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NMR-based structural characterization of transthyretin in its aggregation-prone state

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Abstract Transthyretin (TTR) is an abundant protein in blood plasma and cerebrospinal fluid (CSF), working as a homo-tetrameric complex to transport thyroxine (T_4) and a holo-retinol binding protein. TTR is well-known for its amyloidogenic property; several types of systemic amyloidosis diseases are caused by aggregation of either wild-type TTR or its variants, for which more than 100 mutations were reported to increase the amyloidogenicity of TTR. The rate-limiting step of TTR aggregation is the dissociation of a monomeric subunit from a tetrameric complex. A wide range of biochemical and biophysical techniques have been employed to elucidate the TTR aggregation processes, among which nuclear magnetic resonance (NMR) spectroscopy contributed much to characterize the structural and functional features of TTR during its aggregation processes. The present review focuses on discussing the recent advances of our understanding to the amyloidosis mechanism of TTR and to the structural features of its monomeric aggregation-prone state in solution. We expect that the present review provides novel insights to appreciate the molecular basis of TTR amyloidosis and to develop novel therapeutic strategies to treat diverse TTR-related diseases.

Keywords transthyretin, transthyretin amyloidosis, amyloid, protein aggregation, NMR spectroscopy

Introduction

Transthyretin (TTR) is a transporter protein of the thyroid hormone, thyroxine (T_4), and the retinol-binding protein that is bound to a retinol molecule.[1] It was originally found in human cerebrospinal fluid (CSF), and named prealbumin due to its faster mobility than albumin in protein electrophoresis gel analysis.[2] TTR is synthesized in the liver and the choroid plexus, which results in its prevalence in the blood plasma and CSF, respectively.[3]

TTR is a homo-tetrameric protein, whose monomeric subunit is ~ 14 kDa, consisting of 127 amino acids. Its first X-ray crystallographic study was conducted in 1971,[4] and the first atomic-resolution crystal structure was deposited in 1977.[5] Since then, numerous structural studies were conducted to investigate various aspects of this protein.[6] TTR has two β-sheets; the β-strands D/A/G/H and C/B/E/F (Fig 1). Two T_4 binding sites that are formed in the tetrameric complex are constructed by the strands D/A/G/H, where several hydrophobic residues (e.g. Leu17, Ala108, Ala109, and Leu110) are located to mediate the interaction with T_4.[7]

TTR has attracted many attention as it is responsible for several systemic amyloidosis diseases, such as senile systemic amyloidosis and familial amyloid polyneuropathy/cardiomyopathy.[8,9] To date, more than 100 mutations of TTR have been reported, many of which are related with familial amyloidogenic

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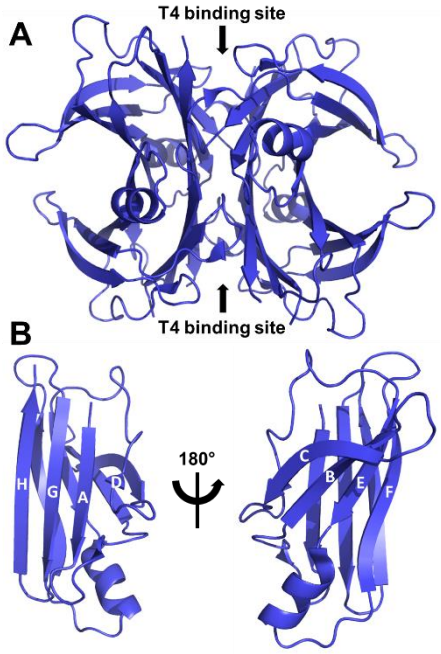

Figure 1. Structural model of transthyretin determined by X-ray crystallography (PDB 4TLT). (A) Tetrameric complex where two central T_4 binding sites (noted with arrows) are formed, (B) Monomeric subunit in which β-strands are marked as described in the text.

