Bioactivity characteristics of trypsin immobilized by affinity interaction and covalent conjugation

Dae Ho Jang and Eun Kyu Lee[+,1]
Bioprocessing Research Lab., Dept. of Chemical Engineering,
[1]Micro Biochip Center,
Hanyang University,
Ansan, Korea 425-791
Tel: 031-400-4072, FAX: 031-408-3779, eklee@hanyang.ac.kr

For technical applications enzymes are usually immobilized to a large variety of supports 1). Also, non-denaturing immobilization of proteins is an important issue in protein chip applications 2). Protein functionality usually decreases when it is immobilized on solid surface because the tertiary structures of a protein could be distorted by immobilization. In order to solve this problem, many researchers have focused on using 'spacer arms' and exploiting 'oriented' immobilization 1~4).

We investigated the effects of immobilization chemistry on the yield of immobilization and the enzymatic activity of the immobilized enzymes, using trypsin as a model protein and aldehyde-decorated macroporous polymer beads (Toyopearl AF Formyl 650M, Tosho co., Japan) as a model matrix. Trypsin was immobilized by two methods; covalent conjugation by reductive amination (at pH 10.0) and affinity interaction via streptavidin-biotin system. The amount of covalently immobilized trypsin was 3.2 mg/ml-gel. It was about three times higher than that of the affinity method. However, when the specific activity of the immobilized trypsin was evaluated by its ability to digest BAPNA (400 Da), human insulin (5,870 Da), and BSA (66,000 Da), the covalent and affinity immobilized trypsin showed ca. 37% and 50%, respectively,of that of the soluble enzyme (on the BAPNA substrate). While the activity of thecovalently immobilized enzyme decreased as the molecular size of the substrate increased, the affinity immobilized trypsin showed higher ac-

tivity on insulin and BSA. This result seemed to indicate the streptavidin-biotin system allowed more steric flexibility in its interaction with a substrate molecule.

To confirm this, we studied the flexibility of immobilized trypsin using quartz crystal microbalance-dissipation (QCM-D). SAMs (self-assembled monolayers) were formed on the Q-sensor surface by aminoalkanethiols, and gultaraldehyde was attached to the SAMs. Trypsin was immobilized on the modified Q-sensors in two ways: reductive amination (at pH 10.0) and the streptavidin-biotin system. The dissipation shift of the affinity immobilized trypsin was 0.5*10-6, whereas that of the covalently attached enzyme was almost zero. Thisindicated the streptavidin-biotin system allowed higher flexibility than the covalent conjugation. These results suggested that the bioactivity of the immobilized enzyme could be strongly dependent on its molecular flexibility.

References

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