

A Radical Probe of Antigen Structure and Antigen-Antibody Interactions Using Synchrotron Radiation and Mass Spectrometry

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The human immune system is capable of producing antibodies that recognize and bind millions of protein antigens by virtue of the highly variable sequences within the paratopes. These paratopes contact and bind antigen in a conformation-dependent process. While X-ray crystallography has played an important role in the study of immune complexes, the technique first requires that the complex is isolated in a pure crystalline form. This is often an impossible task. Once in this state, it is not possible to probe changes in binding in response to the solvent environment. Furthermore, crystallographic methods identify residues in close proximity in the antigen-antibody complex irrespective of whether they are functional in the immune response. The union of synchrotron radiation and mass spectrometry represents a powerful approach for probing structural aspects of the immune response on millisecond timescales. The X-ray radiolysis of aqueous solutions generates a hydroxyl radicals that react with protein antigens through the oxidation of particular amino acid residues. The rate of reaction is influenced in part by the accessibility of these residues to the bulk solvent. By monitoring the rate of oxidation at these residue markers, a three-dimensional footprint of an antigen's structure can be constructed. Some residues will be shielded from solvent in the antigen-antibody complex thus allowing the contact surface of the antigen to be mapped. Mass spectrometry has been used to both identify the site of modification and provide a quantitative measure of the oxidation rate.