Structural Analysis of the TxCo-1 Antibody Effective Against the Toxic Conformer of Amyloid β

Amyloid β (Aβ) oligomers are key elements in the pathogenesis of Alzheimer's disease (AD). Antibodies that recognize the tertiary structure characteristic of the toxic Aβ oligomers—which have a toxic turn at positions 22/23—could become new pharmacologic seeds for AD diagnosis and therapy. We have developed a conformationally restricted analog of Aβ42 with an intramolecular disulfide bond at positions 17/28 (SS-Aβ42). X-ray crystallography of the TxCo-1 Fab domain in the complex with the truncated SS-Aβ42(15–30) clarified the recognition site, giving a structural basis for its low affinity for the wild-type Aβ42 monomer and creating selectivity for its aggregates with a turn at positions 22/23.

Alzheimer's disease (AD) is characterized by the abnormal aggregation of amyloid β (Aβ) protein, followed by the deposition of hyperphosphorylated tau proteins. Recent investigations have suggested that oligomeric Aβs rather than high-molecular-weight Aβ aggregates (fibrils) play a significant role in the progression of AD pathology. The key structures of the Aβ oligomers involved in this cytotoxicity and their aggregative ability remain unknown, although the specific structures of the Aβ fibrils have been solved by solid-state NMR and cryo-electron microscopy [1, 2].

In order to obtain information on the structure–activity relationship of Aβ oligomers, systematic proline replacement of Aβ42 was conducted and the relationship between the secondary structure (β-sheet) and cytotoxicity was examined in detail. The results clearly showed the existence of turns at positions 22/23, 25/26, and 38/39. We focused on positions 22/23 since mutation sites in Aβ of familial AD are concentrated at position 22 in the Arctic, Italian, Dutch, and Osaka mutations. Further studies on the turn at positions 22/23, using solid-state NMR and electron spin resonance, suggested a new mechanism of Aβ42 aggregation induced by radicalization as shown in **Fig. 1** [3]. In brief, phenoxy radicals derived from Tyr10 through oxidation by Cu(II) effectively oxidize Met35 by the formation of the toxic turn at positions 22/23 to bring Tyr10 and Met35 closer together. The generated Met radical cation is ionically stabilized by the carboxylate anion of C-terminal Ala42, resulting in the formation of a hydrophobic core to give a dimer, trimer, and oligomers with cytotoxicity. Oxidative stress by Aβ aggregates can also be explained by liberation of carboxy radicals incorporated in the aggregates.

Although a proline mutation at position 22 enhanced the aggregative ability and cytotoxicity of Aβ42 [3], such a mutation does not occur in nature. Recently, we have found an Aβ42 analog crosslinked intramolecularly at positions 17/28 (SS-Aβ42) [**Fig. 2(A)**] to show high aggregative ability and potent cytotoxicity comparable to that of E22P-Aβ42 [4]. Since the monoclonal antibody

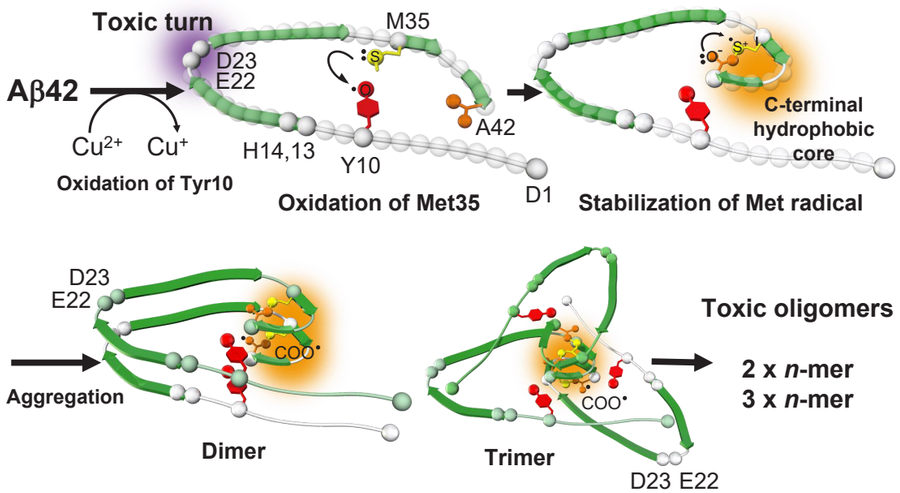

Figure 1: Formation of toxic oligomers of Aβ42 through radicalization.