pathology.[10] For example, the V122I variant was first identified in the TTR-derived fibrils that were extracted from the heart of a patient with cardiac amyloidosis, and it was subsequently shown that about 3% of the African American population in United States may have this mutation.[11] In addition, a series of recent studies identified several amyloidogenic mutations of TTR in South Korea. To date, total 18 patients were found, among which the most common TTR mutation was D38A.[12] Notably, one recent study done in 2018 reported that the global population having hereditary defects on TTR was estimated at 10,186,[13] indicating that TTR-related amyloidogenic disease is a severe challenge that needs urgent attention for development of diverse therapeutic strategies. In this minireview, therefore, we discussed the current understanding to the TTR aggregation and amyloidosis mechanism. In particular, we reviewed a couple of recent studies that have elucidated the structural features of TTR monomers, the aggregation-prone species in the TTR amyloidosis mechanism.

Aggregation mechanism of TTR

The amyloidogenic property of TTR was first identified in 1978,[14] which was followed by the initial in vitro study showing that two synthetic peptides, which corresponds to the residues 10-20 (strand A) and 105-115 (strand G) of TTR, exhibited high amyloidogenicity.[15] Subsequently, by employing various techniques including NMR spectroscopy, Kelly et al. found that native structure of TTR needs to be denatured in order to initiate the aggregation mechanism, and the monomeric state is the important intermediate in this process.[16–18] A series of additional studies have successfully confirmed that monomer dissociation from the native tetrameric complex is a critical rate-limiting step for TTR aggregation and fibril formation (Fig. 2).[19] Indeed, it was found that TTR amyloid fibrils are composed not only of full-length proteins but also of truncated forms.[20,21] In addition, it was shown that many TTR mutations, which are related with TTR amyloidosis pathology,

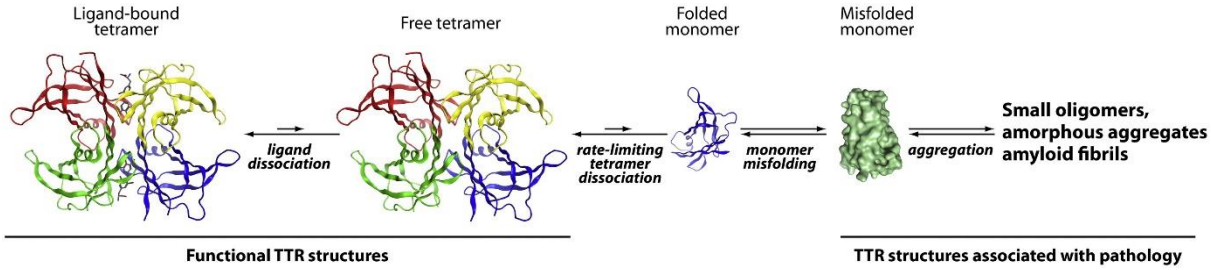

Figure 2. The aggregation mechanism of transthyretin (TTR). The amyloidogenicity of TTR manifests upon monomer dissociation from the native tetrameric complex, initiating the aggregation cascade to amyloid fibril formation. Reprinted with permission by Elsevier.[19]

facilitate tetramer dissociation or monomer stabilization.[22,23] Notably, based on these findings, Kelly et al. developed benzoxazole-derivatives that binds to the T_4 binding site and stabilizes the native quaternary structure of TTR.[24] They confirmed that their small ligand molecules behaved as a 'kinetic stabilizer' of the TTR tetramer, thus reducing monomer dissociation and inhibiting amyloid formation.[25] Finally, they succeeded to develop the drug, called tafamidis [2-(3,5-dichloro-phenyl)-benzoxazole-6-carboxylic acid], which is currently being used to treat TTR familial amyloid polyneuropathy and cardiomyopathy.[26,27] Despite its overall efficacy against TTR amyloidosis pathology, however, there were several reports where tafamidis was not sufficiently effective,[28] making it necessary to develop novel therapeutic approach to complement it.

