Development of Immunoassay-Based Methods for the Analysis of Polychlorinated Dibenzo-*p*-Dioxins

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1. Abstract

The chemical and toxicological properties of polychlorinated dibenzo-*p*-dioxins (PCDDs), along with the apparent pervasiveness of these chemicals in the environment, has created a demand for new analytical methods for these compounds. These new analytical methods for PCDDs ideally would be extremely selective, would exhibit very low detection limits, and would be applicable to very large numbers and wide varieties of samples in a cost-effective manner. Immunoassays (IAs) are well-suited to address many of the extreme analytical challenges presented by PCDDs. In this paper we will present the results of our efforts to develop improved immunoassay-based methods for polychlorinated dibenzo-*p*-dioxin analysis. This research effort involves the design and synthesis of new haptens for improving the performance of already existing IAs, the development of new antibodies and IA's using these haptens, and examination of the toxicological properties of compounds synthesized as part of this research.

2. Introduction

Immunoassays have been developed and successfully applied to the analysis of PCDDs[1-4]. This early work has demonstrated the strengths of immunoassay as a method for analysis of small molecules in environmental samples, and it has pointed out areas where improvements in IA-based methods for PCDD analysis can be realized. Selectivity is a critical factor in the development of analytical methods for analysis of PCDDs. The primary analytical target of this research and the most toxic PCDD cogener is 2,3,7,8-tetrachlorodioxin (I, TCDD); however, there are a large number of PCDD cogeners, polychlorinated dibenzofurans and polychlorinated biphenyls which are toxic and are present in the environment at levels similar to or significantly greater than TCDD levels[5,6]. Two ideal selectivity patterns for PCDD analysis are either high selectivity for only one specific PCCD cogener or a pattern of crossreactivity for a wide range of PCDD-like compounds with a crossreactivity pattern that mimics the relative toxicity of these compounds. Low detection limits are also a critical requirement of IAs for PCDDs due to

the low concentrations of these compounds which are thought to result in toxic effects and due to the low levels at which they are found in the environment[5,6].

3. Approach and Results

In order to produce antibodies to small molecules, synthesis of haptens mimicking the target analyte is necessary. These haptens are then coupled to marcromolecules such as proteins in order to produce immunogenic compounds featuring the exposed structure of the target analyte for development of antibodies. Effective hapten design and synthesis is critical for determining the binding affinity and specificity of antibodies produced for immunoassay development[7]. Compounds (**II-VIII**) have been synthesized. These were coupled to proteins (keyhole limpet hemocyanin, KLH and bovine serum albumin, BSA) using either a mixed anhydride[8] or diazotization methods[9]. The resulting conjugates were used to develop polyclonal sera for immunoassay development. Titer experiments were run using the ELISA protocol described by Gee et al.[10]. Table I shows the titers that resulted from these immunizations. Initial screening of these sera indicate that each hapten with the exception of **VII** produced high-affinity antibodies which may prove useful in the development of IAs to PCDDs. The antibodies bound to both homologous and heterologous coating antigens.

These haptens have been rationally designed with the analytical goals of the desired IAs in mind. These compounds more closely mimic the chemical structure of TCDD compared to haptens employed previously by other researchers[1-4]. In addition, the spacer arms for these haptens have been designed with double bonds and aromatic rings in an attempt to introduce rigidity into the spacer. It is hoped that these haptens with rigid spacers will better present the target analyte in solution during antibody formation by reducing the likelihood of the highly lipophilic PCDD portion of the hapten imbedding itself in a hydrophobic region of the protein portion of the immunogen. The high titers found indicate that this strategy may have been successful. Further assay development in which the haptens and analyte are used as inhibitors will give more definitive information on this hypothesis.

The selection of haptens used as coating antigens and tracer molecules in IA's also greatly influences the selectivity and sensitivity of the IA[7]. Haptens (**II-VIII**) are being used with previously developed monoclonal antibodies to PCDDs[2,3] to see if improved immunoassays can be developed with these antibodies. The monoclonal antibodies were raised to hapten **IX**. They show strong affinity for a coating antigen containing hapten **VIII** and moderate affinity for coating antigens containing haptens **IV** and **VI**. Haptens **II-VIII** are also being evaluated for use as coating antigens in both homologous and heterologous IA systems using the polyclonal antibodies generated with these haptens.

