

Noncompetitive Detection of Low Molecular Weight Peptides by Open Sandwich Immunoassay

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Small peptides with less than 1000 in molecular weight are not considered amenable to sandwich immunoassays due to their difficulty of simultaneous recognition by two antibodies. As an alternative, we attempted noncompetitive detection of small peptides by open sandwich enzyme-linked immunosorbent assay (OS-ELISA) utilizing the antigen-induced enhancement of antibody V_H/V_L interaction. Taking fragments of human osteocalcin (BGP), a major non-collagen peptide produced in bone, as model peptides, OS immunoassay was performed using the cloned V_H and V_L cDNAs from two anti-BGP monoclonal antibodies either recognizing the N- or C-terminal fragment, respectively. When the clones were used for OS-ELISA with immobilized V_L fragment and phage-displayed V_H fragment, enhanced V_H / V_L interaction upon BGP addition was observed. Especially the clone for the C-terminal fragment showed superior detection limit as well as a wider working range than those of competitive assay. The result was reproducible with either purified V_H-alkaline phosphatase or peroxidase-conjugated MBP-V_H, together with immobilized MBP-V_L fusion proteins, and in the latter case the assay can be performed on microplate wells and also in microfluidics. The minimum detectable fragment was the hexamer including the C-terminus, implying hapten-like terminal recognition. To further improve the sensitivity of the assay, a phage-displayed PCR-randomized V_H library was subjected to repeated selections on MBP-V_L in the presence of reduced amount

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of peptide (OS selection). The selection successfully gave a clone with ten-fold lower detection limit, which was well below the serum peptide level in healthy human. This simple approach with a single antibody with a short measurement time may prove a useful tool in immunodiagnostics as well as in proteomics research.

Reference

S. -L. Lim et al., *Anal. Chem.* **79**, 6193 (2007)

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