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High-pressure NMR application for α-synuclein

Jin Hae Kim*

Department of New Biology, Daegu Gyeongbuk Institute of Science and Technology (DGIST), Daegu 42988, Republic of Korea

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Abstract High-pressure (HP) NMR is a powerful method to elucidate various structural features of amyloidogenic proteins. Following the previous mini-review recapitulating the HP-NMR application for amyloid-β peptides of the last issue [J. H. Kim, *J. Kor. Mag. Reson. Soc.* **26**, 17 (2022)], the recent advancements in the HP NMR application for α-synuclein (α-Syn) are briefly summarized and discussed here. Although α-Syn is a well-known intrinsically disordered protein (IDP), several studies have shown that it can also exhibit heterogeneous yet partially folded conformations, which may correlate with its amyloid-forming propensity. HP NMR has been a valuable tool for investigating the dynamic and transient structural features of α-Syn and has provided unique insights to appreciate its aggregation-prone characters.

Keywords high-pressure NMR, α-Synuclein, amyloid, protein aggregation, NMR spectroscopy

Introduction

α-Synuclein (α-Syn) is a 140-residue intrinsically disordered protein (IDP),[1] indicating that this protein does not exhibit any well-defined secondary or tertiary structural feature. α-Syn is famous for its amyloid-forming (amyloidogenic) propensity and the resultant pathological features, such as α-synucleinopathy.[2] In particular, it was found that α-Syn is one of the major constituents of the Lewy body, the intracellular inclusion body found in Parkinson's disease patients.[3] This observation was followed by numerous studies to understand its various features, such as physiological functions,[4,5] pathological characters,[6] and related structural states.[1,7,8] Among these trials, biophysical methodologies focusing on the structural features of α-Syn in its aggregation pathway have been particularly powerful in revealing mechanistic details of its physiological and pathological processes. For example, a fiber diffraction study revealed the cross-β structures of α-Syn amyloid fibrils.[9] Solution NMR spectroscopy contributed to appreciating structural dynamics of α-Syn monomers,[7,8,10] and solid-state NMR provided information on α-Syn fibrils.[11,12] Recently, a series of cryo-electron microscopic studies successfully determined the atomic-resolution structural models of α-Syn amyloid fibrils and identified its polymorphic nature.[13–15]

However, despite all of these innovative studies, the detailed mechanisms of α-Syn aggregation and amyloid formation is still elusive, mainly due to the absence of structural details on the aggregation-prone intermediates. Among several advanced techniques to tackle this issue, high-pressure (HP) NMR has been proven helpful because the pressure application is rather a gentle way to denature a native fold while accessing a physiologically relevant conformation that other conditions cannot easily stabilize.

* Address correspondence to: **Jin Hae Kim,** Department of New Biology, Daegu Gyeongbuk Institute of Science & Technology (DGIST), Daegu 42988, Republic of Korea, Tel: 82-53-785-1770; E-mail: jinhaekim@dgist.ac.kr

