**Development of algorisms to introduce a catalytic function into normal antibodies.**

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**Highlights**

* 1. Amino acids of Thr-Arg at 29th and 30th in CDR1 can exhibit a catalytic function.
* 2. Deletion of Pro95 in CDR3 contributes to exhibit a catalytic function.
* 3. Several catalytic antibodies to cleave of Amyloid-beta (A) were found.
* 4. Several algorisms how to make catalytic antibodies from normal antibody are developed.

**1. Introduction**

Catalytic antibodies capable of hydrolyzing the targeting peptides and proteins are potentially useful agents for therapeutics, through the specific elimination of pathogenic peptides and the essential proteins in microorganisms. Over the course of the last few decades, several groups have reported catalytic antibodies hydrolyzing antigens such as HIV envelop glycoprotein, helicobacter pylori urease, rabies virus, influenza virus and amyloid β peptide (Aβ). However, it is not easy to find out the catalytic antibody from a huge number of normal (non-catalytic) antibodies. It has been desired that the development of an algorism how to easily and effectively introduce a catalytic function into the antibodies. Therefore, for these ten years, the authors have devoted to prepare a protein bank of human antibody light chains having a catalytic triad-like structure. The light chains were cloned from human antibody library and highly purified after the expression in *E. coli* f*.* The number of the stored light chains has reached to several hundreds. Therefore, in this study, the screening whether or not the stored light chains can possess a catalytic activity for a synthetic substrate, Arg-pNA, and FRET-Amyloid-beta (A), was carried out to obtain important information about the algorism.

**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 Arg(R)-pNA and FRET-Amyloid-beta (Aβ).

**3. Results and discussion**

 The authors screened the catalytic activity for more than 100 light chains stored in the above protein bank. Out of them, #7wt and #7TR light chains showed interesting results. The former showed the low activity to cleave synthetic substrate Arg-pNA. In contrast, the latter light chain showed the high catalytic activity to cleave the substrate. For FRET-Aβ (26-33) substrate, the former light chain did not decompose at all but the latter did. The difference of both light chains is in the amino acids at 29th and 30th position in CDR1. The former is Gly-Tyr and the latter Thr-Arg. By being replaced with Thr-Arg, the antibody light chain can gain the catalytic function. Interestingly, #7TR light chain could cleave not only FRET-Aβ (26-33) peptide but also Aβ41 of the full size molecule.

 In addition, we found other unique human antibody light chains, S35 and S38. The amino acid sequence of both light chains is identical except for Pro95 in CDR3. S35 has the Pro95 residue but S38 deleted the amino acid. The former did not decompose Arg-pNA at all. In contrast, the latter showed high decomposition activity (Fig. 1).

 These results are noteworthy, because it enables for us to design a catalytic antibody from the non-catalytic antibody on molecular basis.



**Figure 1.** **Cleavage reaction of a synthetic substrate, Arg-pNA**

**4. Conclusions**

On molecular basis, several algorisms how to make a catalytic antibody from the general antibody are found.

**References**

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