In addition to selectivity and sensitivity, tolerance to organic solvents and surfactants is another important parameter in development of the assays presented. This is another consequence of the lipophilic nature of PCDDs which are soluble to only a very limited degree in polar solvent mixtures. Assays are screened for their tolerance to organic solvents and surfactants.

Another important obstacle to PCDD analysis is the potential hazard posed by chemical standards required for analysis, especially for TCDD. One potential means for circumventing this difficulty is to synthesize TCDD analogs which have properties which result in a TCDD-like analytical response but are less toxic than TCDD. Compounds (**X-XIII**) are TCDD analogs which have been synthesized and are being evaluated as potential surrogate standards for TCDD. *In vitro* bioassays and cell-based bioassays have been developed which allow the assessment of aromatic hydrocarbon (Ah) receptor-mediated toxicity of compounds in the presence and absence of cellular detoxification mechanisms[11]. Compound **X** has been found to activate Ah receptor mediated pathways only 1/50th as effectively as TCDD. Compounds (**X-XIII**) are currently being evaluated for both toxicity and analytical response.

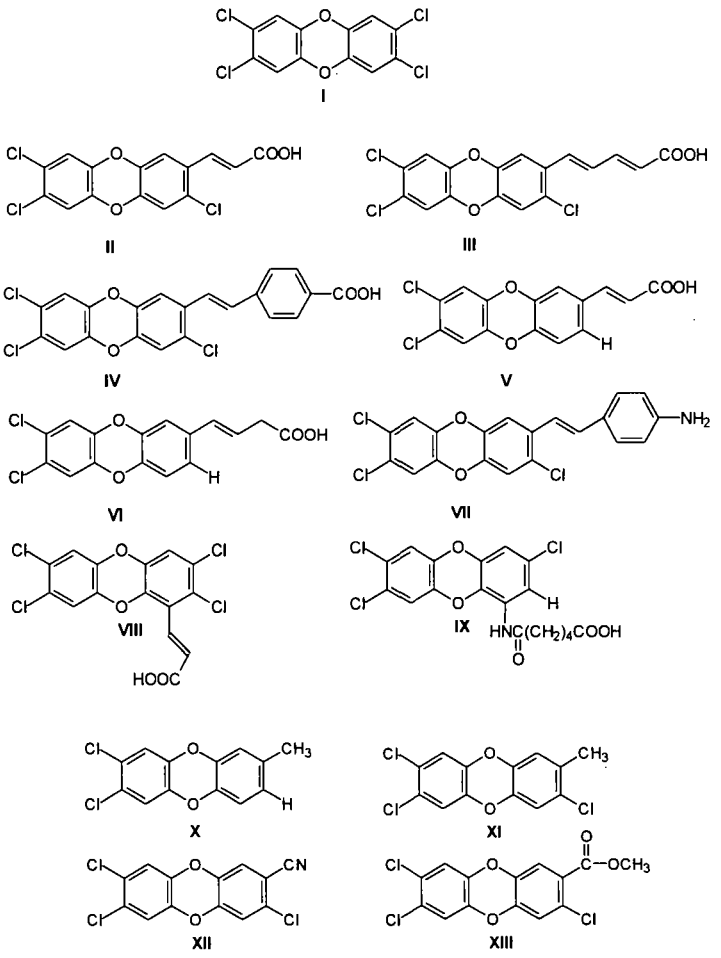

Table I. Rabbits were immunized with the indicated antigen. The resulting serum was tested for ability to bind the respective coating antigen. (-) indicates that no signal was generated. (+) absorbance values 0.3-0.6, (++) absorbance values 0.6-0.9, (+++) absorbance values 0.9-1.2, (++++) absorbance values > 1.2 under the ELISA conditions described above.

Coating Antigen	Immunizing Antigen				
	IV-KLH	VII-KLH	V-KLH	VI-KLH	VIII-KLH
II-BSA	++	-	+	++	+
III-BSA	+++	-	++	+++	+++
IV-BSA	++++	-	+++	+++	+++
IX-BSA	+	-	-	+	+++
VII-BSA	-	-	-	-	-
VIII-BSA	+	-	-	+++	++++

3. References

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