MODELING OF ANTIGEN RECEPTOR SITES IN MOLECULAR IMMUNOLOGY

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ABSTRACT: The process of locating and identifying the sites on the surface of antigens where the corresponding antibodies can bind is known as Epitope mapping. The phenomena of epitope mapping can form an integral measure in the discovery and development of new vaccines, therapeutics and diagnostics (Gershoni, 2007; Ganguly, 2013).

Keywords: Epitope mapping, Vaccines.

INTRODUCTION

Structure of epitope can be linear or conformational model. Linear epitopes are formed by a continuous sequence of amino acids in a protein, while conformational epitopes are composed of amino acids that are discontinuous in the protein sequence but are brought together upon three-dimensional protein folding (Westwood and Hay, 2001; Ganguly, 2013).

CONVENTIONAL METHOD

Many methods are known for epitope mapping among which X-ray crystallography method is the significant one. However, it requires large amounts of purified protein and is expensive and time-consuming. Another method for this purpose is peptide scanning. This technique uses a library of short peptide sequences from overlapping segments of a target protein and tests for their ability to bind the antibody of interest. This method is faster and relatively inexpensive, but is primarily used for mapping linear, not conformational, epitopes. Site directed mutagenesis is the other significant method in which systematic mutations of amino acids are introduced into a protein sequence followed by measurement of antibody binding in order to identify amino acids that comprise an epitope. Amino acids are mutated systematically in this method for introduction in a protein sequence and quantification of antibody binding in order to identify amino acids that comprise an epitope is also possible. This technique has the advantage of mapping both linear and conformational epitopes, but is
labor-intensive and slow, typically limiting analysis to a small number of amino acid residues. The process of mapping epitope structures of complex target antigens like integral membrane proteins or multi-subunit proteins is quite difficult as expression and purification of these types of antigens poses a challenge.

**OTHER EXPERIMENTAL METHODS**

Another method known as overlapping peptide scan or *pepscan analysis* in which a library of oligopeptide sequences from overlapping and non-overlapping segments of a target protein is used and their ability for binding the antibody of interested in analyzed. This method is less time consuming and economical to test large array of candidate antibodies and proteins of interest by combining non-adjacent peptide sequences from different parts of the target protein and enforcing conformational rigidity onto this combined peptide such as by using CLIPS scaffolds (Timmerman *et al.*, 2009). Even randomly distributed epitopes can be engineered with high sensitivity and precision (Cragg, 2011).

By measuring the extent of antibody binding in order to identify amino acids that comprise an epitope, site directed mutagenesis is designed by which both conformational as well as linear epitopes can be modeled. The major drawback of this technique is that it is lengthy and time consuming and is limited to be used on limited number of amino acid and protein residues (Linnebacher *et al.*, 2012).

In mutagenesis mapping, clones containing unique amino acid mutation and the entire library covering every amino acid in the target protein are utilized in a comprehensive mutation library (Gaseitsiwe *et al.*, 2009). There occurs loss in reactivity of amino acids and protein structures are modeled for epitope visualization and measurements. Panel of antibodies against human CCR5, a GPCR co-receptor for HIV entry (Paes *et al.*, 2009) has been used by this methodology.

Other commonly used methods for epitope engineering and modeling include phage display with limited proteolysis, providing high throughput but lack reliability, especially for conformational epitopes (Timmerman *et al.*, 2009).

**NOVEL METHOD OF EPITOPE MAPPING**

Recently, a novel method for epitope mapping of membrane proteins has been developed by Integral Molecular, a biotechnology company in Philadelphia dedicated to developing platform based technologies for the advancement of membrane protein research. This method is called *Shotgun Mutagenesis Mapping* (Integral molecular Membrane protein...
solutions Report, 2012). The technique uses comprehensive mutation library, with each clone containing a unique amino acid mutation and the entire library covering every amino acid in the target protein. In this method, numerous clones are individually arrayed in microplates, expressed in human cells and simultaneously tested for binding to an antibody of interest. Amino acids that are required for antibody binding can be identified by a loss of reactivity and mapped onto protein structures to visualize epitopes (Banik and Doranz, 2010). By this procedure, target proteins can be expressed in mammalian cell lines without purifying them, so epitope maps of antibodies against complex proteins can also be conveniently identified. This approach has recently been used for epitope mapping a panel of antibodies in human HIV infection to detect the virus entry into the host (Linnebacher et al., 2012).

REFERENCES