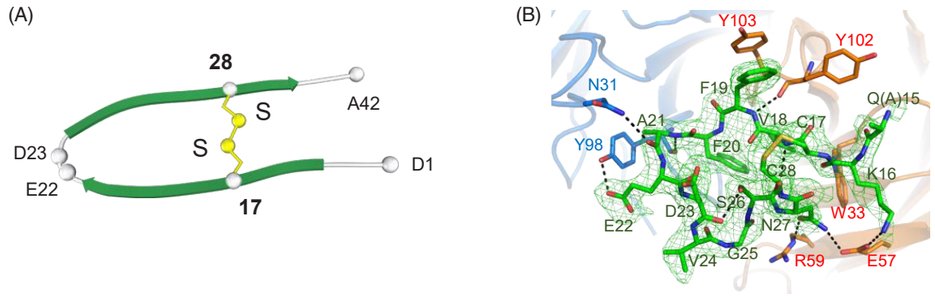

Figure 2: (A) Structure of SS-Aβ42. **(B)** SS-Aβ42(15–30) binding manner in the crystal structure of TxCo-1 Fab - SS-Aβ42(15–30) complex. Electron density of Aβ (contoured at 2 sigma, green) is also drawn. SS-Aβ42(15–30), heavy chain of TxCo-1 Fab, and light chain of TxCo-1 Fab are shown in green, orange, and blue, respectively.

obtained from the immunization of E22P-Aβ9-36 in mice (24B3 antibody) was useful for diagnosis of AD using human cerebrospinal fluid [3], we produced a monoclonal antibody (TxCo-1) using SS-Aβ42 as an immunogen in collaboration with Immuno-Biological Laboratories [5].

To elucidate the binding manner of SS-Aβ42 to the TxCo-1 Fab domain, crystal structure analyses were performed. For the co-crystallization, the truncated SS-Aβ42(15–30) was added to the purified Fab fragment solution to adjust the molar ratio of the Fab and the Aβ peptide to 1:3. The crystals of the TxCo-1 Fab domain complexed with SS-Aβ42(15–30) were obtained from a 1:1 mixture of the protein solution and precipitant solution [18% (w/v) polyethylene glycol monomethyl ether 5,000 and 0.2 M magnesium formate in 0.1 M sodium acetate buffer (pH 4.0)] by the sitting-drop vapor diffusion method at 293 K. X-ray diffraction work was carried out under a nitrogen gas stream at 100 K using synchrotron radiation with an EIGER X4M detector on the BL-1A beamline. The diffraction data were processed and scaled with the XDS package [6] and Aimless program [7] from the CCP4 suite [8]. The crystals belong to the space group *C*2 with cell dimensions of a = 185.0 Å, b = 40.5 Å, c = 69.4 Å, and β = 97.2°. The structure was solved at 2.5 Å resolution by the molecular replacement method using the program Molrep [9] from the CCP4 suite [8], with the coordinates of anti-E22P-Aβ antibody 24B3 (unpublished data) as a search model. The electron densities ascribable to the residues 16 to 28 of SS-Aβ42(15–30) were clearly observed, indicating that TxCo-1 Fab recognizes the region including the toxic turn at positions 22/23 [**Fig. 2(B)**] [5]. To our knowledge, this is the first identified antibody that binds to the toxic turn structure of Aβ42.

Immunohistochemical staining of human brain tissue suggested the presence of Aβ with structural similarity to SS-Aβ42 in the brains of patients with AD [5]. It is noteworthy that the TxCo-1 immunoreactive pattern in the brain samples did not overlap with that identified by conventional Aβ antibodies (6E10 and mOC64). In summary, the conformationally restricted SS-Aβ42 and its monoclonal antibody TxCo-1 could become useful tools to understand the pathological role of Aβ with the toxic conformation at positions 22/23 in the progression of AD.

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BEAMLINE

BL-1A

K. Irie,[1] Y. Irie,[1] Y. Matsushima,[1] A. Kita,[1] Y. Kageyama[2] and I. Tooyama[2] ([1]Kyoto Univ., [2]Shiga Univ. of Med. Sci.)