PROTOCOL

Epitope Mapping by Surface Plasmon Resonance in the BIAcore

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Abstract

An epitope may be defined as a specific site on an antigen module characterized by the binding of one monoclonal antibody (MAb). Epitope mapping by surface plasmon resonance in the BIAcore biosensor may be performed to characterize an antigen or a group of specific MAbs or both. This article describes the BIAcore instrument and methods for such mapping. Examples include molecular interaction studies with simple and complex proteins, such as myoglobin and calprotectin, respectively.

Index entries: Epitope mapping; surface plasmon resonance; biomolecular interaction; monoclonal antibody; myoglobin; calprotectin.

1. Introduction

1.1. The BIAcore Instrument

The BIAcore (biomolecular interaction analysis) analytical system consists of a detection unit, an autosampler, and a liquid delivery system, controlled by a computer. Karlsson et al. (1) have given a detailed description of the analytical system, the detection principle, and the theoretical background for binding measurements. The system combines a microfluidic unit in contact with a sensor for surface plasmon resonance (SPR) detection. Figure 1 shows the principle of SPR detection. The sensor chip consists of a glass slide mounted in a plastic frame. On one side of the glass, a thin film, approx 50 nm, of gold is deposited, and the dextran matrix is attached on top of this film. The sensor chip is inserted into the instrument with the dextran/gold side in contact with the flow cells. When the injected sample is passing through the flow cell, antigen binds to immobilized antibody in the dextran matrix. Light covering a span of angles of incidence falls on the glass side and is reflected into a 2D array detector where the intensity of the reflected light is measured. SPR occurs at a certain angle and is seen as a minimum in reflected light intensity. When the refractive index close to the gold film is changed, for example, when immobilized antibody binds antigen, the angle at which SPR occurs is changed. This change is proportional to the amount of bound protein, and is expressed as refractive units (RU) on the Y-axis of a sensorgram; 1000 RU corresponds to 1 ng protein/mm² of the 100-nm thick dextran layer. Reproducibility of the system has been validated by Fagerstam et al. (2).

1.2. Antigen–Antibody Interaction

An epitope is a specific site on an antigen molecule defined by the binding characteristics of one monoclonal antibody (MAb) (3,4). Structural and functional approaches to the study of protein antigenicity have led to two different perceptions of the nature of protein epitopes (4). The structural approach concentrates on the spatial arrangement of atoms found in the antigen–antibody complex and shows that at least 15 amino acid residues may be implicated in each epitope. The functional approach, which introduces the additional dimension of time, takes the form of crossreactive binding measurements and leads to the view that a smaller number of residues are implicated in each epitope. Functional binding assays are operational in character, and it must be considered that differ-
Fig. 1. The principle of SPR signal detection. Surface plasmon resonance detects changes in refractive index of the surface layer of a solution in contact with the sensor chip. The change in refractive index is caused by variation of the mass on the sensor chip surface owing to interactions of the biomolecules (A,B). A dip in the intensity of the reflected light occurs at a certain angle, which is referred to as the resonance signal (RU) (C). The shift in resonance signal is plotted against time and displayed in a sensorgram (from ref. 1).

different types of epitopes are identified by the use of different probes (4). With the BIAcore, we are taking the functional approach.

1.3. Epitope Mapping

Epitope mapping in the BIAcore may be performed to characterize an antigen or a group of specific monoclonal antibodies, or both (5). High affinity antibodies are important in most immunochemical techniques and essential to immunoassay sensitivity (6). However, binding kinetics are affected by binding conditions, immobilization, labeling, or conjugation of the reactants as well as the flexible nature of proteins (6–8), as we have shown in a recent BIAcore study (7) by comparing native and colloidal gold-labeled MAbs.

Epitope mapping with conventional EIA or RIA is time-consuming, including elaborate labeling methods, and considerable amounts of purified reactants are needed. With the BIAcore, there is no labeling of the reactants, purification is not necessary (cell supernatants or ascites can be used), and small amounts of the reactants are sufficient for epitope mapping. The fully automated system can handle large mapping matrices overnight. The association and dissociation rate