On Bead Detection Of Dioxin Using Dioxin Binding Pentapeptide

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Dioxins are highly toxic and persistent, so it is very important to detect these chemicals in environmental samples, especially from soil and sediment. The purposes of this study are to screen peptides that bind dioxin and to develop a dioxin detection method using the obtained peptide sequence. Using the technique of combinatorial chemistry, we succeeded in the screening of dioxin binding pentapeptide. A pentapeptide library was constructed using a solid phase split synthesis approach. Peptide beads were suspended in a buffer (20 % 1,4,-dioxiane, 10 mM phosphate buffer (pH8.2)) containing florescent-labeled 3,4-dichlorophenoxypropylamine as a fluorescent bait for the screening. Fluorescence intensities of the stained beads were determined using a fluorescence microscopy equipped with a CCD camera and an image analyzing software. Sixty fluorescent peptide beads which bound to fluorescent bait from 2.5 million beads library were selected. The beads were then suspended in a solution containing 1 nM fluorescent bait and 10 nM 2,3,7-trichlorodibenzo-*p*-dioxin (2,3,7-TriCDD). Disappearance of fluorescence on beads is an evidence of the competitive binding of dioxin against the fluorescent bait to peptide on bead. Two peptide beads were finally selected.

The binding affinities of the two screened peptides to 2,3,7-TriCDD or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TeCDD) were analyzed by on beads competitive assay using fluorescent bait. Schematics of dioxin detection were shown in Fig. 1. The dioxin binding peptide beads were incubated with the fluorescent bait containing various concentration of dioxin in the detection buffer.

The calibration graph representing the correlation between 2,3,7-TriCDD concentration and fluorescent intensity is shown in Fig. 2. The fluorescent intensity on bead decreased with the increment of dioxin concentration. The calibration was fitted by a equation often used for competitive ELISA, $y=(a-d)/(1+(x/c)^b)+d$. The value of IC_{50} against 1 nM fluorescent bait was 1.3 nM. And the detection limit of the dioxin concentration was 0.7 nM (0.2 ng/ml). These are almost the same level as the monoclonal anti-dioxin antibodies. It is marvelous that the only five amino acid peptide achieves sub-ppb level detection of dioxin without any special sensitizing apparatus. The significant advantages of synthetic peptide are usability in organic solvent and ease of quality control of the material compared to immuno antibodies. We are expecting the potential of this peptide to be an alternative for natural immuno antibodies.

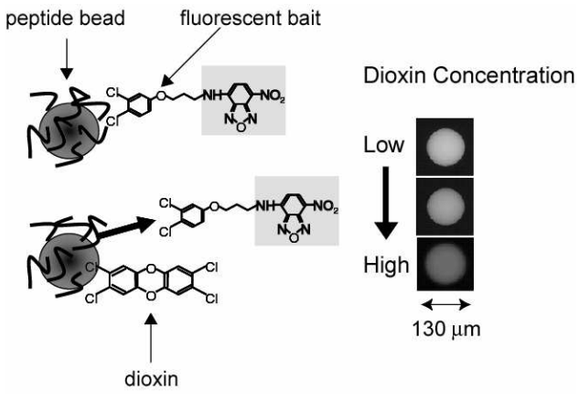

Fig. 1. Schematic of dioxin detection

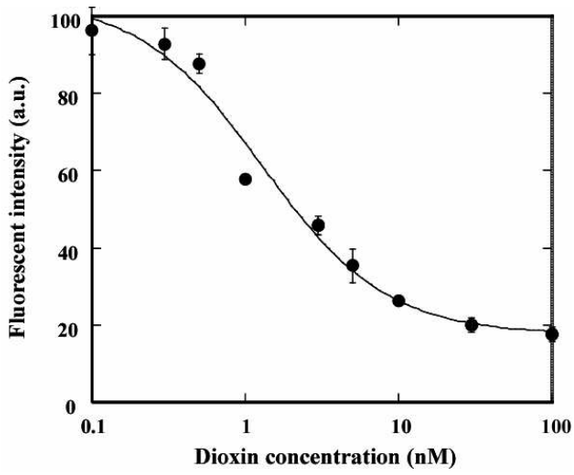

Fig. 2. Calibration graph between fluorescent intensity and concentration of 2,3,7-TriCDD