Journal of the Korean Magnetic Resonance Society **2022**, *26*, 17-20
DOI 10.6564/JKMRS.2022.26.1.017

High-pressure NMR application for amyloid-beta peptides

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Received Mar 19, 2022; Revised Mar 20, 2022; Accepted Mar 20, 2022

Abstract High-pressure (HP) NMR is a versatile tool to investigate diverse features of proteins. This technique has been particularly powerful to elucidate structural dynamics that only populates sufficiently in a pressurized condition. Amyloidogenic proteins, which are prone to aggregate and form amyloid fibrils, often maintains highly dynamic states in its native or aggregation-prone states, and HP NMR contributed much to advance our understandings of the dynamic behaviors of amyloidogenic proteins and the molecular mechanisms of their aggregation. In this mini review, we therefore summarize recent HP NMR studies on amyloid-beta (Aβ), the representative amyloidogenic intrinsically disordered protein (IDP).

Keywords high-pressure NMR, amyloid-beta, protein aggregation, protein dynamics, NMR spectroscopy

Introduction

Proteins often exhibit dynamic structural features. For example, there is a class of proteins, often called metamorphic proteins, which manifest multiple conformations interconverting in a physiological condition.[1,2] In addition, it is now known that many human proteins do not have a well-defined secondary or tertiary structure in their entire chain or at its certain regions, hence referred to respectively as intrinsically disordered proteins (IDPs) or proteins containing intrinsically disordered regions (IDRs).[3,4] And, it is not surprising to find that these highly dynamic properties heavily correlate with their physiological and pathological characteristics. A number of studies were conducted to characterize the structural dynamicity of proteins and appreciate the underlying molecular mechanisms, yet our understanding to this is still shallow. There are several challenges to investigate the dynamic features of proteins, such as conformational heterogeneity and instability of certain conformational states, which often results in a low population in its native state.

Among several strategies to overcome these challenges, high-pressure (HP) based approaches were proven effective.[5] Most proteins have lower molar volume in their unfolded states than that of folded states because of the presence of cavities in folded proteins.[6] Therefore, pressure application can efficiently perturb energetic states of native conformational states, which often results in manifestation of lowly populating states. In practice, a pressurized condition is considered mildly denaturing for most proteins; local structural changes, reflecting energetic perturbation and subsequent stabilization of alternative conformations, are first observed in a gently pressurized protein, while a further increase of pressure can cause partial or full denaturation of proteins.[7] Although HP application can be accompanied by various spectroscopic techniques, NMR spectroscopy has posed unique advantages, because it can monitor structural and mobile features of proteins in an atom-specific fashion.[5,7,8] Recent

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development of the commercially available instruments for HP NMR boosts up its application for a wider range of proteins.[9] In addition, novel applications using a synchronized pressure application with NMR pulses have paved the way to characterize pressure-induced protein folding or denaturation events in atomistic details.[10,11]

In this mini-review, we discuss a few recent application examples of HP NMR to characterize structural characteristics of an amyloidogenic protein. Amyloidogenic propensities often correlate with native or non-native dynamic features of proteins,[12] yet their structural elucidation is elusive due to the aggregation-prone and structurally heterogeneous nature. Here, we focus on discussing the application examples of HP NMR on amyloid-beta (Aβ). Aβ is famous for its close relatedness with pathological processes of Alzheimer's disease.[12] In addition, this protein exhibits highly dynamic structural features, thus being classified as an IDP, while it can also aggregate to form amyloid fibrils in a physiological condition. Therefore, HP NMR is a unique and appropriate tool to investigate the dynamic features of Aβ in its aggregation pathway.[13]

HP NMR with Aβ monomers

The effects of HP application on IDPs are usually less significant than those on folded proteins, because molar volumes of IDPs, even in their native states, are comparable with those in the unfolded states. However, if a IDP has a tendency to form a partial/transient secondary or tertiary structure, careful examination on pressure-induced chemical shift changes of NMR signals can provide residue-specific information regarding its residual structures.

Notable examples of this are a series of HP NMR studies on Aβ peptides. Aβ peptides are one of the representative IDPs, and are famous for its highly efficient aggregation and amyloid-forming propensity. Although it was reported that Aβ maintains highly dynamic state in its monomeric state, several HP NMR-based investigations also proved the presence of residual folded conformations. Munte et al. applied an HP NMR method to investigate the native structural states of Aβ.[14] By carefully monitoring ^{1}H-^{15}N HSQC signals of Aβ(1-40) in the pressure and temperature titration experiments and analyzing the resultant signal movements, they concluded that Aβ(1-40) may have two conformational states: one highly dynamic state and the other relatively compact state. Consistently, Rosenman et al. reported that a couple of Aβ(1-40) variants, whose amyloidogenic propensities are higher than wild-type, exhibited more sensitive NMR signal perturbations upon pressure application in the stretch of the residues Q15-L17.[15] Previous studies showed that the region encompassing these residues is important for Aβ aggregation.[16] This observation again indicates that Aβ maintains highly dynamic, yet still residual folded conformations, e.g., β-hairpin structures, which was indeed reported from a prior simulation study.[17] More recently, Vemulapalli et al. employed HP NMR and identified salt bridges at the N-terminal region of Aβ peptides.[18] In this study, the N-terminal residues, such as E3, R5-S8, E11-E22, showed pressure-sensitive signal movements, and subsequent analyses indicated that these regions also experienced changes in their secondary structures. They also performed a ^{13}C-detected multi-quantum chemical exchange saturation transfer experiment along with molecular dynamics simulations to propose that R5 and a few nearby residues are engaged in salt bridge formations.

