HAPten IMMUNODETECTION WITH NANOPEPTAMERS CONSTRUCTED WITH THE PENTAMERIC PROTEIN VEROROTOXIN

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Small compounds cannot bind simultaneously to two antibodies, and thus, their immunodetection is limited to competitive formats in which the analyte is indirectly quantitated by measuring the unoccupied antibody binding sites using a competing reporter. This limitation can be circumvented by using phage-borne peptides selected for their ability to specifically react with the analyte-antibody immunocomplex, which allows the detection of these small molecules in a noncompetitive format (PHAIA) with increased sensitivity and a positive readout. In an effort to find substitutes for the phage particles in PHAIA, we explore the use of the B subunit of the Shiga-like toxin of Escherichia coli, also known as verotoxin (VTX), as a scaffold for multivalent display of anti-immunocomplex peptides. Using the herbicides molinate and clomazone as model compounds, we built peptide-VTX recombinant chimeras that were produced in the periplasmic space of E. coli as soluble pentamers, as confirmed by multiangle light scattering analysis. These multivalent constructs, which we termed nanopeptamers, were conjugated to a tracer enzyme and used to detect the herbicide-antibody complex in an ELISA format. The VTX-nanopeptamer assays performed with over a 10-fold increased sensitivity and excellent recovery from spiked surface and mineral water samples. The carbon black-labeled peptide-VTX nanopeptamers showed great potential for the development of a lateral-flow test for small molecules with a visual positive readout that allowed the detection of up to 2.5 ng/mL of clomazone. In this work, we built VTX-based nanopeptamers using antiimmunocomplex peptides selected against the herbicides molinate and clomazone bound to their cognate antibodies. These nanopeptamers were produced at high yields as soluble recombinant proteins and showed assay performances similar to the respective phage-borne peptides.

Keywords: nanopeptamers, PHAIA, haptens