Engineered Antibodies for Monitoring of Polynuclear Aromatic Hydrocarbons

Alexander E. Karu, Bitao Zhao, Hui-I Kao, University of California. Berkeley, CA 94720.
Victoria A. Roberts, Jean-Luc Pellequer, The Scripps Research Institute. La Jolla, CA 92037.
Qing X. Li, Kai Li, Steve Thomas, University of Hawaii. Honolulu, HI 96822.

RESEARCH OBJECTIVE

The long-term goal of this project is developing antibodies and antibody-based methods for detection and recovery of polynuclear aromatic hydrocarbons (PAHs) and PAH adducts that are potential biomarkers in environmental and biological samples.

SIGNIFICANCE

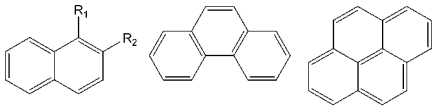
Rapid, inexpensive, high-throughput methods to identify and monitor PAHs are needed in several DOE focus areas, including human and ecosystem health effects, risk and exposure assessment, decontamination and decommissioning, and remediation.

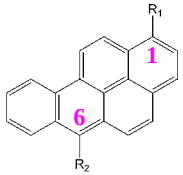
PROJECT COMPONENTS

Karu Lab: Uses new haptens that represent key PAHs to derive recombinant Fab (rFab) and single-chain Fv (scFv) antibodies from hybridoma lines and combinatorial phage display libraries.
Roberts Lab: Builds computational models of antibody binding sites and PAH haptens and parent compounds to guide antibody engineering by mutagenesis.
Li Lab: Designs and synthesizes haptens and develops enzyme immunoassays (EIAs), sensors, and immunoaffinity methods to apply novel antibodies for practical analyses in support of DOE's mission.

Project ID: 54546

PAHs





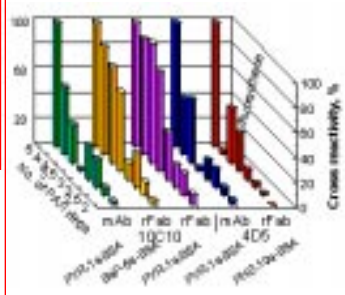
Benzo[a]pyrene (BaP)

PAHs are structurally similar, planar, hydrophobic compounds that lack hydrogen-bonding groups, making them particularly difficult to distinguish immunologically.

GENETIC ENGINEERING

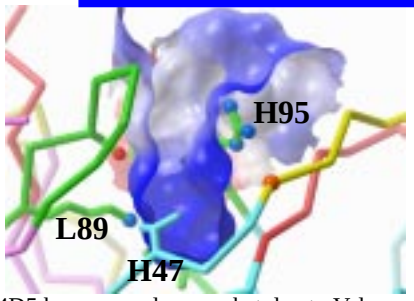
Using large combinatorial libraries should be more successful than conventional polyclonal and monoclonal antibody methods for developing panels of antibodies with subtle differences in cross-reactivity that will distinguish among the PAHs.

Large Phage Libraries → Human scFv → **NAPH-2**
Large Phage Libraries → Human Fab → **Ø**
PAH-specific Hybridoma → Mouse rFabs 4D5, 10C10 → **Engineered Variants**

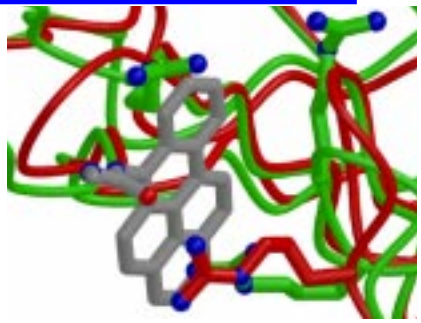


4D5 and 10C10, evoked by a 6-BaP conjugate, show different cross-reactivity profiles depending on the PAH hapten conjugate used as competitor in indirect competition EIAs. NAPH-2, selected from a human scFv phage library using a naphthalene hapten, competitively binds soluble naphthalene (data not shown). Selections from human rFab libraries using BaP or fluoranthrene were unsuccessful.

