A novel strategy for the rapid preparation and isolation of intact immune complexes from peptide mixtures[†]

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Abstract

The development and application of a miniaturized affinity system for the preparation and release of intact immune complexes are demonstrated. Antibodies were reversibly affinity-adsorbed on pipette tips containing protein G´ and protein A, respectively. Antigen proteins were digested with proteases and peptide mixtures were exposed to attached antibodies; forming antibody–epitope complexes, that is, immune complexes. Elution with millimolar indole propionic acid (IPA)-containing buffers under neutral pH conditions allowed to effectively isolate the intact immune complexes in purified form. Size exclusion chromatography was performed to determine the integrity of the antibody–epitope complexes. Mass spectrometric analysis identified the epitope peptides in the respective SEC fractions. His-tag-containing recombinant human glucose-6-phosphate isomerase in combination with an anti-His-tag monoclonal antibody was instrumental to develop the method. Application was extended to the isolation of the intact antibody–epitope complex of a recombinant human tripartite motif 21 (rhTRIM21) auto-antigen in combination with a rabbit polyclonal anti-TRIM21 antibody. Peptide chip analysis showed that antibody–epitope binding of rhTRIM21 peptide antibody complexes was not affected by the presence of IPA in the elution buffer. By contrast, protein G´ showed an ion charge structure by electrospray mass spectrometry that resembled a denatured conformation when exposed to IPA-containing buffers. The advantages of this novel isolation strategy are low sample consumption and short experimental duration in addition to the direct and robust methodology that provides easy access to intact antibody–antigen complexes under neutral pH and low salt conditions for subsequent investigations.