A New Generation of Reagents for Immunosensors: Recombinant Antibodies that Recognize Metal–Chelate Complexes

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A subgoal of our present project is to develop new protein reagents that will provide superior performance in the hand-held immunosensor. In support of this specific aim, we have expressed two antibodies, 5B2 and E5, as recombinant $F(ab)'_2$ fragments and subsequently used these constructs to identify residues important in the recognition of metal–chelate complexes. Antibody 5B2 was raised to a Pb(II)-loaded isothiocyanatobenzyl-diethylenetriamine pentaacetic acid (DTPA)-protein conjugate. The native antibody bound to complexes of Pb(II)-*p*-aminobenzyl-DTPA with an affinity of 4.6×10^{-9} M. A monovalent Fab fragment prepared from the native protein and the bivalent recombinant fragment exhibited comparable affinities for the same Pb(II)–chelate complex, approximately six times lower than that of the intact antibody. Covalent modification of lysine residues and molecular modeling predicted that Lys^{58} in the heavy chain contacted the Pb(II)–chelate ligand. Mutational analysis supported a role for Lys^{58} in ion pair or hydrogen bond formation with the carboxylate groups on the chelate. Antibody E5 was directed toward an isothiocyanatobenzyl-ethylenediamine tetraacetic acid (EDTA)-protein conjugate that was loaded with ionic Cd(II). The native immunoglobulin recognized Cd(II)-*p*-aminobenzyl-EDTA with an affinity of 8.2×10^{-12} M. A proteolytically derived $F(ab)'_2$ fragment and the bivalent recombinant fragment bound to the same Cd(II)–chelate complex, with affinities that were comparable to that of the native antibody. Homology modeling and mutagenesis identified three residues (Trp^{52} and His^{96} in the heavy chain and Arg^{96} in the light chain) that were important for Cd(II)–chelate recognition. His^{96} likely mediates a direct ligation to the Cd(II) ion, and Trp^{52} appears to be involved in hydrophobic stacking with the benzyl moiety of the chelator. Arg^{96} appeared to mediate an electrostatic or hydrogen bond to the chelate portion of the complex. These modeling/mutagenesis studies provide insight into the molecular interactions most important in antigen recognition and will ultimately lead to engineered antibodies specifically designed to provide superior performance in immunosensor applications.