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Biosensor immunoassay for the screening of dioxin-like polychlorinated biphenyls in retail fish

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ABSTRACT

Dioxin-like polychlorinated biphenyls (DL-PCBs) often make up the majority of the toxic equivalent (TEQ) contribution of dioxins found in fish samples. For the purpose of making risk assessments, it is therefore important to develop screening methods for determining TEQ concentrations of DL-PCBs in retail fish. We have developed a rapid biosensor immunoassay (BIA) for DL-PCBs that uses a surface plasmon resonance sensor (Biacore 3000). The BIA is highly specific for 2,3′,4,4′,5-pentachlorobiphenyl (PCB 118) that is generally the most abundant DL-PCB isomer found in fish. The fish extracts were first cleaned up on a multilayer silica gel column followed by an alumina column, then subjected to the assay. The quantitative limit of the assay was 1 ng PCB 118 per gram of tested sample. Dilution and recovery tests using purified fish extracts suggested that the matrix effect was minimized in the assay by diluting the analyzed samples. The assay results for retail fish samples ($n = 7$) agreed well with those obtained by an enzyme-linked immunoassay (ELISA) using the same monoclonal antibody: ELISA has been already validated for determining DL-PCBs in fish samples, so BIA performs well in this analysis. Finally, BIA results for the TEQ concentrations of DL-PCBs in retail fish samples ($n = 10$) correlated well with those obtained by high-resolution gas chromatography coupled to high-resolution mass spectrometry ($r = 0.89$). Our method is therefore useful for screening retail fish to determine the TEQ concentrations of DL-PCBs.

1. Introduction

Dioxin-like polychlorinated biphenyls (DL-PCBs) together with polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are collectively known as dioxins. Four non-*ortho* PCBs and eight mono-*ortho* PCBs are currently classified as DL-PCBs, and these have been assigned toxicity equivalent factors (TEFs) relative to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) [1,2]. People living in Japan are subject to exposure to high levels of dioxins through consumption of fish [3–5]. The DL-PCBs often make up the majority of the toxic equivalent (TEQ) contribution of dioxins in fish samples [6–9]. Therefore, it is important to determine the TEQ concentrations of DL-PCBs, in addition to those of PCDD/Fs, in retail fish.

High-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) is widely accepted as the most reliable method for determining the TEQ levels of dioxins. Although this technique is reliable and sensitive, it is time-consuming, requires expensive equipment, and must be performed by highly trained staff. Reporter-gene assays, such as the chemical-activated luciferase gene expression (CALUX) assay, are currently considered to be the best method for screening TEQ concentrations of dioxins in food [for review, see 10]. However, its drawbacks include the need for cell culture, which requires skilled personnel and elaborate

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equipment, and the probable need for a license to conduct the assay.

An enzyme-linked immunosorbent assay (ELISA) could be a simpler alternative that does not require cell culture. 2,3′,4,4′,5-Pentachlorobiphenyl (PCB 118) is generally the most abundant DL-PCB isomer found in fish [7,11,12]. On the basis of HRGC/HRMS data obtained in our national survey of dioxins in Japan [13], we found that the concentrations of PCB 118 correlates well with the TEQ concentrations of DL-PCBs in retail fish, although PCB 118 makes a relatively small contribution to the total TEQ of DL-PCBs as a result of its low TEF. Therefore, we recently developed an ELISA that uses a monoclonal antibody (Mab) that is highly specific for PCB 118 to screen retail fish for the TEQs of DL-PCBs [13]. The ELISA is simple, quick, and suitable for screening DL-PCBs in retail fish samples.

Various immunosensor techniques are growing in popularity for residue analysis because of their speed. Although electrochemical immmunosensors, like screen-printed electrodes, are widely used and cost-effective and allowing on site analysis, surface plasmon resonance (SPR) immunosensors have proved to be the most widely reported for immunosensor applications [for review, see 14]. Additionally, SPR techniques can eliminate the need for enzyme-labeled reagents and are mostly highly automated, they are quite simple methods. In particular, SPR-based biosensor immunoassays (BIAs) using Biacore instruments have been proposed, mainly for the determination of residues of veterinary drugs in foodstuffs [for reviews, see 15 and 16]. BIA has also been applied in the detection of dioxins and PCBs [17]; however, there are no previously published reports on their performance in determining these compounds in food samples. We developed an SPR-based BIA by using a Mab that is specific to PCB 118, and we assessed the performance of the BIA in screening TEQ concentrations of DL-PCBs in retail fish.

