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Project ID: **54546**

Project Title: **Engineered Antibodies for Monitoring of Polynuclear Aromatic Hydrocarbons**

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YEAR 3 PROGRESS REPORT
AWARD NO. DE-FG07-96ER62316

PROJECT TITLE: **Engineered Antibodies for Monitoring of Polynuclear Aromatic Hydrocarbons**

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REPORTING PERIOD:	1 Aug. 1998— 1 Aug., 1999
AWARD PERIOD:	15 Sept. 1996 — 15 Sept. 1999

RESEARCH OBJECTIVE

The objective is to develop improved antibody-based methods for detection of multiple polynuclear aromatic hydrocarbons (PAHs), to fill several needs in DOE's remediation, regulatory monitoring, ecotoxicology, and human health effects missions. Present-generation immunochemical detection methods have already proven to be useful and cost-effective in DOE applications. The problem being addressed is that the unique properties of PAHs make it impractical to generate antibodies with the required diversity, specificity and selectivity, by the previous techniques. The scientific goals are to determine the mechanisms by which antibodies bind PAHs, use genetic engineering and computational chemistry techniques to construct improved antibodies, and to devise methods for making immunochemical and instrumental analysis more compatible. The potential relevance is that our results should provide a rational basis by which immunochemical and other molecular recognition systems for PAHs and other large classes of toxic pollutants such as PCBs could be produced and deployed with substantially less cost, labor, and development time.

PROGRESS

This report summarizes work completed after two years and 10 months of the 3-year project period. It is a composite of accomplishments and problems encountered in our three laboratories between August 1998 and August 1999, following our previous report and poster at the July 1998 EMSP Workshop in Chicago.

Results from the previous period indicated that PAH binding in two recombinant mouse Fab antibodies (rFabs), 4D5 and 10C10, may at least partly involve interaction with positively charged amino acid side chains on either side of the binding site (Lysine 89 on the L chain and Arginine 95 on the H chain). Single and double mutants with uncharged side chains of about the same size were prepared, verified, and characterized by enzyme immunoassay (EIA). The L89 mutant and wild-type 4D5 bound eleven different PAH haptens identically, within experimental error, but the H95 single- and L89-H95 double mutants abolished PAH binding. In indirect EIAs the L89 mutant competitively bound soluble benzo[a]pyrene (BaP), pyrene, and fluoranthene, with half-maximal inhibition (I_{50}) values comparable to that of wild-type 4D5, but phenanthrene, anthracene, fluorene, and chrysene were not bound.

The Li lab characterized, and the Roberts lab modeled the binding sites of two single-chain Fv antibodies (scFvs) that bound naphthalene (designated Nap2 and Nap16), and two that bound phenanthrene haptens (Phen42 and Phen57), that we derived from the Nissim combinatorial phage display library in the previous reporting period. These scFvs had high nonspecific binding, probably because all had the same V_L sequence from a BSA-binding antibody. Indirect competition EIAs with these scFvs were not sensitive enough for practical purposes, but a direct

EIA format was developed in which Nap2 scFv competitively bound naphthalene from aqueous solution with I_{50} values of 150-600 ppb.

The Roberts Lab prepared computational structure models of PAH binding sites in the Nap and Phen scFvs, and compared them with the binding site in mouse rFabs 4D5 and 10C10. Although the scFvs had much shallower binding pockets, they had positively charged side chains on both sides, as in the rFabs.

Dr. Li's laboratory developed a method for benzo[a]pyrene concentration and cleanup from environmental and biological samples, using purified MAb 4D5 coupled to cyanogen bromide-activated Sepharose beads packed in 1.5 mL columns or added to the samples directly. Recoveries of 94-96% were obtained from samples containing 200 ng of benzo[a]pyrene, and the beads could be regenerated at least three times. This method provided extracts from water, urine, and marine coral samples for PAH analysis by gas chromatography-mass spectrometry. In addition, supercritical fluid (CO_2) procedures were developed for extraction of PAHs and related compounds from clay soils, harbor sediment, marine coral, crabs, and fish, and pilot studies of PAH bioavailability were begun.

SIGNIFICANCE AND IMPLICATIONS

Our immunoassay and computational modeling results depict a novel mechanism different from the general assumption that PAH binding is determined by ligand size, complementary shape of the binding pocket, and hydrophobic interactions. In the mouse rFab and human scFv antibodies we have studied, recognition involves a general motif in which (a) the PAH ring is bound approximately parallel to the V_L—V_H interface, (b) cationic amino acid side chains on each side interact with the ring's • electrons, and (c) the precise geometry and flexibility of the interface influences affinity and cross-reactivity. These findings also pose intriguing possibilities for how PAHs may be bound by molecular signaling proteins such as the aryl hydrocarbon receptor, and PAH-metabolizing enzymes important in bioremediation.

