In vitro selection of VHH antibodies targeting DNA aptamers

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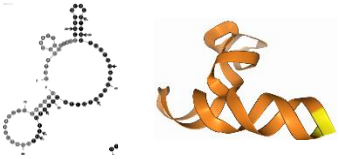
DNA-protein interaction is one of the most important intermolecular systems in organisms, such as transcriptional regulation. However, DNA used in living organisms is basically a double helix structure, and there has been no search for proteins that interact with DNA aptamers, which have a unique tertiary structure consisting of single-stranded DNA. In this study, we will use an artificial library of VHH antibody, which is attracting attention as a next-generation antibody as a protein, to investigate the possibility of molecular recognition by the various surface geometries of its CDR3 portion in particular.

The aim of this study was to obtain VHH antibodies that bind specifically to DNA aptamers that capture the antibiotic oxytetracycline (OTC). Small molecules such as OTC are known to be difficult to produce conventional antibodies because of their small molecular size. On the other hand, many DNA aptamers have been obtained against small molecules.

The DNA aptamer used in this study was the one shown on the right, considering its dissociation constant with OTC (KD =9.61 nM) and its selectivity (binding to OTC approximately 18.3-fold stronger than tetracycline and 36.6-fold stronger than doxytetracycline, which are also tetracycline groups).[1)]



Secondary (left) and tertiary (right) structures of DNA aptamer.

Specifically, four rounds of in vitro selection with an artificial VHH antibody library targeting OTC-binding DNA aptamers were performed using the cDNA display method. The recovery of DNA encoding the VHH antibody eluted in the final round was measured as 1.31% by quantitative PCR. The same selection was also performed under the conditions of targeting only streptavidin beads and streptavidin beads - DNA aptamer as a negative selection. The respective DNA recovery rates were 0.002% and 0.05%. Based on these results, we believe that we have successfully selected VHH antibodies that bind specifically only to the OTC - DNA aptamer.

DNA sequencing by NGS was then used to identify the DNA sequence encoding VHH, which binds only to the OTC - DNA aptamer.

Amino acid sequence of the CDR region of VHH that binds to the target.

	CDR1	CDR2	CDR3
VHH 1	GSVSSINAMG	AISRSGGSTYYADSVKG	WRFHKWHWLRQHVDY
VHH 2	GFTFDDYAMS	TITSGGRTNYADSVKG-	KGEKHWWRWHDY
VHH 3	GRTFNDYAMG	AINWSGDTTYYADSVKG	WHYYNVWSWQRY

In the future, we plan to use the DNA to express VHH that binds only to the OTC - DNA aptamer and measure affinity to the OTC - DNA aptamer.

〈References〉

(1) Man Bock Gu et al. ssDNA aptamers that selectively bind oxytetracycline. Bioorganic & Medicinal Chemistry 16 (2008) 1254–1261