SITE-SPECIFIC MODIFICATION AND CONTROLLED IMMOBILIZATION OF THE NANOBODY TARGETING VASCULAR CELL ADHESION MOLECULE 1 USING SORTEASE A FOR NEXT-GENERATION SURFACE BIOFUNCTIONALIZATION

Duy Tien Ta1, Erik Steen Redeker1, Wanda Guedens1 and Peter Adriaensens1,2

1) Biomolecule Design Group, Institute for Materials Research (IMO), Hasselt University, B-3590 Diepenbeek, Belgium
2) Applied and Analytical Chemistry, Institute for Materials Research (IMO), Hasselt University, B-3590 Diepenbeek, Belgium

Introduction

Nanobodies – the minimized camelid variable domains of single-domain antibodies – have numerous advantages over the conventional antibodies: small size (~15 kDa) (Fig. 1), ease of genetic manipulation and expression in E. coli, high stability and strong antigen binding capacity compared to the full-length antibody [1].

Staphylococcus aureus sortase A catalyzes the transpeptidation process by recognizing the specific LPETG recognition motif in the target protein, then removing the glycine and establishing a peptide bond between the carboxyl group of threonine and the amine group of an oligoglycine containing co-substrate [2].

In this study, we use sortase A as an in vitro strategy for site-specific protein modification and demonstrate this for the nanobody targeting Vascular Cell Adhesion Molecule 1 (NbVCAM1) as a model protein.

Expression of NbVCAM1-LPETG(His)6 and Sortase A

The NbVCAM1 gene with the LPETG signal and the S. aureus sortase A gene are separately expressed in E. coli. Both genes are engineered with a His6-tag sequence to enhance subsequent purification.

In vitro modification and immobilization of NbVCAM1-LPETG(His)6

The recombinant nanobody is subject to a sortase A-mediated ligation to contrast labeling probes [4], such as biotin, or further ligated to other oligoglycine containing targets including ‘clickable’ biomolecules or material surfaces (Fig. 2). The activity of the modified or immobilized nanobodies will be tested by measuring their antigen-binding capacity using ELISA, ellipsometry and SPR.

Results

The NbVCAM1 containing the LPETG motif is successfully and site-specifically labeled with a biotin molecule in a sortase-A catalyzed transpeptidation reaction with triglycine-biotin (Fig. 3). A product yield of approximately 80% is achieved after 24 hours incubation at 37°C when using 64 µM enzyme (Fig. 4).

![Figure 3. Sortase A-mediated biotinylation of the VCAM1-targeting nanobody. A reaction mixture of 20 µM NbVCAM1-LPETG-His6 is incubated with 16 µM sortase A, 0-1000 µM CaCl2, and 40 µM triglycine-biotin for 24 hours at 37°C. Treatments without nanobody or enzyme are used as controls. The nanobody without the LPETG signal sequence and the crude cell extract containing the NbVCAM1-LPETG-His6 are also included as references (Ca2+ concentration is fixed at 100 µM). The reaction mixtures are then resolved on SDS-PAGE gel, and then blotted on a PVDF membrane and visualized with streptavidin-alkaline phosphatase (upper panel). The yield is quantified by densitometric analysis using ImageJ software (http://rsb.info.nih.gov/ij/).](image)

![Figure 4. Time-course kinetic study of the in vitro biotinylation of NbVCAM1-LPETG-His6 under sortase A catalysis. The reaction was performed with different concentrations of sortase A. The product was detected by Western blot and the yield was quantified by densitometric analysis using ImageJ software (http://rsb.info.nih.gov/ij/).](image)

Conclusion

The enzymatic biotinylation of NbVCAM1 using sortase A is performed at mild conditions with promisingly high specificity. This study paves the way to a site-specific modification of the nanobody with a ‘click’ function, resulting in homogeneously modified proteins for future ‘click’ chemistry-mediated coupling onto material surfaces. This is in order to obtain a surface covered with homogeneously oriented nanobodies via stable covalent bonds.

Acknowledgement

This work is funded by the FWO project G.0581.12N. The authors gratefully thank Prof. Dr. Muylde manners (Vrije Universiteit Brussel, Belgium) for kindly supplying the vector pHEN6(c):pEL8-NbVCAM1-His6 used in this research. Further, this study was a part of the Interreg IV-A project “BioIMMEdics” (www.bionimedics.org). In the framework of Interreg IV-A, the financial contribution from the EU and the province Limburg (Belgium) is kindly acknowledged. We further want to acknowledge the financial support from the Interuniversity Attraction Poles Programme (P705) initiated by the Belgian Science Policy Office (BELSPO).

References


duytienta@uhasselt.be