Research Brief 181: Lower Detection of Toxic Substances by Phage Library–enhanced Immunoassays

Release Date: 01/05/2010

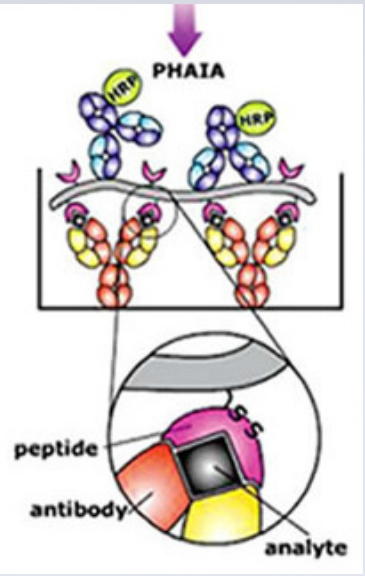
Superfund Research Program
http://www.niehs.nih.gov/srp/

Background

Studies to link environmental contamination to human health effects require quantification of contaminant levels in the environment and internal doses of the chemicals. These studies involve analyses of large numbers of samples in a variety of matrices. Because of their sensitivity, specificity, simplicity, speed, and low cost, immunoassays (analytical methods that use antibodies as detectors) are useful in such investigations.

At the University of California, Davis (UC-Davis), Ms. Shirley Gee and Dr. Bruce Hammock focus their research on developing assays for chemicals that have the potential for widespread exposure and potential impact on human health. The researchers have developed more than 40 assays for parent compounds and/or metabolites in the course of their work.

The UC-Davis group also works to identify novel approaches and reagents to improve the sensitivity of existing immunoassays; increase the throughput of the methods; and make the assays more robust for analysis of environmental (e.g., soil, water, sediment) or biological (e.g., urine, blood, saliva) samples. They have adapted some of their assays for use in the field and are working to overcome the challenges of detecting and quantifying small molecules.



Interactions in the PHAIA process

Advances

Immunoassays for large molecules (e.g., proteins) are conducted in a "sandwich format": one antibody captures the protein and a second antibody detects the captured protein. However, small molecules (e.g., pesticide metabolites) are not large enough to accommodate two antibody binding events. To quantify small molecules, the Gee-Hammock lab group enhanced immunoassays using a phage anti-immune complex assay (PHAIA). In this method, an analyte-antibody complex is formed and then rather than a second antibody, a filamentous phage particle bearing a short peptide group is used as the detector. The phage displaying the appropriate peptides is selected from a large library via a rapid process called panning that identifies the small peptides on the phage coat that have high affinity for the analyte-antibody complex.

The researchers then worked to enhance their previously developed immunoassays using this process. Application of PHAIA techniques resulted in:

- 3-fold increases in sensitivity for atrazine, a heavily used herbicide in maize production in the United States and South America,
- 30-fold increases in sensitivity for molinate, a herbicide widely used rice production
- 10-fold increases in sensitivity for digoxin, a drug used to treat congestive heart failure

This research has benefited from an effective collaborative relationship with Professor Gualberto Gonzalez-Sapienza, Laboratory of Immunology, College of Chemistry, Institute of Hygiene, University of the Republic of Uruguay, Montevideo, Uruguay. Several doctoral students from Uruguay came to UC Davis to continue their research on the phage-enhanced immunoassays.

With this increased sensitivity for molinate and atrazine, the researchers were able to test water samples from agricultural areas of Uruguay. No environmental matrix interferences affected the analyses. The UC-Davis lab group then developed a dipstick method for cost-effective field detection of molinate in river water. The dipstick method is sensitive to 2.5 ng/ml, which is below drinking water standards for WHO (6 ng/ml) or the State of California (20 ng/ml). These analytical tools may also be useful to monitor emerging exposure-risk issues related to occurrences of triazine herbicides in drinking water.

Several years ago, the researchers developed an immunoassay to monitor agricultural and residential exposures by detecting a common metabolite of many synthetic pyrethroid insecticides (3-phenoxybenzoic acid or 3-PBA) in urine samples. When they enhanced the assay for 3-PBA with the phage displayed-peptide methodology, they lowered the level of detection 5-400-fold (depending on which immunoassay is used for the comparison) and matrix effects were minimal. In this work, the researchers used magnetic particles as the solid phase in place of the typical 96 well microtiter plate. This may have contributed to increased sensitivity due to the larger surface area and the greater solution of the components of the assay. They also developed a dipstick for 3-PBA detection. With a detection limit of 0.25 ng/ml, the dipstick is applicable to detect 3-PBA in samples at levels near the geometric mean concentration of 0.300-0.500 ng/ml observed in the CDC's 2001-2002 National Health and Nutrition Examination Survey (NHANES).

Significance

These new tools, which the UC-Davis group has exported to state governments and universities in the United States and several foreign countries, allow researchers to gather more comprehensive information on the relationship of human exposure to environmental contaminants. The PHAIA methods have considerably improved sensitivity compared to other immunoassay methods, and the approach is a major shortcut in the development of sensitive assays as the phage anti-immune complexes can be isolated from extensive phage-display libraries rather than immunizing animals to generate antibodies.

For more information, contact:

Shirley J. Gee
Department of Entomology
University of California, Davis
Davis, CA 95616
530-752-8465
Email: Sjgee@ucdavis.edu

Bruce D. Hammock
Department of Entomology
University of California, Davis
Davis, CA 95616
530-752-8465
Email: Bdhammock@ucdavis.edu

To learn more about this research, please refer to the following sources:

Gonzalez-Techera, A.; Kim, H. J.; Gee, S. J.; Last, J. A.; Hammock, B. D.; Gonzalez-Sapienza, G. 2007. Polyclonal Antibody-Based Noncompetitive Immunoassay for Small Analytes Developed with Short Peptide Loops Isolated from Phage Libraries. *Anal. Chem.* **79**:9191-9196.
Available online: DOI:10.1021/ac7016713

Gonzalez-Techera, A., Vanrell, L., Last, J. A.; Hammock, B. D.; Gonzalez-Sapienza, G. 2007. Phage Anti-Immune Complex Assay: General Strategy for Noncompetitive Immunodetection of Small Molecules. *Anal. Chem.* **79**: 7799-7806.
Available online: DOI:10.1021/ac071323h

Kim, H.J., Ahn, K.C., González-Techera, A., González-Sapienza, G.G., Gee, S.J., Hammock, B.D. 2009. Magnetic bead-based phage anti-immunocomplex assay (PHAIA) for the detection of the urinary biomarker 3-phenoxybenzoic acid to assess human exposure to pyrethroid insecticides. *Anal. Biochem.* **86**:45-52.
Available online: DOI:10.1016/j.ab.2008.12.003

Kim, H.J., González-Techera, A., González-Sapienza, G.G., Ahn, K.C., Gee, S.J., Hammock, B.D. 2008. Phage-borne peptidomimetics accelerate the development of polyclonal antibody-based heterologous immunoassays for the detection of pesticide metabolites. *Environ Sci Technol.* **42**:2047-2053.
Available online: DOI:10.1021/es702219a

NIEHS
National Institute of Environmental Health Sciences

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health

SRP_ResearchBrief_181_01.10