Primary screening of capturing antibody for purification of sugar-modified IFN by surface plasmon resonance biosensor

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Abstract

Coupling sugar moiety to proteins is one of the methods to improve the biological circulation stability in blood[1]. We need to purify the sugar-modified proteins from cultured media in a short time. Our aim was to screen the effective antibody for capturing sugar-modified proteins[2]. ELISA and affinity chromatography itself were tried to screen the antibodies. However, these methods have critical defects: long process time and hard to get the kinetic information. We used SPR to solve these problems. SPR was used to figure out "interactions dynamics" between proteins and surface-immobilized ligands. Proteins (100 fmol/mm^2) are affinity-captured from solution to ligands covalently attached to the SPR-sensor chip surface. In this study, we tried to screen the most effective antibody among several antibody culture media. Moreover, we tried to figure out the specific antibody for sugar-modified protein. IFN (30 µg/mL, 22 KD) and anti-IFN antibody culture media (60 different antibodies media, 40 µL) were used. We immobilized native IFN on SPR sensor chip surface (CM5, BIACORE AB, Sweden). Sixty culture media containing 60 types of antibodies were injected to screen out ca. 10 best coupling antibodies. The bound antibodies were recovered from the chip, and immobilized to affinity resin. Then the mixture containing both native and sugar-modified IFNs were introduced to the column to select the antibody that best captured the sugar-modified IFNs. By this way, we can identify the antibody with the highest affinity toward the sugar-modified IFN. It was demonstrated that the SPR was a powerful tool for primary screening of antibody based on its functional affinity with the target protein.

References

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