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Hydrogen-bond network and pH sensitivity in transthyretin: Neutron crystal structure of human transthyretin

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ABSTRACT

Transthyretin (TTR) is a tetrameric protein associated with human amyloidosis. *In vitro*, the formation of amyloid fibrils by TTR is known to be promoted by low pH. Here we show the neutron structure of TTR, focusing on the hydrogen bonds, protonation states and pH sensitivities. A large crystal was prepared at pD 7.4 for neutron protein crystallography. Neutron diffraction studies were conducted using the IBARAKI Biological Crystal Diffractometer with the time-of-flight method. The neutron structure solved at 2.0 Å resolution revealed the protonation states of His88 and the detailed hydrogen-bond network depending on the protonation states of His88. This hydrogen-bond network is composed of Thr75, Trp79, His88, Ser112, Pro113, Thr118-B and four water molecules, and is involved in both monomer–monomer and dimer–dimer interactions, suggesting that the double protonation of His88 by acidification breaks the hydrogen-bond network and causes the destabilization of the TTR tetramer. In addition, the comparison with X-ray structure at pH 4.0 indicated that the protonation occurred to Asp74, His88 and Glu89 at pH 4.0. Our neutron model provides insights into the molecular stability of TTR related to the hydrogen-bond network, the pH sensitivity and the CH···O weak hydrogen bond.

1. Introduction

Transthyretin (TTR) is found in human plasma at a concentration of approximately 0.20 mg/ml, where it binds to the retinol-binding protein and to the thyroid hormone thyroxine (Bartalena and Robbins, 1993). In addition, TTR is the major carrier of T4 in cerebrospinal fluid, where it is present at a concentration of 0.02 mg/ml (Aldred et al., 1995). TTR is a homo-tetrameric β-sheet-rich protein composed of four subunits termed A, B, C and D. The TTR monomer is composed of 127 amino acids and has a molecular mass of 14 kDa and eight β-strands designated as A–H and a short α-helix termed the EF-helix (Fig. 1A). In each dimer, association of two monomers (A and B or C and D) occurs through hydrogen bonds and hydrophobic interactions. Hydrogen bonds between the two H strands of neighboring monomers create a hydrogen-bonded monomer–monomer-interface. The association of two dimers (AB dimer and CD dimer) results in a tetrameric structure. The dimer–dimer contacts predominantly involve several hydrogen bonds and hydrophobic interactions of residues in two loops (i.e., the AB and GH loops) at the edge of the strands (Fig. 1B).

TTR is one of more than 30 amyloidogenic proteins that, when partially denatured, can misassemble into several aggregate structures, including amyloid fibril with cross-β sheet quaternary structure (Buxbaum and Tagoe, 2000; Kelly, 1996). The TTR amyloid fibrils are found in patients afflicted with either familial amyloidotic polyneuropathy (FAP) or senile systemic amyloidosis (SSA) (Benson, 1989; Rochet and Lansbury, 2000). Deposits of WT-TTR amyloids accumulate in the heart in SSA. FAP occurs at a much younger age than SSA, in some cases during the second decade of life, and affected individuals have a mutation of TTR. The full length polypeptide is a predominant product of the amyloid fibrils in FAP

Abbreviations: TTR, transthyretin; WT-TTR, wild-type TTR; FAP, familial amyloidotic polyneuropathy; SSA, senile systemic amyloidosis; iBIX, IBARAKI Biological Crystal Diffractometer; MLF, Materials and Life Science Experimental Facility; J-PARC, Japan Proton Accelerator Research Complex.

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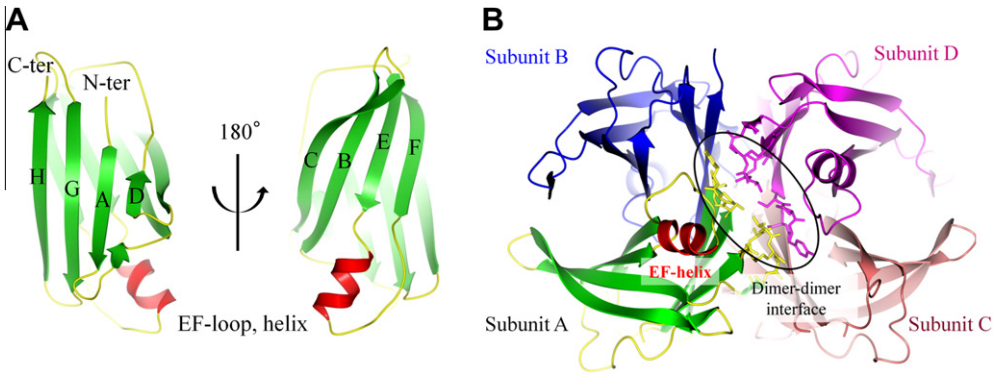

Fig. 1. Overall structure of TTR. (A) Ribbon diagram of the monomer. The α-helices are shown as ribbons, the β-strands as arrows and the loops as tubes colored in red, green and yellow, respectively. (B) Ribbon diagram of the tetramer. Subunit A is colored the same as in Fig. 1A, subunit B is colored in blue, C is in pink and D is in magenta. The residues at the dimer–dimer interface between subunit A and subunit D are circled and shown as sticks.

