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# Foreword

Physiological responses arise from and are transmitted via molecular interactions in living body between various compounds including endogenous molecules (amino acids, peptides, proteins, nucleotides, lipids, hormones, transporters etc.) and biologically and pharmacologically active exogenous compounds (drugs, pesticides etc.). Therefore, it is a fundamental issue in biomedical and pharmaceutical studies to investigate these molecular interactions qualitatively and quantitatively. So far, several methods of binding analyses have been used for various purposes, such as measurement of binding affinity, identification of specific binding site, investigation of competitive binding and/or allosteric effect, and screening of novel drugs. These methods have their own features, and can be categorized into several groups based on methodological viewpoint. The first group is for the analytical methods utilizing semipermeable membrane. Equilibrium dialysis method and ultrafiltration metehod are classical and are still widely used. These methods are applicable to the binding study between compounds with much different molecular size, and give us the unbound concentration of the compound with the smaller molecular size. The binding parameters can be determined by the following mathematical data-treatment such as Scatchard analysis. The binding analyses based on spectroscopy form the second group. Several fluorescent, ultraviolet or visible binding probes possibly give us both quantitative information of binding affinity as well as qualitative information such as the location of specific binding site on ligand molecule. Other spectroscopic methods such as circular dichroism, NMR, and Raman spectroscopy are also available to binding study. The third group is for the binding analysis utilizing liquid phase separation system such as liquid chromatography and electrophoresis. This group involves several different modes such as frontal analysis, Hummel-Dreyer method and affinity CE mode. In addition, some other types of binding analyses are also available, such as ultracentrifugation and differential scanning calorimetry. Surface plasmon resonance (SPR), which determines kinetic binding rate constants as well as the binding constant, has become popular very rapidly in this decade.

Although HPLC was originally developed as a powerful tool of separation, very soon it was found that HPLC is also applicable to evaluate physicochemical properties. Because chromatographic retention is governed by the physicochemical properties of solutes, stationary phase and mobile phase, these properties can be conversely evaluated by the retention behavior. For example, some molecular characteristics of solute such as hydrophobicity, planarity and specific hydrophilic and lipophilic surface can be estimated by measuring the dependence of capacity factor upon organic modifier content in mobile phase. Enthalpy effect and entropy effect are evaluate by van't Hoff plot. The binding constant between solute and additive in mobile phase is determined by measuring the dependence of the capacity factor of the solute upon the additive concentration.

So far, bioaffinities have been successfully incorporated into HPLC system to achieve highly selective separation. Affinity chromatography using antigen-antibody interaction and chiral separation using protein-immobilized column are typical examples. Because the biological and biochemical affinities are the driving force of separation, they can be conversely evaluated by measuring the retention

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behavior. Same as in case of HPLC, biological and biochemical affinities have been incorporated into CE system, and these binding affinities can be evaluated by monitoring migration behavior.

The binding analyses utilizing liquid phase separation systems have unique features. They enable high throughput analyses because of their simple procedure, rapid analytical time, and, in case of affintiy HPLC mode, the repeated use of ligand-immobilized columns. This feature is advantageous to large-scale screening works such as drug development. Some of these methods enable binding analyses with samples which are not very pure because of the fundamental separation ability of the system. In addition, binding affinities of two or more compounds may be evaluated simultaneously by using their mixed sample. For example, the protein binding affinity of each enantiomer of a chiral drug can be determined separately by affinity CE mode using racemic mixture. Another feature is an easy access and on-line connection to other separation mode and/or analytical instrument. For example, highly sensitive and stereoselective binding study can be achieved by the hyphenization of frontal analysis with preconcentration and chiral HPLC separation. Because the binding analyses utilizing separation system do not use any membrane, they are free from adsorption of compounds onto membrane and leakage of bound form from membrane, which are terrible problems in widely used equilibrium dialysis and ultrafiltration methods. Micro-scale binding analyses using CE system is a beneficial feature, especially for the binding study of rare compounds of which the large-scale preparation is difficult. The advance of micro-TAS will bring further advantage to the micro-scale analysis. In addition, the liquid phase separation systems have the potential advantage to mimic the circumstance of biological fluid.

The present topical issue is focused on the analysis of bioaffinity using separation system. The principle and the features of representative methods are reviewed, and their recent application studies are reported. We hope that this topical issue serves to the better understanding of binding analysis, provides updated overview of the recent state in this research field, and gives information useful and helpful to the scientific research of readers.

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