Applications of Protein Separation Characeristics of SPR Biosensor

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Abstract

Recent developments of surface plasmon resonance (SPR) biosensor technology for biospecific interaction analysis enables us to monitor a variety of biomolecular reactions in real time without labeling. This optical technique detects and quantifies the changes in refractive index in the very vicinity of the sensor chip surface to which ligands are immobilized. Since the changes in refractive index are proportional to the changes in adsorbed mass, the SPR technology allows the detection of minute amount of biomolecules (analyte) interacting with the immobilized ligand. Consequently, SPR biosensor can be used to study the interactions of various biological systems from proteins, oligonucleotides, liposaccharides, and lipids to small molecules, phage, viral particle, and cells.[1)]

We applied this 'quantitative biorecognition' capability of SPR technology to various applications. First, we attempted to use it as an assay method to replace ELISA. Immobilizing the anti-HBsAg polyclonal antibody to the dextran layer on a CM5 chip surface, we could measure HBsAg concentration in an analyte. The binding characteristics between HBsAg and its antibody followed a typical monolayer adsorption isotherm. This study showed the potential of SPR as a rapid, simple, multi-sample, on-line assay tool for HBsAg quantification replacing the current ELISA method.[2)] Also, anchoring the interferon antibody to the sensor chip, we extended this concept to quantify rhIFN and validated this specific bioassay. The result showed that the SPR-based assay method had excellent precision (or reproducibility) and accuracy. Coefficient of variation value was much less than that of ELISA. It also showed strong ruggedness, a wider range of linearity and a lower limit of detection.

Second, we tried to indirectly evaluate the bioactivity and immunogenicity of rhIFN and its surface-modified analogs, e.g., PEGylated IFNs, using this assay. IFN antibody was immobilized and the amount of IFN binding was correlated with the bioactivity and immunogenicity. The result was very close to the antiviral bioactivity result obtained from

the cell proliferation test of MBDK (Madin-Darby bovine kidney) cell culture. The immunogenicity result also coincided well with the ELISA result. It suggested the SPR technology could be applied for in-vitro, rapid and quantitative screening of those biological characteristics.

Finally, we confirmed that the SPR biosensor could detect conformational changes of covalently immobilized proteins on a chip, and used it to monitor the process of solid-phase refolding of the immobilized proteins. In this study, we first observed a positive relationship between the resonance value and a protein's 'degree of unfolding,' induced by a chaotropic denaturant such as urea or guanidine-HCl. When the effect of disulfide bonds on the refolding yield was investigated, we could observe the difference between urokinase (containing 12 disulfides) and disulfide-free myoglobin. Unlike myoglobin, the resonance value of urokinase was not fully recovered after refolding, which suggested that the unrecovered value might represent the mis-shuffled structures. We proposed that this method could monitor the unfolding/refolding status on-line and identify the optimal condition for solid-phase refolding process of immobilized proteins.[3-4)]

References

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