patients, while fragments of TTR as well as full-length polypeptide are found in SSA amyloid deposits (Westermark et al., 1990). Dissociation of TTR tetramer appears to be the rate-determining step that allows subsequent partial misfolding and misassembly, leading to several aggregate morphologies (Colon and Kelly, 1992; Hurshman et al., 2004; Jiang et al., 2001; Kelly et al., 1997; Lashuel et al., 1999; Liu et al., 2000a; McCutchen et al., 1993, 1995). At acidic pH, TTR undergoes dissociation into a partially folded monomeric intermediate, which self assembles to give rise to amorphous aggregates and/or amyloid fibrils (Colon and Kelly, 1992; Kelly, 1996; Lai et al., 1996; Lashuel et al., 1998; Liu et al., 2000b). However, the precise molecular mechanisms underlying TTR aggregation remain elusive.

Recent structural studies have begun to reveal the structural changes by the lowered pH in both wild-type and amyloidogenic mutant TTR. Lowering the pH from 7.0 to 5.3 does not induce significant conformational changes in the WT-TTR crystal structure (Hamilton et al., 1993; Hornberg et al., 2000). Moreover, the crystal structure of the WT-TTR determined at pH 4.6 is virtually identical with that of the pH 7.0 structure (Pasquato et al., 2007). In contrast, the crystal structure of the I84A amyloidogenic mutant showed notable conformational changes at pH 4.6 compared to that of the I84A structure determined at pH 7.5; the I84S pH 4.6 structure also showed conformational changes similar to those of I84A at pH 4.6 (Pasquato et al., 2007). In these structures, a large conformational change is found at the EF-helix and loop, in the area of the short α-helix (75–81) and of the contiguous long loop (residues 82–87) that connects the helix to the F-strand. Furthermore, the crystal structure of the WT-TTR determined at pH 4.0 and 3.5 also showed conformational changes in the same region as that of both I84A and I84S, but in a different manner (Palaninathan et al., 2008). The pH-dependent effects in proteins are mainly electrostatic in nature and originate from changes in the protonation states of acidic and basic residues (Schaefer et al., 1997; Ullmann and Knapp, 1999; Yang and Honig, 1993). To further investigate the structural explanation for the pH-dependent effects of TTR, information on the hydrogens and protonation states will be required. The X-ray atomic form factors of hydrogen and deuterium are much smaller than those of other elements, and so it is hard to determine protonation states with X-rays. In contrast, neutron protein crystallography is preferred as a tool to determine the hydrogen bonding, protonation states and hydration of biomacromolecules, since the neutron-scattering length of hydrogen and deuterium are comparable to those of other elements (Niimura and Bau, 2008).

In this study, we expressed, purified and crystallized the N-terminal truncated mutant TTR which lacks flexible residues 1–11, and carried out a time-of-flight neutron diffraction study using the IBARAKI Biological Crystal Diffractometer (iBIX) installed at the pulsed neutron source of the Materials and Life Science Experimental Facility (MLF) at the Japan Proton Accelerator Research Complex (J-PARC) to obtain a complete data set from TTR (Tanaka et al., 2010). The neutron structure of TTR solved at 2.0 Å resolution provides the protonation states and the detailed information about the hydrogen bonds. We discuss the networks of hydrogen bonds, the protonation states at pD 7.4 and the origin of pH sensitivity related to the structural stability of TTR.

2. Materials and methods

2.1. Protein expression and purification

Deletion of the N-terminal regions was achieved using the PCR-based mutagenesis method as previously described (Mizuguchi et al., 2008). ΔN11-TTR (residues 12–127) was subcloned into the *NdeI* and *SalI* sites of a modified pQE-30 vector (*Qiagen*) for the expression of TTR without a histidine-tag, and the sequence of inserted DNA segments was verified by DNA sequencing. ΔN11-TTR was expressed in the E.coli host strain M-15 (*Qiagen*) using a pQE-30 system. The expression and purification of ΔN11-TTR were carried out as previously described (Miyata et al., 2010).

2.2. Crystallization experiments

Initial crystallization conditions were screened with the crystallization screen kits WIZARD I and II (*Emerald Biostructures*) by the hanging drop vapor-diffusion method at 293 K. As the results of the screening showed that crystals were grown only from the magnesium ion-containing conditions, the crystallization screenings were carried out again using protein solution supplemented with 0.2 M $MgCl_2$ using the crystallization screen kits WIZARD I, II, III (*Emerald Biostructures*) and Salt RX (*Hampton Research*). Within a few days, single crystals were observed using *tri*-ammonium citrate pH 7.0 as the precipitating agent. Crystallization conditions were optimized manually by refinement of the concentration of the precipitant and the protein solution. In neutron protein crystallography, the incoherent scattering of hydrogen atoms produces a high background in the diffraction pattern from the protein crystal, making it difficult to see weak diffraction peaks. In order to avoid the incoherent scattering from hydrogen atoms, the crystals for the neutron diffraction experiments were grown using the protein solution exchanged by heavy water and precipitating agents prepared with the heavy water. The large deuterated crystals of