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Creation of Novel Catalytic Antibodies Using Yeast Cell Surface Display System Without Mammalian Immune System

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Research aims

Catalytic antibodies have the diversity of antibody molecules in the immune system *in vivo* as well as catalytic functions of enzyme molecules. Catalytic antibodies have generally been prepared from mouse by immunization with a haptenic transition-state analog. However, the preparation of catalytic antibodies by the traditional immunization method is time-consuming and labor-intensive at the present state. The aim of this study was to create tailor-made artificial enzymes with the desired catalytic ability *in vitro* using the yeast molecular display method, which has several advantages over the immunization method, including the ability to generate catalytic antibodies more rapidly and easily and the ability to adapt more flexibly to high-throughput screening systems. In this study I attempted to create a novel antibody with serine protease-like activity by introducing the catalytic triad composed of Ser-His-Asp into the amino acid sequence of an antibody molecule, since catalytic antibodies with a protease activity should have great application potential in medical fields.

Methods and Results

1. Introduction of catalytic triad into the light chain of catalytic antibody 6D9

Using the plasmid to display the catalytic antibody 6D9[1] already constructed, the catalytic triad was designed in its light chain (Lc). The design of the catalytic triad was performed on the basis of the sequence of anti-VIP antibody prepared by immunization with the vasoactive intestinal peptide (VIP). The anti-VIP antibody moderately catalyzes the hydrolysis of VIP[2] and its catalytic mechanism is similar to that of serine proteases[3]. Interestingly, Ser27a, His93 and Asp1 in the light chain variable region can form a serine protease-like catalytic triad[4,5]. From the result of alignment of amino acid sequences of light chain variable regions between the anti-VIP antibody and the catalytic antibody 6D9, it was assumed that the residues Ser27a, His93 and Asp1 in the anti-VIP antibody light chain probably form a serine protease-like catalytic triad. In order to introduce a similar catalytic triad into the light chain of the antibody at the identical position, we carried out site-directed mutagenesis in the light chain variable region of 6D9 to replace Glu1 and Thr27a with Asp and Ser, respectively. This double mutant was addressed as Lc (Triad) to distinguish from the wild-type Lc (WT). The plasmid for display of Lc (Triad) on the yeast cell surface was introduced into *Saccharomyces cerevisiae* BJ2168 strain, which was defective in multiple proteases. Display of Lc (Triad) on the yeast cell surface was confirmed by observing the fluorescence around yeast cells under a fluorescence microscope using the immunostaining method.

2. Activity measurement of Lc (Triad) displayed on the yeast cell surface

Since the yeast molecular display method permits to display proteins on the yeast cell surface, it is possible to deal with protein- or enzyme-displaying yeast as a protein cluster and a whole-cell biocatalyst in the case of an enzyme in particular. Activity of Lc (Triad) displayed on the cell surface was measured without the isolation and purification of Lc (Triad).

Lc (Triad)-displaying yeast cells were cultivated at 30°C for 24 h and then harvested by centrifugation (3000 rpm, 5 min). After cells were washed twice with 50 mM Tris-HCl (pH 8.0), and resuspended for 500 μl of a cell suspension (OD_{600}=80) with the same buffer, 500 μl of 10 μg/ml BODIPY FL casein (Molecular Probes, Eugene, OR, USA) or seven kinds of 200 μM peptide-MCA (Peptide Institute, Osaka, Japan) was added. After centrifugation of the reaction mixture, aliquot of the cell-free supernatant was transferred into a 96-well plate, and then fluorescence was measured using the Fluoroskan Ascent FL plate reader (Labsystems, Helsinki, Finland) with a filter pair of 485 nm/527 nm for BODIPY FL casein and 390 nm/460 nm for peptide-MCA, respectively. Lc (WT)-displaying yeasts were also measured to compare with the activity of Lc (Triad)-displaying yeasts. When each cell suspension was reacted with Suc-GPLGP-MCA, a substrate for collagenase-like peptidase, a remarkable difference in hydrolytic activities was observed between them: Lc (Triad)-displaying cells showed higher catalytic activity than Lc (WT)-displaying cells. The difference disappeared in the presence of the serine protease inhibitor DFP (diisopropylfluorophosphate), suggesting that the three amino acid residues, Ser27a, His93 and Asp1, functioned as a catalytic triad responsible for the proteolytic activity in a similar manner to the anti-VIP antibody light chain. These results indicate that collagenase-like protease could be created by the introduction of the catalytic triad into the light chain of 6D9[6].

Conclusion

Lc (WT), the wild-type light chain of the catalytic antibody, and Lc (Triad), its mutant with Ser, His and Asp at positions 27a, 93, and 1, respectively, were displayed on the cell surface of the protease-deficient yeast strain BJ2168. When each cell suspension was reacted with some peptide substrates, hydrolytic activity of Lc (Triad)-displaying cells toward a substrate for collagenase-like peptidase was higher than that of Lc (WT)-displaying cells. The result with the serine protease inhibitor DFP indicates that the three amino acid residues, Ser27a, His93, and Asp1, function as a catalytic triad responsible for the proteolytic activity in a similar manner to the anti-VIP antibody light chain. Although the molecular recognition mechanism of the Lc remains to be investigated in detail, the introduction of additional amino acid alternations into the light chain variable region will enable us to further regulate the substrate specificity of Lc (Triad).

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