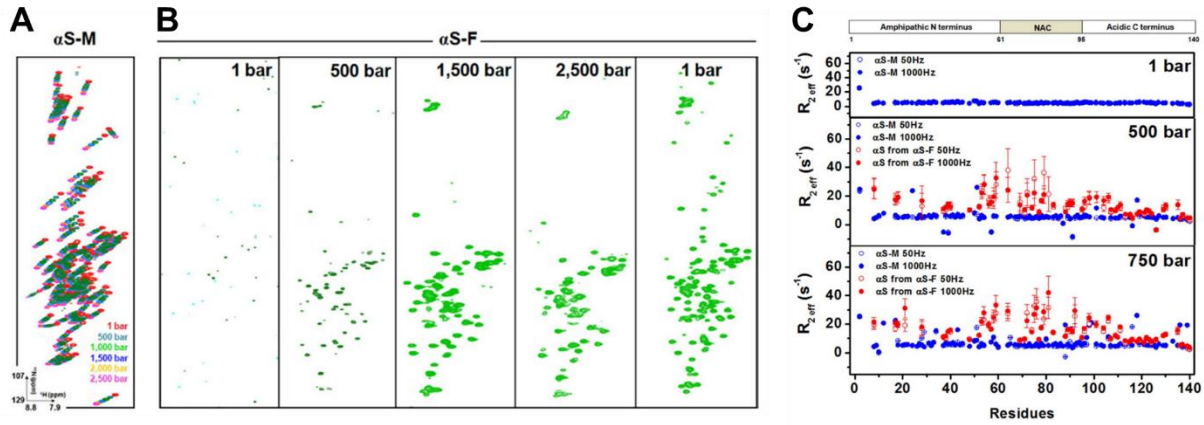

Figure 1. High-pressure (HP) NMR application on α-Syn. (**A**) ^{1}H-^{15}N HSQC NMR spectra of α-Syn monomers (αS-M) at various pressurized conditions. (**B**) ^{1}H-^{15}N HSQC NMR spectra of α-Syn fibrils (αS-F) at various pressurized conditions. (**C**) R_2 relaxation rate measurement results of αS-M (blue) and αS-F (red) at different pressure conditions as indicated in each panel. Note that R_2 rates of αS-F significantly differed from those of αS-M, implying their different structural and dynamic characters. Adapted from the figures of [18].

HP NMR for α-Syn monomers and fibrils

One of the initial HP studies on α-Syn was done by Foguel et al. with in-vitro preformed α-Syn fibrils.[16] In this study, the authors observed with light scattering and circular dichroism (CD) spectroscopy that pressure application caused dissociation of α-Syn fibrils into soluble monomers. This dissociation was reversible; upon decompression and re-incubation at the fibrillization condition (37 °C and pH 7.5), a similar fibril could be formed, suggesting that the pressure-dissociated α-Syn monomer still maintains at least a part of fresh monomer-like. However, due to the technical limitations, this study could not provide further atomistic details for possible pressure-induced structural transition.

Roche et al. overcame this difficulty by employing HP NMR and monitoring structural transitions of N-terminal-acetylated α-Syn at various pressurized conditions.[17] They observed that some NMR signals in 2D ^{1}H-^{15}N HSQC spectra exhibited non-linear movements along with the pressure titration, implying the existence of a low-populated 'excited state.'

A subsequent study done by de Oliveira et al. succeeded in observing the structural differences between fresh α-Syn monomers and the monomers dissociated from a fibril (Fig. 1).[18] In this study, the authors employed several spectroscopic techniques, such as CD, small-angle X-ray scattering (SAXS), and HP NMR (Fig. 1AB), and compared structural and dynamic changes in α-Syn after fibrillization. Intriguingly, the dissociated monomers from a fibril showed narrower radius of gyration (R_g) distribution at SAXS and altered R_2 relaxation rates at HP NMR analysis (Fig. 1C). The subsequent NMR-based mapping indicated that the hydrophobic buried residues in the α-Syn fibril are mainly affected by pressure-induced dissociation, suggesting irreversible structural transition by fibrillization. Seeding experiments further confirmed this observation; the pressure-dissociated monomer could facilitate the aggregation of fresh α-Syn monomers. Indeed, Golebiewska et al. consistently reported that the HP condition could facilitate aggregation of α-Syn in cultured neuronal cells.[19]

Notably, some independent HP-based studies indicated that the α-Syn amyloid fibril contains hydrophobic cavities, which explains how α-Syn fibrils were disassembled in a pressurized condition.[18,20] In addition, the aggressive mutants of α-Syn, A30P and A53T, was shown to constitute

different hydrophobic cavities in their fibrils, thus being capable of inducing formation of distinctive intermediates in their pressure-induced fibril dissociation.[20,21]

Conclusions

α-Syn is one of the most famous IDPs and pathogenic amyloidogenic proteins. Due to these intriguing and physiologically critical features, this protein has been an important target for numerous structural studies. Among them, HP NMR-based approaches have been beneficial in providing unique information for the structural states of α-Syn in its monomeric form as well as its oligomeric intermediate and fibril states. Compared to the other denaturation methodologies, HP application provides a relatively mild denaturing condition, thus enabling to observe conformations more similar and relevant to the physiological state. The studies discussed in this review clearly show that HP NMR effectively provides unique information regarding the structural states of aggregation-prone proteins and their amyloidogenic mechanisms.[22] Along with continued technical development, we believe that HP NMR stands as a unique and indispensable tool for important yet challenging proteins, such as IDPs and amyloidogenic proteins.[23]

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