2. Experimental

2.1. Materials

Dioxin-analysis-grade acetone, dichloromethane, and *n*-hexane were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). A multilayer silica gel column filled from bottom to top with 0.9 g silica gel, 3 g of 2% (w/w) potassium hydroxide-impregnated silica gel, 0.9 g silica gel, 4.5 g of 44% (w/w) sulfuric acid-impregnated silica gel, 6 g of 22% (w/w) sulfuric acid-impregnated silica gel, 0.9 g silica gel, 3 g of 10% (w/w) silver nitrate-impregnated silica gel, and 6 g of anhydrous sodium sulfate was purchased from GL Science Inc. (Tokyo, Japan). Alumina B-Super I was obtained from ICN Pharmaceuticals Inc. (Costa Mesa, CA, USA). Dimethyl sulfoxide (DMSO) was purchased from Sigma–Aldrich (Milwaukee, USA). An activated carbon/dispersed silica gel reversible column was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). DL-PCBs were obtained from AccuStandard Inc. (New Haven, CT, USA). PCB analogue–bovine serum albumin (BSA) conjugate, a Mab against PCB 118, and the ELISA kit using the Mab were purchased from EnBioTec Laboratories (Tokyo, Japan).

Sensor chips (CM5), HBS-EP buffer [0.01 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 0.15 M NaCl, 3 mM EDTA, 0.005% surfactant polysorbate 20; pH 7.4], and an amine coupling kit were supplied by Biacore AB (Uppsala, Sweden).

Fish samples were purchased during 2002 and 2004 from supermarkets in Tokyo, Japan. The samples (muscle tissue) were homogenized by using a food processor and stored at −20 °C until analysis.

2.2. Equipment

The Biacore 3000 was supplied by Biacore AB (Uppsala, Sweden). HRGC/HRMS was performed by using an HP-6890 plus gas chromatograph coupled to a JEOL JMS-700 MStation mass spectrometer supplied by JEOL Ltd. (Tokyo, Japan).

2.3. Purification of fish tissue for the BIA

The homogenized fish sample (20 g) was added to aqueous 2 M KOH (100 mL) and subjected to alkali digestion at room temperature for 16–20 h. The alkaline hydrolysate was added to methanol (150 mL) and extracted three times by mechanically shaking (10 min) with *n*-hexane (100 mL). The extract was washed twice with 2% (w/v) aqueous NaCl (150 mL), treated several times with concentrated sulfuric acid, and then passed through the multilayer silica gel column (see above). The eluate obtained with *n*-hexane (200 mL) was then loaded onto an alumina column (15 g) and washed with *n*-hexane (150 mL). The mono-*ortho*-PCBs containing PCB 118 was eluted with 2% (v/v) dichloromethane/*n*-hexane (150 mL). The fraction was dried by using a nitrogen stream, and the residue was dissolved in DMSO (200 μL). Alternatively, the eluate from the alumina column was loaded onto an active carbon-dispersed silica gel reversible column and washed with *n*-hexane (50 mL). The mono-*ortho*-PCBs containing PCB 118 were then eluted with 25% (v/v) dichloromethane/*n*-hexane (50 mL). The fraction was dried using a nitrogen stream and the residue was dissolved into DMSO (200 μL). The solution was centrifuged at 10,000 rpm for 5 min and the supernatant was subjected to BIA.

2.4. BIA

2.4.1. Preparation of the biosensor chip

A PCB analogue–BSA conjugate was immobilized (yielding approximately 1500 relative response (RU)) on the surface of a CM5 sensor chip by using an amine coupling kit, following the manufacturer's instructions. Briefly, the chip surface was activated by injecting a 1:1 (v/v) mixture of 0.4 M 1-ethyl-3-(3-demethylaminopropyl) carbodiimide hydrochloride (EDC) and 0.1 M *N*-hydroxysuccinimide (NHS) at a flow rate of 10 μL min^{-1} and with a contact time of 7 min. The reactants were removed and the PCB analogue–BSA conjugate (50 μg mL^{-1} in 10 mM acetate buffer, pH 4.0) was injected over the surface during 10 min at a flow rate of 10 μL min^{-1}. To deactivate the remaining active sites, 1 M ethanolamine was injected during 7 min at a flow rate 10 μL min^{-1}. The sensor chip surface was washed repeatedly with 50 mM NaOH for 5 s at a flow rate of 60 μL min^{-1}.