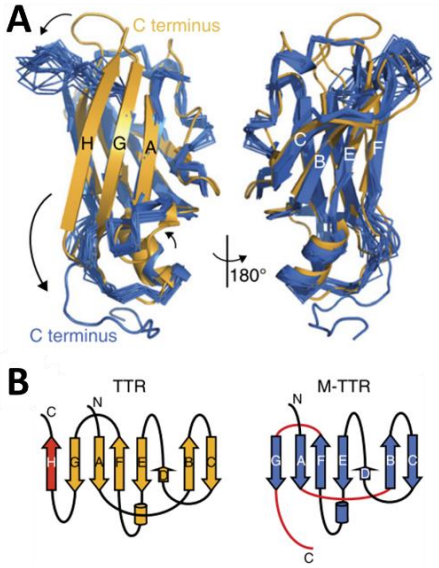

Figure 3. The solution structure of M-TTR determined by NMR spectroscopy in a pressurized condition. (A) The structural model of M-TTR monomers determined by NMR spectroscopy (blue) is overlaid with the tetrameric structural model obtained by X-ray crystallography (orange; PDB 1GKO). (B) The secondary structural motifs that were observed in the tetrameric state (X-ray; orange) and the monomeric state (NMR; blue). Note that the β-strand H in the tetrameric state disappeared in the monomeric state. Modified from [29] with permission by Springer Nature.

NMR-based structural characterization of the monomeric state

While current treatment strategies for TTR-related pathologies have been mostly focused on targeting the native tetrameric state, the main culprit of TTR aggregation is rather the monomeric species which could be a better target for therapeutic intervention. Recent NMR-based studies succeeded to determine structural features of TTR monomers, whose information will be applicable to develop novel therapeutics targeting TTR monomers, the actual aggregation-prone species. In order to investigate the monomeric state of TTR, Zweckstetter et al. first constructed the monomeric variant of TTR, F87M/L110M (M-TTR).[29] M-TTR is an engineered variant of TTR, maintaining a monomeric state in solution.[30] It is notable that X-ray crystallographic study of M-TTR showed that this protein forms a tetrameric complex in a crystallization condition, implicating rather a dynamic quaternary structure of this protein.[31] Therefore, in order to stabilize homogeneous and monomeric structural state of M-TTR, Zweckstetter et al. obtained NMR signals of M-TTR in a pressurized condition, and determined its atomic-resolution solution structure (Fig. 3). The major difference of this NMR-based structural models from the X-ray crystallographic model of the same protein is that the C-terminal β-strand H is disordered in solution. In the tetrameric complex, the β-strand H constitutes the dimeric interface by forming an extensive hydrogen bond network with a neighboring subunit. The NMR analysis results therefore indicate that the stability of the β-strand H in M-TTR is highly dependent on tetramer formation. It is also notable that destabilization of the β-strand H causes exposure the β-strand G, where the highly amyloidogenic motif (the residues 105-115) resides, indicating that this structural transition may explain the increased aggregation propensity of M-TTR compared to the wild-type protein.

In addition, Zweckstetter et al. determined the solution structure of F87M/L110M/T119M (T119M M-TTR) with NMR spectroscopy.[32] T119M mutation is known

to suppress the amyloidogenic activity of TTR. NMR spectroscopic analysis confirmed that T119M M-TTR maintains homogeneous and stable monomeric state, which enabled its structure determination in an ambient condition. Notably, while the C-terminal β-strand H was not structured in M-TTR, the structural models of T119M M-TTR showed that it restored at least a portion of the β-strand H structure, providing wild-type like protection to the β-strand G. This is indeed consistent with the reduced aggregation tendency of T119M M-TTR.

Conclusions

Despite urgent importance of elucidating atomistic details of the TTR amyloidosis mechanism, its structural characterization has been hampered due to the highly dynamic and heterogeneous nature. In order to overcome these challenges, several research groups employed cutting-edge NMR-based techniques, which were successful to reveal various structural features of pathogenic TTR species.[22,33,34] In particular, the unique structural features of TTR monomers were revealed with NMR spectroscopy, providing novel insights to appreciate the molecular mechanisms of TTR amyloidosis and to develop novel therapeutic strategies to treat TTR-related diseases. We expect that various NMR-based techniques will constitute an essential toolbox to investigate various native and non-native structural features of amyloidogenic proteins.

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