctive signal perturbation patterns observed in the pressure titration (Fig. 1). On the other hand, the second structural transition was evidenced by overall increase of the signals at the central regions, which seemingly corresponds to stabilization of the unfolded state of M-TTR.

Taken together, the present work demonstrated that M-TTR has heterogeneous and dynamic structural states at ambient condition, which may be related with its highly amyloidogenic propensity. The consistent observation was made from several previous studies, indicating that the mobile structural state of M-TTR is indeed related with its amyloidogenesis.[12–14] We expect that this study may contribute to reveal the structural heterogeneity and dynamics of TTR and to appreciate the molecular mechanisms of TTR aggregation and fibrillization.

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