Synthetic studies of functional molecules by regioselective protein modification (Ⅲ). ~Development and evaluation of a streptavidin-based tetravalent VHH antibody~

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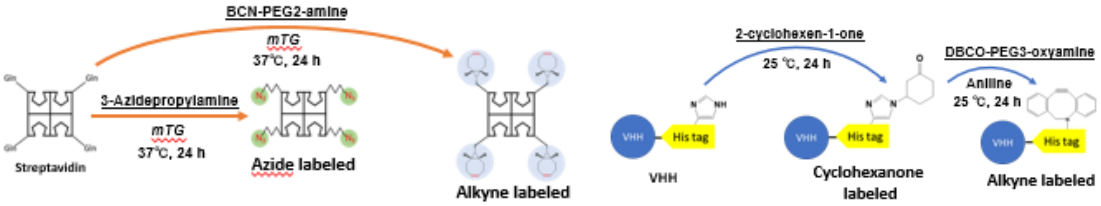
VHH antibodies from the family Camelidae, which have antigenic activity against viruses, are smaller in size than that of conventional antibodies, and their excellent refolding function makes them suitable for the creation of new functional molecules.

In our ongoing studies, our objective is preparation of tetravalent VHH antibodies by means of the glutamine residue of streptavidin. In addition, the histidine tag is added to VHH antibodies for purification and a simple modification method using regioselective click reaction will be applied to the tetravalent VHH antibodies.

In this study, introductions of azide and alkyne functions via amino groups on the glutamine residue of streptavidin were tried using microbial-derived transglutaminase enzyme, and a fluorescent label for the streptavidin derivative was carried out using the click reaction. In addition, cyclohexanone was regioselectively introduced into the histidine tag site of the VHH antibody using an aza-Michael addition reaction, followed by binding of an alkyne compound by an oxime formation reaction and introduction of a fluorescent probe by a click reaction. Structures of these compounds were confirmed by SDS-PAGE.

The results of SDS-PAGE showed that enzymatically azide-labeled or alkyne-labeled streptavidin reacted with fluorescent substances by click reaction. The VHH antibodies were also alkyne-labeled by using two chemical methods and then fluorescently labeled by the click reaction.



1) J. Pralhad, R. Vishal, *Chem. Commun*., **2019**, *55*, 1100-1103. 2) Abdolkarim Sheikhi, Mohammad Hojjat-Farsangi, *Taylor&Francis*., **2020**. 3) Pavel Strop, *Bioconjugate Chem*., **2014**, *25*, 855-862. 4) B. Spolaore, *et al*., *Bioconjugate Chem*., **2014**, *25*, 470-480. 5) Akshay Jain, Kun Cheng, J. Control. Release, **2017**, 245, 27-40.