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Synthesis of haptens and selective enzyme-linked immunosorbent assay of octachlorostyrene ☆

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ABSTRACT

A sensitive, competitive indirect enzyme-linked immunosorbent assay (ELISA) was developed for the detection of octachlorostyrene (OCS), a persistent and bioaccumulative toxicant. To achieve the most sensitive antibody, several haptens with different linkers that simulated the special structure of OCS were synthesized and conjugated to carrier proteins. Polyclonal rabbit antibodies against different immunizing antigens were obtained and screened against different coating antigens. Under the optimized conditions, this indirect ELISA shows a linear detection range from 1.4 to 86.3 ng/mL, with an IC_{50} value of 4.46 ng/mL and a limit of detections (LOD) of 0.1 ng/mL. Twelve kinds of compounds were tested for calculating cross-reactivities, and almost all of them showed little cross-reactivity ($<5\%$). Water and sera samples spiked with OCS were analyzed by ELISA and the achieved recoveries were satisfied with a mean recovery of 92%. This immunoassay can be used as a rapid and convenient tool to monitoring OCS in environmental samples.

1. Introduction

Octachlorostyrene (OCS), a toxic halogenated aromatic compound, belongs to the primary level I priority of the persistent and bioaccumulative toxicants (PBTs) categorized by the US Environmental Protection Agency (EPA). OCS is not commercially manufactured but to be an inadvertent byproduct of processes that combine carbon and chlorine at high temperature [1]. Since firstly being found in dead cormorants [2], OCS has been occasionally detected in environmental samples such as sediments, fish, marine mammals and even in human being [3–8].

Concerns over the occurrence of OCS in the environment are probably caused by two main factors: its persistence and its high bioaccumulation. Judging from its special chemical structure, it is not difficult to see that OCS is a fully chlorinated aromatic compound and is likely to be a highly persistent substance. The half-life time of OCS in liver of the artificially raised rainbow trout was nearly twice longer as that of hexachlorobenzene, and the elimination half-life time of OCS in yellow eel was 1.7 and 2.3 fold, respectively, longer as that of hexachlorobenzene and pentachlorobenzene under natural conditions [4,8]. Moreover, the very low water solubility and the bioconcentration factor ranging from 8100 to 1,400,000 implied that OCS could bioaccumulate easily in aquatic food webs and would have an influence on aquatic organisms [9,10]. In 1984, Kamlnsky et. al [3] conducted a study about the sources of OCS in Lake Ontario and analyzed sediments of different places. OCS was detected in 8 of the 11 cores, and its concentration ranged from 20 ng/g to 140 ng/g (dry weight). By an investigation of fish and fish oils of the North Atlantic [7], OCS and its homologs were quantified. OCS and (*E*)-*β*,2,3,4,5,6-hexachlorostyrene were dominating in fish samples, with concentration of 0.49~24.0 ng/g fat and 0.65~41.0 ng/g fat, respectively. In 1992, OCS was detected in 7% of the breast milk samples collected from 497 Canadian women, and the mean concentration of OCS in all samples was 0.05 ng/g breast milk [5]. Seldén [6] conducted an analysis about the plasma of Sweden workers who had the past experience of degassing with hexachloroethane in an aluminum foundry. The mean concentrations of OCS in their plasma was found at 54.6 ng/g (lipid), nearly 78 folds higher than that in the control group and the OCS concentration increased with the duration of exposure to hexachloroethane. Besides the urinary porphyrin excretion of these workers was about 2 fold higher than that in the control group, which probably indicated the toxic effects of OCS on porphyrin metabolism of human [11].

Regarding above researches, a routine assay for screening and monitoring OCS in environmental or biological samples is of increasing significance and necessity. Current analytical methods

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for detection of OCS are mainly based on gas liquid chromatography (GC), GC–MS, or high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS) [2,4–8]. No immunoassay method was reported for the detection of OCS for the reason of probably shorting of the antibody. Comparing with these instrumental methods, immunoassay methods are well-known for their selectivity, detectability, reliability, analysis speed, less pretreatment and low cost. Besides, they offer an easy way to simultaneously detect a single analyte of interest in many samples. So immunoassay methods have been widely used in the field of clinical diagnostics, environmental monitoring, food quality, agriculture and testing of personnel exposed to toxic chemicals [12–15].

The aim of this study was to develop an ELISA for analysis of OCS based on polyclonal antibodies. Thus, the synthesis of new haptens to elicit antibodies, the characterization of antibodies and the analysis of OCS in water and sera samples are described. To our knowledge, this is the first paper reporting the method for detection of OCS in samples by immunoassay method.

2. Materials and methods

2.1. Chemicals, apparatus, buffers and solutions

2.1.1. Chemicals and apparatus

Bovine serum albumin (BSA), ovalbumin (OVA), Goat anti-rabbit IgG peroxidase conjugate, Tween 20, 3,3′,5,5′-tetramethylbenzidine (TMB), incomplete Freund's adjuvant and complete Freund's adjuvant were purchased from Sigma-Aldrich Company. Hexachlorobenzene, octachlorostyrene (10 ng/μL), pentachloronitrobenzene and $Pd(PPh_3)_4$ were purchased from J&K Company. Propargyl alcohol and 3-mercaptopropanoic acid was obtained from Alfa Aesar Company. Flash column chromatography was performed on 300–400 mesh silica gel.

