Abstract

Antibodies stand as a cornerstone of many laboratory assays and are used as indispensable tools in biomedicine. iBodies polymer conjugates have been proposed as a possible alternative to antibodies in several traditionally immunological methods. iBodies are based on methacrylamide copolymers decorated with affinity anchors, reporters and targeting ligands which mediate conjugate specificity. The necessity to design ligands and their linkage is a significant obstacle to the generalization of this technology. One way of overcoming this obstacle is the development of secondary antibody mimetics which will use antibodies to obtain specificity for target identification and/or visualization.

Based on the literature, several cyclic peptide Fc-binding ligands were designed with linker moieties for attachment to the polymer conjugate. Free and conjugated ligands were compared based on their interaction constants with human IgG. Conjugation with polymer carrier in all cases led to an increase in affinity. Human isotype specificity is dependent on the cyclization method, with single disulfide bridge cyclization exhibiting selectivity for IgG1-4 and IgM, and N→C cyclization leading to abolition of IgM binding.

The polymer conjugates have been compared in terms of sensitivity and selectivity using dot blot with several commercial secondary antibodies. As a visualization agent, conjugates exhibit selectivity and sensitivity throughout a panel of 16 antibody species comparable to protein G. However, secondary antibodies in comparison to polymers still dominate in their respective idiotype with sensitivity higher by one to four orders of magnitude.

Based on the available data, further optimization of targeting peptide ligand is necessary in order to obtain performance comparable to secondary antibodies. Amino acid substitution preferences in targeting peptide for antibodies of selected species were obtained through analysis of a combinatorial peptide library. Uncovered motifs will be used as a basis for further optimization of Fc-binding ligands and their conjugates.

Keywords: iBody; antibody mimetics; molecular recognition; binding constants; cell receptors