ARCHITECTURE OF ANTI-PAH BINDING SITES

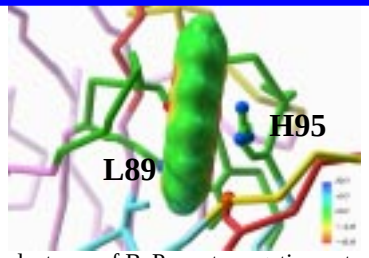


4D5 has a very deep pocket due to Val H47 (usually Trp) that shows strong positive charge (blue surface) due to Lys L89 and Arg H95. 10C10 is similar (Leu H47).

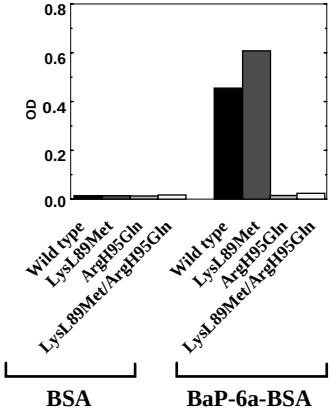


Like 4D5 (red with gray bound BaP), NAPH-2 (green) has Arg side chains in the binding pocket that can contact bound antigen.

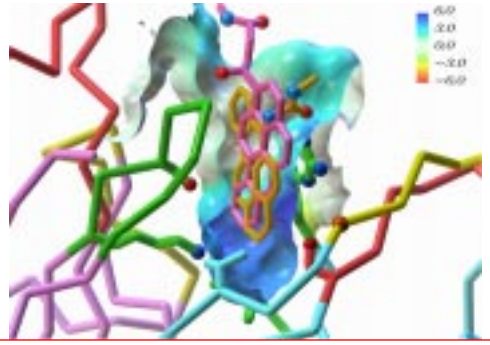
MECHANISM FOR STABILIZING BOUND PAHs: CATION-π



The π electrons of BaP create negative potential (red and yellow) above and below the plane of the aromatic ring system that complements the positive potential in the 4D5 binding pocket. Arg H95 is essential for strong binding; replacement by a neutral side chain abolishes BaP binding in immunoassays (right).

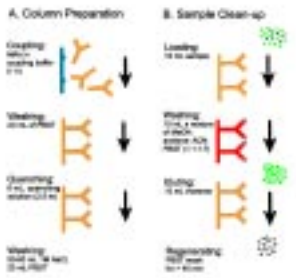


EXPLAINING CROSS REACTIVITY

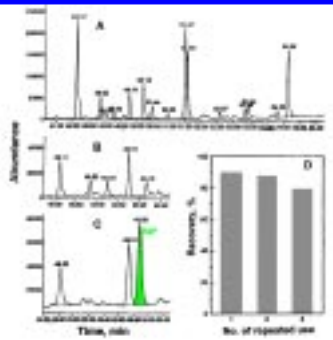


Both the 1- (pink) and the 6-linked (orange) BaP haptens dock with favorable energetics to the 4D5 binding pocket because of the almost symmetrical shape of BaP. Thus, the shape of the 4D5 pocket can accommodate multiple orientations of BaP itself as well as other smaller PAHs.

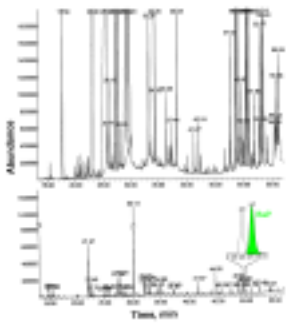
EXTRACTION AND RECOVERY METHODS



Immunoaffinity column preparation and sample cleanup.



GC-MS total ion chromatograms of the loading (A), washing (B), and elution (C) fractions of coral extract fortified with BaP show that all BaP remains trapped on the column until the elution step. Recovery remains high through 3 repeated uses of the immunoaffinity column (D).



