Epitope Mapping: B-cell Epitopes

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Advanced article

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doi: 10.1002/9780470015902.a0002624.pub2

Immunoglobulin molecules are folded to present a surface structure complementary to a surface feature on the antigen – the epitope is this feature of the antigen. Epitope mapping is the process of locating the antibody-binding site on the antigen, although the term is also applied more broadly to receptor–ligand interactions unrelated to the immune system.

Introduction

Immunoglobulin molecules are folded in a way that assembles sequences from the variable regions of both the heavy and light chains into a surface feature (comprised of up to six complementarity-determining regions (CDRs)) that is complementary in shape to a surface structure on the antigen. These two surface features, the 'paratope' on the antibody and the 'epitope' on the antigen, may have a certain amount of flexibility to allow an 'induced fit' between them. In the narrow sense, epitope mapping is the process of locating the antibody-binding site on the antigen. The term is also applied in a broader sense to T-cell epitopes (Stern and Wiley, 1994) and has even been applied to receptor–ligand interactions unrelated to the immune system. Only B-cell epitopes are relevant to the biochemical understanding of antibody–antigen interactions. **See also**: Antibodies; Immunoglobulin Fold: Structures of Proteins in the Immunoglobulin Superfamily

What Is a B-cell Epitope?

In the case of protein antigens, B-cell epitopes consist of groups of amino acids that lie close together on the protein surface and that determine antigenicity. Epitope mapping is usually done with monoclonal antibodies (mAbs), though it can be done with polyclonal antisera in a rather less specific way. **See also**: Antigens; Epitopes; Monoclonal Antibodies

Van Regenmortel 1989 made the important distinction between 'structural' epitopes that are 'in contact' with the antibody, as defined by X-ray crystallography and 'functional' epitopes defined by amino acid residues that are important for binding the antibody and cannot be replaced.

It is also essential to distinguish between conformational ('discontinuous', 'assembled') epitopes, in which amino acids far apart in the protein sequence are brought together by protein folding, and linear ('continuous', 'sequential') epitopes, which can often be mimicked by simple peptide sequences (Berzoksky, 1985). Most native proteins are formed of highly convoluted peptide chains, so that residues that lie close together on the protein surface are often far apart in the amino acid sequence (Barlow *et al.,* 1986). Consequently, most epitopes on native, globular proteins are conformation-dependent and they disappear if the protein is denatured or fragmented. Sometimes, by accident or design, antibodies are produced against linear (sequential) epitopes that survive denaturation, though such antibodies usually fail to recognize the native protein. The simplest way to find out whether an epitope is conformational is by Western blotting after SDS-PAGE. If the antibody still binds after the protein has been denatured, the epitope is unlikely to be highly conformational. It must be remembered, however, that few proteins are completely linearized by denaturation and some epitopes identified by Western blotting may still have a conformational element. **See also**: Proteins: Fundamental Chemical Properties; Protein Folding *In Vivo*; Western Blotting

Epitope Mapping Methods

X-ray crystallography is a 'structural' approach to epitope mapping that can identify contact residues in antigen–antibody complexes, but needs good crystals of complexes and has usually been used for highly conformational epitopes on the surface of soluble proteins. Nuclear magnetic resonance (NMR) is another 'structural' approach that is performed in solution and thus avoids the need for crystals. Since NMR is limited to peptides and small proteins, it may be unsuitable for highly assembled epitopes. Electron microscopy is a low-resolution method of 'structural mapping' which, although unable to identify contact residues, can be used to identify the location of epitopes on very large antigens such as whole viruses. **See also**: Electron Microscopy; Macromolecular Structure Determination by X-ray Crystallography; Nuclear Magnetic Resonance (NMR) Spectroscopy of Proteins

The remaining epitope mapping methods are essentially 'functional' in approach and can be divided into four groups: (1) competition methods, (2) antigen modification methods, (3) fragmentation methods and (4) the use of synthetic peptides or peptide libraries.

Competition methods can be very useful when a relatively low degree of mapping resolution is adequate. You may want to establish, for example, that two mAbs recognize different, nonoverlapping epitopes for a two-site immunoassay, or to find mAbs against several different epitopes on the same antigen so that results due to cross-reactions with other proteins can be rigorously excluded. The principle behind competition methods is to determine whether two different mAbs can bind to a monovalent antigen at the same time (in which case they must recognize different epitopes) or whether they compete with each other for the same antigen epitope. **See also**: Immunoassay

Protection of the antigen from acetylation by the presence of antibody is applicable to conformational epitopes and is considered more reliable than direct chemical modification, which may affect antigen structure and give false positive results. Protection from proteolytic digestion, also known as 'protein footprinting', is similar in principle; antigens are exposed to proteases in the presence or absence of antibody (which is fairly protease-resistant) and differences in digestion are detected by gel electrophoresis. If the antigen can be expressed from recombinant cDNA and the approximate position of the epitope is known, specific mutations can be introduced by site-directed mutagenesis methods. Alternatively, random mutations can be introduced into part of the antigen by polymerase chain reaction (PCR), followed by screening to detect epitope-negative mutants. The 'escape mutant' approach for viral surface epitopes recognized by neutralizing antibodies involves selection and sequencing of spontaneous mutants whose infectivity is no longer blocked by the antibody.

