Unique antigenases to enzymatically cleave Tau peptides at C- and N-terminal moieties

Taizo Uda1, Hiroaki Taguchi2, Emi Hifumi3

*1 Research Promotion Institute, Oita University, Oita, 870-1192, Japan; 2 Faculty of Pharmaceuticals Sciences, Suzuka University of Medical Science, Suzuka, Mie 513-8670, Japan; 3 Nano-tech Lab, ISIT, Fukuoka 819-0388 Japan*

*\*Corresponding author: uda@isit.or.jp*

**Highlights**

* 1. FRET-peptide system was a strong tool for screening catalytic antibodies (antigenases).
* 2. Several antigenases to cleave FRET-Tau peptides were found in this study.
* 3. Each antigenase exhibited a little different feature in the cleavage of the Tau peptide.
* 4. Antigenases against Tau-peptide are involved in human antibody genes.

**1. Introduction**

 Most common cause of dementia among older adults is Alzheimer’s disease (AD). In the disease, two kinds of molecules, amyloid β peptide (Aβ) and Tau protein, are considered as the main factors relating with the cause of the disease by the accumulation with neurofibrillary tangles (NFT) or phosphorylated filamentous tau protein, which works as proteinopathies on the brain.

 Antigenases (catalytic antibodies) capable of hydrolyzing peptides and proteins (antigenase) are potentially useful agents for therapeutics, through the specific elimination of pathogenic peptides and the essential proteins in microorganisms. Actually, antigenases such as HIV envelope glycoprotein, helicobacter pylori urease, influenza viruses, and amyloid β (Aβ) have been developed in a last few decades.

 In this study, Tau protein was targeted in order to make the antigenases capable of enzymatically degrading the protein. For this objective, we designed and synthesized a substrate based on Förster resonance energy transfer (FRET) to efficiently find out the desired antigenases from our protein bank, in which hundreds of human antibody light chains possessing a catalytic triad–like structure are stored as the protein.

**2. Methods**

The fluorescence-quenched substrates were synthesized by conventional Fmoc SPPS using Rink amide resin. The structure of the substrate was confirmed by ESI-MS after purification by HPLC. After a human antibody light chain gene inserted into pET20b (+), it was transformed into *E. coli* and induced by IPTG. After recovering the soluble fraction of the culture supernatant, it was purified by Ni-NTA chromatography, cation exchange chromatography or size exclusion chromatography. Catalytic activity was measured using synthetic substrates of FRET-Tau (391-408) at C-terminal side and FRET-Tau (19-30) at N-terminal side.

**3. Results and discussion**

The number of the stored light chains in our protein bank reached to hundreds. Thus, we screened the antigenases capable of cleaving the Tau peptides using the synthesized FRET-Tau peptide substrates. As the results, two antigenases (C1 & C2) to cleave the FRET-Tau (391-408) peptide at C-terminal side were found in 111 light chains examined. On the other hand, one antigenase (N1) to hydrolyze the FRET-Tau (19-30) peptide at N-terminal side was obtained in 96 light chains examined. The C1 antigenase cut the peptide bond between Val399-Ser400 (Fig. 1) and C2 between Gly401-Asp492. The two antigenases showed different enzymatic feature. Regarding the N1 antigenase, the peptide bond between Asp22 and Arg30 was cleaved. It is interesting that there were several antigenases capable of cleaving several sites of Tau protein in the protein bank of antibody light chains, which were prepared from human antibody library. This suggests that the catalytic antibody light chains against Tau molecule can naturally be produced.



**Figure 1. HPLC chromatogram for the products after the reaction of Tau peptide at C-terminal side and C1 antigenase. The peptide bond between Gly401 and Asp402 was digested by C1 antigenase.**

**4. Conclusions**

We found several antigenases capable of cleaving Tau peptides. C1 & C2 antigenases digested the C-terminal (391-408) side peptides. N1 antigenase hydrolyzed the N-terminal side peptide (19-30). These antigenases were present in human antibody genes.

**References**

1. E. Hifumi, Y. Mitsuda, K. Ohara, T. Uda, J. Immunol. Methods **269** (2002) 283-298.
2. E. Hifumi, F. Morihara, K. Hatiuchi, T. Okuda, A. Nishizono, T. Uda, J. Biol. Chem. **283** (2008), 899–907.
3. E. Hifumi, S. Takao, N. Fujimoto, T. Uda, J. Am. Chem. Soc. **133** (2011), 15015-15024.
4. E. Hifumi, N. Fujimoto, M. Arakawa, E. Saito, S. Matsumoto, N. Kobayashi, T. Uda, J. Biol. Chem. **288** (2013) 19558-19568.
5. E. Hifumi, M. Arakawa, S. Matsumoto, T. Yamamoto Y. Katayama,T. Uda, FASEB J. **29** (2015) 2347-2358.
6. E. Hifumi, S. Matsumoto, H. Nakashima, S. Itonaga, M. Arakawa, Y. Katayama, R. Kato, T. Uda, FASEB J. **30** **(**2016) 895-908.