Facile fabrication and instant application of miniaturized antibody-decorated affinity columns for higher-order structure and functional characterization of TRIM21 epitope peptides.

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Abstract

Both epitope excision and epitope extraction methods, combined with mass spectrometry, generate precise informations on binding surfaces of full-length proteins, identifying sequential (linear) or assembled (conformational) epitopes, respectively. Here, we describe the one-step fabrication and application of affinity columns using reversibly immobilized antibodies with highest flexibility with respect to antibody sources and lowest sample amount requirements (fmol range). Depending on the antibody source, we made use of protein G- or protein A-coated resins as support materials. These materials are packed in pipet tips and in combination with a programmable multichannel pipet form a highly efficient epitope mapping system. In addition to epitope identification, the influence of epitope structure modifications on antibody binding specificities could be studied in detail with synthetic peptides. Elution of epitope peptides was optimized such that mass spectrometric analysis was feasible after a single desalting step. Epitope peptides were identified by accurate molecular mass determinations or by partial amino acid sequence analysis. In addition, charge state comparison or ion mobility analysis of eluted epitope peptides enabled investigation of higher-order structures. The epitope peptide of the TRIM21 (TRIM: tripartite motif) autoantigen that is recognized by a polyclonal antibody was determined as assembling an "L-E-Q-L" motif on an α-helix. Secondary structure determination by circular dichroism spectroscopy and structure modeling are in accordance with the mass spectrometric results and the antigenic behavior of the 17-mer epitope peptide variants from the full-length autoantigen.