2.1.2. Buffers and solutions

The buffers used in this study were as follows: (1) phosphate-buffered saline (PBS, pH 7.4): 138 mM NaCl, 1.5 mM KH_2PO_4, 7 mM Na_2HPO_4 and 2.7 mM KCl; (2) washing buffer (PBST): a PBS solution containing 0.05% (v/v) of Tween 20; (3) coating buffer (0.05 M carbonate buffer): 15 mM Na_2CO_3 and 35 mM $NaHCO_3$, pH 9.6; (4) blocking buffer: PBS mixed with 1% of OVA and 0.05% (v/v) Tween 20; (5) substrate buffer (TMB+H_2O_2): 400 mL of 0.6% TMB–DMSO mixed with 100 mL of 1% H_2O_2 in citrate–acetate buffer (pH 5.5); (6) enzymatic stopping solution: 2.0 M H_2SO_4; and (7) OCS standard solution: the octachlorostyrene dissolved with CH_3CN of different volumes to get the standard solutions at different concentrations.

2.2. Instrumentation

All melting points were determined with a Taike XT-4 micro-melting-point apparatus. ^1H and ^{13}C NMR spectra were recorded on a Varian INOVA-400 instrument using tetramethylsilane (TMS) as internal standard. Mass spectra were obtained by SHIMAZU GC-17 QP-5000 or ThermoFinnigan MAT95XP. Ultraviolet–visible (UV–vis) spectra were obtained by UV-2100 spectrophotometer (LabTech). Polystyrene microtiter plates (96-well) were purchased from Jet Biofil Company. Absorbances were measured in a microtiter plate reader (ELx800, BioTek Company Limited). Immunoassay competitive curves were mathematically analyzed by the software of Origin 8.5. GC analysis was conducted by GC-2010 with a RTX-5 column (SHIMADZU).

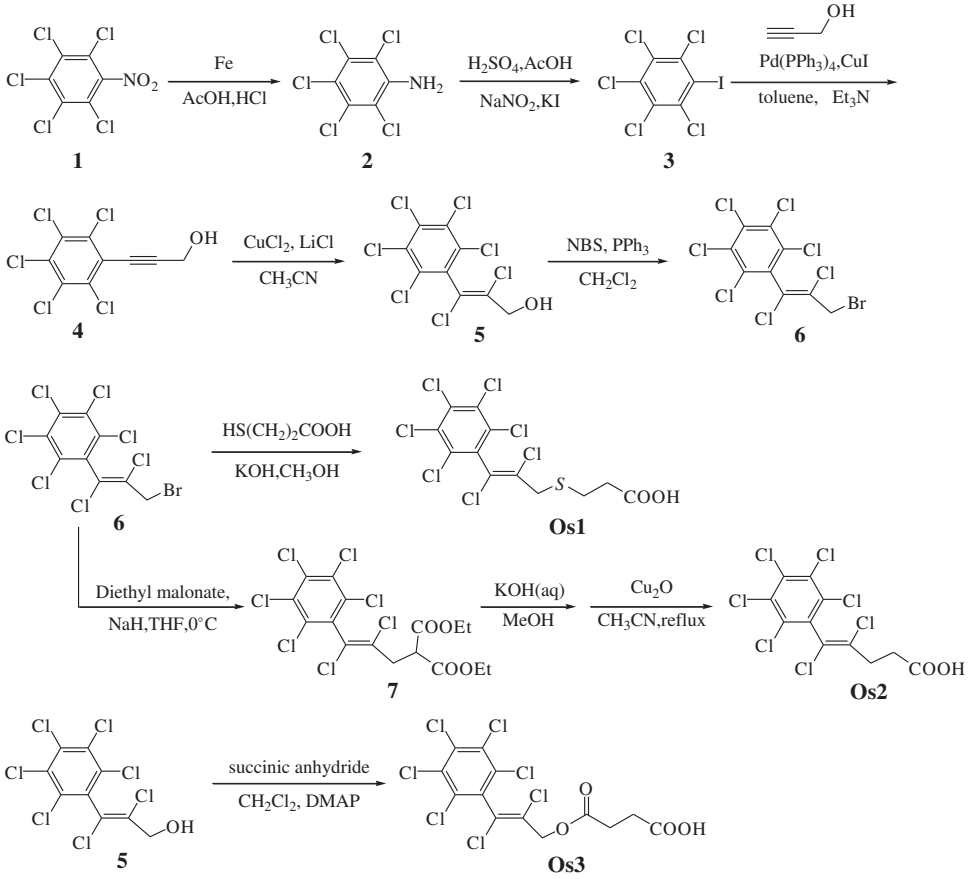

Fig. 1. Synthetic routes of three haptens of octachlorostyrene.