Analysis of binding of fluorescent bait to dioxin binding pentapeptide on resin

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Dioxins are environmental pollutants and well known toxic compounds. Dioxins are formed as an unintentional by-product of many industrial processes such as waste incineration, chemical and pesticide manufacturing and pulp and paper bleaching. Polychlorinated dibenzo-*p*-dioxins (PCDDs) have 75 positional congeners and particularly 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TeCDD) is known as the most toxic congener. Dioxins are highly toxic and persistent, so it is very important to detect these chemicals in environmental samples.

Recently, we succeeded in screening pentapeptide antibody binding dioxin from a combinatorial library. From 2.5million pentapeptide beads library utilizing 19 natural amino acids except for cysteine, we obtained 2 dioxin binding pentapeptide beads. Peptide synthesized by solid phase Fmoc chemistry is uniformly provided and is a cost effective material, thus, it is expected to be a new material as a dioxin binder instead of immuno antibody.

Dioxin detection method using the dioxin binding pentapeptide on resin bead is shown in Fig. 1. Both dioxin and *N*-NBD-3-(3',4',-dichlorophenoxyl)-1-propylamine (fluorescent bait) are bound to peptide bead competitively. So the fluorescent intensity on bead disappears with increment of dioxin concentration.

In this study, binding fluorescent bait to the peptide bead was analyzed. The dioxin binding peptide beads were incubated with fluorescent bait in a buffer (20 % 1,4-dioxiane, 10 mM phosphate buffer (pH 8.2)). Then, a stained bead image was photographed by using a fluorescence microscope equipped with a CCD camera. Time course of fluorescent intensity of an image of a bead was represented in Fig. 2. Evaluation of binding kinetics was done using the equations shown in Fig. 2. A binding constant (K_A) of 8×10^9 M^{-1} was obtained from the curve fitting. IC_{50} of 2,3,7-trichlorodibenzo-*p*-dioxin (2,3,7-TriCDD) against fluorescent bait of 1 nM was 1.3 nM. Thus binding constant of 2,3,7-TriCDD must be more than 10^9 M^{-1}. The peptide bead could be an alternative for natural antibodies in dioxin detection method.

Moreover effect of dye moiety of fluorescent bait on affinity was investigated. Four kinds of fluorescent baits were newly synthesized using coupling dyes, Alexa Fluor 350 (Ex:345nm, Em:440nm), Dansyl chloride (Ex:372 nm, Em:518 nm), FITC-I (Ex:495 nm, Em:520 nm), Alexa Fluor 633 (Ex:630 nm, Em:650 nm). Only Dansyl chloride labeled bait indicated a potential to be bound to the dioxin binding peptide but the competitive binding against 2,3,7-TriCDD could not be observed. This result implies that the structure of fluorescent dye strongly affects binding fluorescent bait to the peptide bead. In other words, NBD structure would be also involved in the molecular recognition of the peptide bead.

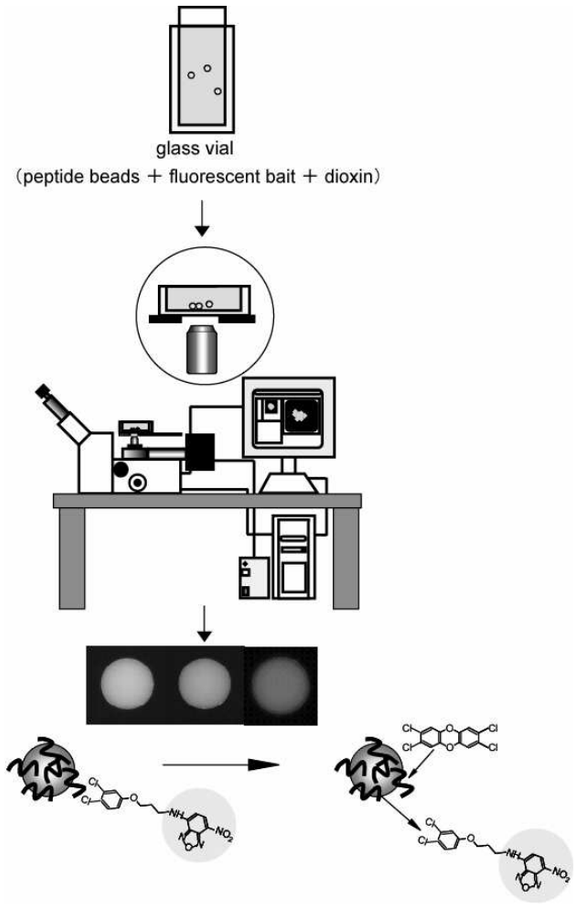
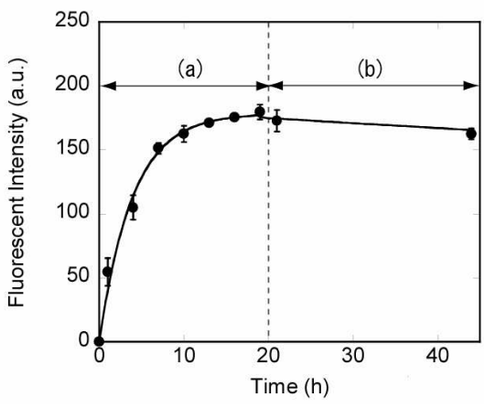


Fig. 1. Detection strategy



(a) $F_{(t)} = F_{max} \cdot e^{-kd \cdot t}$

(b) $F_{(t)} = \dfrac{k_a \cdot C \cdot F_{max}}{k_a \cdot C + k_d}\{1 - e^{-(ka \cdot C + kd) \cdot t}\}$

k_a: binding rate constant
k_d: dissociation rate constant
F_{max}: maximul value of fluorescent intensity

Fig. 2. Time course of binding of fluorescent bait to dioxin binding peptide beads
(a) binding process (b) dissociation process