A simple fragmentation approach for epitopes that survive denaturation is partial protease digestion of the antigen, followed either by Western blotting for larger fragments or by high-performance liquid chromatography (HPLC). The fragments that bind antibody can be identified by *N*-terminal microsequencing or by mass spectrometry. Chemical cleavage at specific amino acids is a commonly used alternative method for generating antigen fragments. **See also:** Liquid Chromatography; Mass Spectrometry in Biology

If the antigen can be expressed from recombinant cDNA, digestion with DNAase I, followed by cloning and expression of the cDNA fragments to create 'epitope libraries', is a popular way of generating overlapping antigenic fragments. Several methods involve random shortening of the antigens produced from plasmid vectors, including transposon mutagenesis and exonuclease III digestion. The power of this approach can be increased by incorporating phage display methodology, in which the antigen fragments are displayed on the surface of filamentous phage. This has the important advantage that antibody-positive clones can be obtained by selection rather than screening. This approach was taken one step further in 'mRNA display', in which advantage is taken of the mechanism of puromycin action to make a direct covalent link between a library of expressed RNA sequences and the peptides they encode, enabling co-selection by antibody of antigenic epitope and its corresponding nucleotide sequence (Wilson *et al*., 2001). **See also**: Phage Display Technologies

Overlapping peptides for mapping sequential epitopes can be synthesized on pins (PEPSCAN), on a cellulose membrane support (SPOTS) or on micro-arrays. Combinatorial libraries of random peptide sequences in solution can also be used. The advent of random peptide libraries displayed on the surface of filamentous bacteriophage took this approach a vital step further by enabling selection of displayed peptides instead of screening. Selection of random peptides is unique in producing a range of sequences that are related, but not identical, to the antigen sequence; this enables inferences to be made about which amino acids in the epitope are most important for antibody binding. Peptide libraries can also be displayed on the surface of *Escherichia coli* in the major flagellum component, flagellin. An advantage shared by all peptide methods is that antigen is not required and this may be important for 'rare' antigens that are difficult to purify. Phage-displayed peptides may also mimic conformational epitopes although they do not correspond to any linear sequence in the antigen ('mimotopes'). Computational methods are being developed both to identify conformational epitopes (Haste Andersen *et al*., 2006) and to predict immunogenic epitopes (Flower, 2003). **See also**: Bacteriophage Display of Combinatorial Antibody Libraries

There is increasing interest in developing mass spectrometric (MS) approaches to epitope mapping because of direct sequencing capability, applicability to conformational and posttranslationally modified epitopes and the possibilities for automation (Hager-Braun and Tomer, 2005). In general, tryptic peptides that bind to antibody, or which are protected by antibody from hydrogen–deuterium exchange, are identified by LC-MS-MS, but 100% coverage of antigen sequence with tryptic peptides is often hard to achieve.

Applications

Apart from its intrinsic value for understanding protein interactions, epitope mapping also has a practical value in generating antibody probes of defined specificity as research tools and in helping to define the immune response to pathogenic proteins and organisms. Mapping of antibodies that inhibit protein function (e.g. enzyme activity) can be used to determine which parts of the protein are involved in that function. Antibodies with known binding sites have also been used to study the topology of transmembrane proteins, the domain structure of proteins, the orientation of proteins in relation to intracellular structures and alternative gene products produced by genetic deletion. **See also**: Antibody Synthesis in Vitro; Protein–Protein Interactions

The term 'epitope mapping' has also been used to describe the attempt to determine all major sites on a protein surface that can elicit an antibody response in mice or humans. This information might be very useful, for example, to someone wishing to produce antiviral vaccines or

diagnostic antigens. However, the map obtained may be influenced by how mAbs are selected and by the mapping method, which may not detect conformational epitopes. The immune response also varies between species, strains and even individuals, but useful epitopes may still be identifiable. Mapping antibodies in human serum samples is a useful approach to characterizing the human immune response (Pereboeva *et al.,* 2000) although it is complicated by the fact that antisera behave as a complex mixture of mAbs.

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Further Reading

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