Molecular Modeling of Dioxins and Related Compounds for the Design of Dioxin Binding Assays

Larry H. Stanker[1], Bruce D. Hammock[2], Shirley J. Gee[2], James R. Sanborn[2] and A. Daniel Jones[3]
[1]Food Animal Protection Research Laboratory, U.S. Department of Agriculture, Agricultural Research Service, 2881 F & B Road, College Station, TX 77845
[2]Department of Entomology, [3]Facility for Advanced Instrumentation, University of California, Davis, CA 95616

1. Introduction

The polychlorinated dibenzo-p-dioxins (PCDDs) are a group of highly toxic, environmentally significant compounds. Analysis for this group of compounds is complicated by both the physical properties of the compound (e.g., the large number of congeners; its ability to strongly bind soil particles) and by the observations that the biological activity of the 75 dioxin congeners varies widely. Thus, analysis for dioxins requires the use of sophisticated analytical methods such as gas chromatography and mass spectroscopy (*1*). In an attempt to simplify the analysis of dioxins, immunochemical methods have been developed. Initially using polyclonal antiserum (*2*) and more recently using monoclonal antibodies (*3*). These latter monoclonal antibodies form the basis of an enzyme- immunoassay that is capable of detecting dioxin, at part-per-billion levels, in a variety of environmental and industrial matrices (*4,5*). We currently are working to improve this assay by the synthesis of new haptens for use as tracers with the current antibodies and by the synthesis of new antigens for improved antibodies.

The central component of any immunoassay is the antibody. Antibodies are complex protein molecules composed of heavy and light chains. The interaction of an analyte with an antibody molecule involves specific chemical interactions between the side chain groups of those amino acids forming the combining site of the antibody and the analyte. A variety of interactions can occur including H-bonding, π-π interactions, salt-bridges, hydrophobic interactions and van der Waals interactions. However covalent bonds are not formed since the antibody-antigen interaction is reversible. Superimposed upon these chemical interactions associated with antigen recognition is a structural component. The tertiary structure of the antigen must correctly fit into the antibody combining site in order for the respective chemical groups to be in the proper relative positions to facilitate the above chemical interactions.

2. Approach and Results

Recently we have employed energy-minimized molecular models of a select number of dioxin congeners as an aid in elucidating those properties of the analyte that might be responsible for the differential antibody binding observed to these congeners (*6*). We have expanded these studies to include modeling the structural and electronic properties of a number of newly synthesized dioxin molecules that have various substitutions that allow conjugation to a carrier protein. These modified dioxins are useful as immunogens since in order to develop antibodies to dioxin, the analyte must first be covalently linked to a carrier molecule, usually a protein. In the example cited above (*3*) the analyte was linked to a carrier protein by introduction of an amino group on the number one carbon and linkage to the carrier protein was achieve via a six carbon adipate linkage (Figure 1). Analysis of the cross reactivity of the subsequent monoclonal antibodies with different PCDDs, polychlorinated dibenzofurans (PCDFs), and with the hapten and its precursor (1-nitro-triCDD) suggests that the antibodies are binding the linkage chemistry as well as the dioxin molecules. Thus, the introduction of a nitrogen off the number one carbon appears to be influencing antibody recognition. Energy-minimized models suggest that the hapten has minimal alterations in its structural and electron density surfaces. However, the effects on the electrostatic potential surface following introduction of this nitrogen are dramatic and are summarized in Figure 2. The discrete electronegative regions associated with the chlorines and the oxygens of 2,3,7,8-TCDD (Figure 2A) are replaced by a large electronegative zone spanning this entire region of the molecule (Figure 2B). Clearly such alterations may have a significant effect on the resulting antibodies and our cross reactivity studies previously described (*2,6*) support this conclusion. The electronic and structural properties of a number of newly synthesized dioxin immunogens will be discussed in relation to their potential usefulness as immunogens and the ability of existing antibodies to bind these compounds. Such models are improving our capability to design improved tracers and antigens.

3. References

1) Crummett, W.B. *Chemosphere* **1983**, 12, 429.

2) Albro, P.W.; Luster, M.I.; Chase, K.; Clark, G.; McKinney, J.D. *Toxicol. Appl. Pharmacol.*, 50, **1979**, 137.

3) Stanker, L.; Watkins, B.; Vanderlaan, M.; Budde, W.L. 1987. *Chemosphere* **1987**, 16, 1635-1639.

4) Vanderlaan, M.; Stanker, L.H.; Watkins, B.E.; Petrovic, P.; Gorbach. S. *Environmental Toxicology and Chemistry* **1988**, 7, 859-870.

5) Watkins, B.E.; Stanker, L.H.; Vanderlaan, M. 1989. An Immunoassay for Chlorinated Dioxins in Soils. *Chemosphere* **1989**, 19, 267-270.

6) Stanker, L.H.; Recinos A.; Linthicum, S. In *Immunoanalysis of Agrochemicals Emerging Technologies.* Nelson, J.O., Karu, A.E., Wong, R.B., Eds. ACS Symposium Series 586, **1995**, American Chemical Society, Washington DC, pp 72-88..

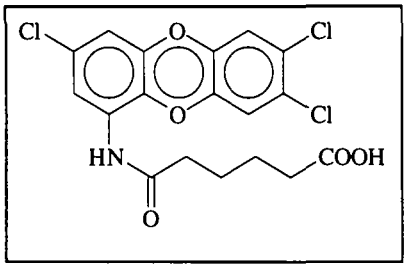

Figure 1: 1-N-(adipamino)-3,7,8-triCDD used as immunogen by Stanker et al. (*3*).

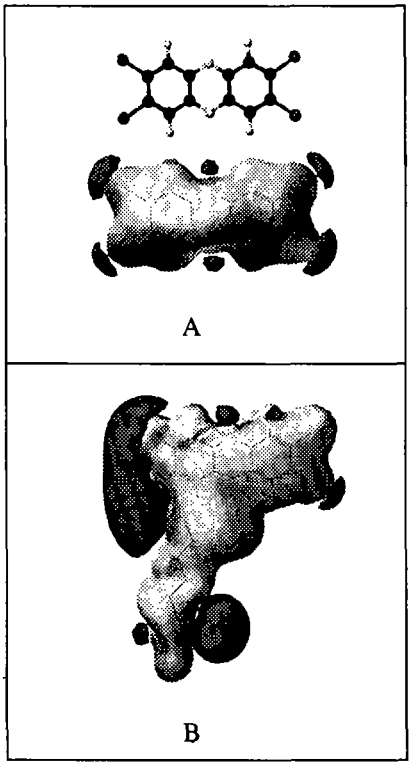

Figure 2: Negative (dark shading) and positive (light shading) electrostatic potential surfaces calculated for low energy conformations of 2,3,7,8-TCDD (panel A), and 1-N-(adipamino)-3,7,8-triCDD (panel B).