HP NMR with Aβ oligomers

On the other hand, there were a couple of successful studies of characterizing oligomerization of Aβ with HP NMR. Cavini et al. was able to characterize de-polymerization and re-polymerization processes of Aβ oligomers in pressurized and de-pressurized conditions, respectively.[19] After applying a rapid pressure jump, they monitored signals of monomeric Aβ, whose intensity was either decreased due to polymerization or increased by de-polymerization. Eventually, acquisition of a series of NMR data in various temperature conditions enables to estimate the thermodynamic parameters of Aβ oligomerization.[20]

Moreover, Barnes et al. developed a novel pressure-jump NMR method, with which structural features of Aβ oligomers were investigated.[21] In this work, the authors first confirmed that Aβ forms oligomers within a few seconds at a high concentration (roughly 1.3 mM), while it goes back to monomeric state upon pressure application. Subsequently, they synchronized

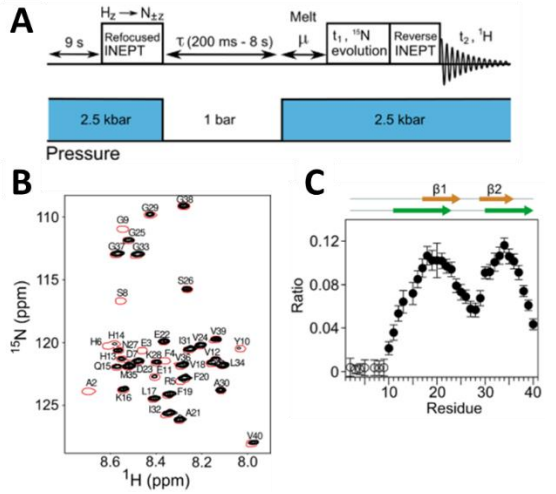

Figure 1. The pressure-jump experiment to investigate the oligomeric states of Aβ(1-40).[21] (A) The NMR pulse program that is synchronized with pressure application. Note that the low pressure delay (τ, 0.2-8 s) is applied to make Aβ(1-40) peptide form oligomers. Upon high pressure application (2.5 kbar), Aβ(1-40) restores the monomeric state. (B) The ^{1}H-^{15}N HSQC spectra of τ = 0.2 s (red) and τ = 5.5 s (black) were compared. Due to the long ^{15}N T_1 relaxation times of oligomeric states, the black signals survived even after long τ, while the signals for A2-G9 broadened because of their dynamic feature even in the oligomeric state. (C) The signal intensity ratio between the spectra of τ = 0.2 s and τ = 5.5 s. It is evident that the signals for the residues 18-21 and 31-34 were maintained better than the other signals, indicating their relative rigidity in the oligomeric state. Modified from [21] with permission by ACS publications.

NMR pulses with pressure application (Fig. 1A), so that the NMR data can be acquired right after monomerization of Aβ oligomers. This procedure is necessary because the signals of oligomeric Aβ is invisible in a regular NMR spectrum, yet the structural features of oligomeric Aβ are still capable of affecting on the signals of monomeric Aβ due to the long ^{15}N T_1 relaxation times of oligomeric species. Indeed, in contrast to the ^{1}H-^{15}N HSQC spectrum acquired after an incubation time of 0.2 s at unpressurized condition, the spectrum with an incubation time of 5.5 s at unpressurized condition showed broadening of a few signals corresponding to the residues A2-G9. This observation indicates that the N-terminal stretch of Aβ remains disordered even after oligomerization. In addition, the researchers identified that ^{15}N T_1 relaxation times for the residues 18-21 and 31-34 are significantly increased, implying their orderedness in the oligomeric state.

Conclusions

The dynamic features of amyloidogenic proteins are an important aspect not only to appreciate their aggregation pathways, but also to devise therapeutic strategies to prevent or alleviate the related pathological mechanisms. However, their detailed elucidation has been challenging because these proteins adopt highly dynamic states and easily aggregate in the amyloid-forming condition. HP NMR is a useful alternative strategy to overcome these difficulties; pressure application maintains a mildly denaturing condition, where aggregation is suppressed, yet residual structural features for amyloidogenesis remains, allowing structural elucidation with NMR spectroscopy. HP NMR application for Aβ has been indeed successful from the characterization of heterogeneous conformational states in its monomeric state to the acquisition of partial, yet exceptional information regarding the oligomeric states. The outstanding advantages of HP NMR has been also evidenced in applications for other IDPs (e.g., α-synuclein[22]) and globular proteins (e.g., transthyretin[23,24]), proving the wide applicability and still-unexplored potential.

Acknowledgements

This research was supported by the National Research Foundation (NRF) funded by the Ministry of Science & ICT (NRF-2020R1I1A2074335).

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