GC-MS total ion chromatograms of crude extracts of coral obtained by supercritical fluid extraction (SFE), before (A) and after (B) cleanup by immunoaffinity column. SFE is a simple, fast, economical, nondestructive extraction method that reduces generation of organic solvent wastes because it utilizes supercritical CO_2.

CONCLUSIONS

- The binding pocket for BaP is unusually deep due to use of seldom-used sequences in the mouse germline. Such sequences are not present in the human germline or in the human synthetic libraries. Therefore, the architecture of the binding site is more important than sheer diversity in recombinant antibody libraries.
- Cation-π interactions reveal that complementarity of charge as well as shape is essential for PAH binding.
- The similarity among the PAHs and the importance of shape fit in recognition indicate that cross-reactivity is inherent among the PAHs.
- A custom-made library based on 4D5 and 10C10 should be a rich source of variants with altered cross-reactivity profiles that will be useful for distinguishing among the structurally similar PAHs.
- Hapten conjugates can be ranked as competitors in various immunoassay formats. This “recognition index” is useful for optimizing assay specificity and sensitivity for various PAHs.
- Immunoaffinity chromatography with MAb and rFab 4D5 removed over 90% of the interferents from supercritical fluid extracts of PAHs in corals.
- Use of EDTA as a modifier in supercritical CO_2 fluid extraction allows concurrent recovery of polar and apolar chemicals and improves recovery efficiency for PAHS and their adducts from soil, sediment, and coral.

PUBLICATIONS

M. Liu, G.A. Rechnitz, K. Li and Q.X. Li (1998) Capacitive immunosensing of polycyclic aromatic hydrocarbon and protein conjugates. Anal. Lett. 31:2025-2038.
K. Li, R. Chen, B. Zhao, M. Liu, A.E. Karu, V.A. Roberts and Q.X. Li (1999) Monoclonal antibody-based enzyme-linked immnunosorbent assays for part-per-billion determination of polycyclic aromatic hydrocarbons: Effects of haptens and formats on sensitivity and specificity. Anal. Chem. 71:302-309.
F. Guo, Q.X. Li, J.P. Alcantara-Licudine (1999) A simple Na4EDTA-assisted sub/supercritical fluid extraction procedure for quantitative recovery of polar analytes in soil. Anal. Chem 71:1309-1315.
M. Liu, G.A. Rechnitz and Q.X. Li (1999) Flow injection immunosensing of polycyclic aromatic hydrocarbon with a quartz crystal microbalance. Analyt. Chim. Acta 387:29-38.
M. Liu, G.A. Rechnitz and Q.X. Li (2000) Gold electrode modification with thiolated hapten for the design of amperometric and piezoelectric immunosensors. Electrochem. Anal. 12, 21-26.
S. Thomas and Q.X. Li (2000) Immunoaffinity chromatography for the analysis of polycyclic aromatic hydrocarbons in coral. Environ. Sci. Technol. In press.
Q.X. Li, A.E. Karu, K. Li and S. Thomas (2000) Refinement of immunochemical methods for environmental analysis of polycyclic aromatic hydrocarbons. ACS Symposium series, In press.
J.L. Pellequer, S.W.W. Chen, A.J. Feeney, B. Zhao, H.I. Kao, A.E. Karu, K. Li, Q.X. Li and V.A. Roberts (2000) Architecture of antibody binding sites for polynuclear aromatic hydrocarbons. ACS Symposium series, In press.
K. Li, L. Woodwood, A.E. Karu and Q.X. Li (2000) Immunochemical detection of polycyclic aromatic hydrocarbons in National Wildlife Refuge in Oahu, Hawaii. Anal. Chim. Acta. In press.
H. Li, R.I. Krieger and Q.X. Li (2000) Improved HPLC method for analysis of 1-hydroxypyrene in urine specimens of cigarette smokers. Sci. Total Environ. In press.
J.L. Pellequer, B. Zhao, H.I. Kao, C.W. Bell, K. Li, Q.X. Li, A.E. Karu and V.A. Roberts (2000) Stabilization of bound polycyclic aromatic hydrocarbons by a π-cation interaction. Submitted.