Notwithstanding published reports of high affinity antibodies to other haptens obtained from the Fab 2LOX and Nissim phage display libraries, these libraries were clearly not optimal for the PAH binders we sought. Our data indicate that constructing and screening a library made from rFab 4D5 should be much more productive.

The supercritical fluid extraction procedures developed by Dr. Li's lab can be used to prepare samples for both immunoassay and instrumental (GC/MS) analysis. This makes confirmatory testing more reliable and less expensive. The immunoaffinity methods they developed could be scaled up and used to process large numbers of samples economically.

In summary, this project has produced novel basic information as well as practical methods, but more time is needed to achieve the goals in the original proposal. A one-year no-cost extension will be requested.

PLANNED ACTIVITIES DURING NO-COST EXTENSION PERIOD

Based on the results to date, we believe that we can best use our remaining time and resources to more precisely define the determinants of PAH binding, and the targets for mutagenesis to improve binding in rFab 4D5. Concurrently, Dr. Li's laboratory will refine and adapt their recombinant antibody-based analytical methods for one or more site-specific DOE applications. For both of these aims, scaled-up expression of the original rFabs and mutants has become an immediate priority. The antibody sequences will be moved into another vector with an affinity tag that will facilitate purification and immobilization on sensors and affinity matrices. The novel implications of the binding site models (specifically, the roles of charge interactions, orientation of the V_L—V_H interface, and flexibility of the pocket) are sufficiently important that Drs. Roberts and Pellequer will attempt to obtain a definitive X-ray crystallographic structure of rFab 4D5, to confirm and adjust the mutagenesis strategies for improving PAH binding. In addition, we have begun discussions with Dr. Tuan Vo-Dinh at Oak

Ridge National Laboratory to identify a sensor system and demonstration project to move our work closer to deployment.

PUBLICATIONS AND ABSTRACTS (LAST 12 MONTHS)

1998 Karu, A. E., Q. X. Li and V. Roberts. Engineered antibodies for monitoring of polynuclear aromatic hydrocarbons. (Poster presented at Dept. of Energy Environmental Science Management Program Workshop, Chicago, IL (July 27-30). http://www.doe.gov/em52/1998posters/id54546.pdf

1998 Liu, M., G. A. Rechnitz, K. Li and Q. X. Li. Capacitive immunosensing of polycyclic aromatic hydrocarbon and protein conjugates. Anal. Lett. 31:2025-2038

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1999 Liu, M., Q. X. Li and G. A. Rechnitz. Gold electrode modification with thiolated hapten for the design of amperometric and piezoelectric immunosensors. *Electrochem. Anal*. (in Press).

1999 Pellequer, J.-L.; Zhao, B.; Kao, H.-I.; Karu, A. E.; Roberts, V. A.; Cation-pi interactions in antibody binding of polynuclear aromatic hydrocarbons (Abstract No. 36750); Symposium on First Accomplishments of the Enviironmental Management Science Program, ACS Div. of Nuclear Chemistry and Technology, 218th National Meeting, American Chemical Society, New Orleans, LA, Aug 22-26

1999 Li, Q. X., K. Li, S. Thomas and H. Li. Application of immunochemical methods for the analysis of polynuclear aromatic hydrocarbons in the environment (Abstract No. NUCL0047). Symposium on First Accomplishments of the Environmental Management Science Program, 218th National Meeting, American Chemical Society, New Orleans, LA, Aug 22-26

1999 Thomas, S. and Q. X. Li. Immunoaffinity chromatography for the analysis of polycyclic aromatic hydrocarbons in coral. Environ. Sci. Technol. (submitted Sept. 99):

PERSONNEL INVOLVED:

University of Calif., Berkeley:
- Alexander E. Karu (PI)
- Christopher W. Bell, Ph.D. (postdoctoral) Oct. 96 — April 97
- Bitao Zhao (M.S. in Medicinal Chemistry) Feb. 97 — June 1998
- Hui-I Kao (Accepted into Ph.D. program in biochemistry, Univ. of Rochester, Sept. 1999.) May 1998 — June 1999

The Scripps research Institute:
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- Rongliang Chen, (chemist, supported in part)
- F. Guo (postdoctoral fellow, supported in part)
- H.Li (graduate student, supported in part)
- Kai Li (postdoctoral fellow, supported in full)
- M. Liu (graduate student, supported in part)
- S. Thomas (graduate student, supported in full)