

Environmental Management Science Program

Project ID Number 55185

New Strategies for Designing Inexpensive but Selective Bioadsorbants for Environmental Pollutants: Selection of Specific Ligands & Their Cell Surface Expression

Brent L. Iverson

University of Texas at Austin

Austin, Texas 78712

Phone: 512-471-5053

E-mail: biverson@utxvms.cc.utexas.edu

George Georgiou

University of Texas at Austin

Austin, Texas 78712

Mohammad M. Ataii

University of Pittsburgh

Pittsburgh, Pennsylvania 15219

Richard R. Koepsel

University of Pittsburgh

Pittsburgh, Pennsylvania 15219

June 1, 1998

New Strategies for Designing Inexpensive but Selective Bioadsorbents for Environmental Pollutants: Selection of Specific Ligands & Their Cell Surface Expression

Brent L. Iverson, University of Texas at Austin

George Georgiou, The University of Texas at Austin

Mohammad M. Ataai, University of Pittsburgh

Richard R. Koepsel, University of Pittsburgh

Research Objective

The broad, long term objective of the research plan is to develop exquisitely selective polypeptide metal chelators for the remediation of aqueous systems. A variety of polypeptide chelators will be developed and optimized ranging from antibodies to small peptides. Then, through unique molecular engineering approaches developed in our laboratories, the polypeptide chelators will be anchored directly on the surface of the cells that produce them. Thus, instead of using isolated biomolecules we will employ inexpensive genetically engineered whole cell adsorbents. Following a simple, easily scaleable treatment, the engineered cells can be used to manufacture an inexpensive, particulate adsorbent for metal removal.

Research Progress and Implications

We are currently in year two of a three year program. Work has been focused on preparing the molecular biology constructs needed to carry out the optimization of a metal complex binding antibody, and on the isolation of a metal binding peptide.

- 1) We have isolated genes for the V regions of the heavy and light chains for the Ru(bpy)₃ specific monoclonal AC1106 (Shreder, K.S. Hariman, A. and Iverson, B.L., J. Am. Chem. Soc. 1996, 118, 3192-3201). This antibody binds Ru(bpy)₃ derivatives with high affinity, and will serve as our generic metal-complex binding pocket.
- 2) A -((gly)₃ser)₄- linker was added between the heavy and light chain to complete the construction on a so-called single chain Fv (scFv). The scFv is the smallest functional unit of an antibody that retains full binding activity.
- 3) The scFv has been cloned into a pET25b(+) vector for soluble expression.
- 4) We have synthesized various metal complexes to be used for a thorough binding analysis and optimization studies of the scFv.
- 5) We have prepared milligram quantities of the AC1106 scFv and characterized the binding properties using a fluorescence enhancement assay. We have measured binding constants for the scFv binding to various Ru(bpy)₃ derivatives.
- 6) We have cloned the scFv into our bacterial surface expression system in preparation for antibody engineering efforts. By using iterative rounds of randomization and selection via fluorescence activated cell sorting (FACS) the affinities for different metal ions will be evolved.
- 7) We have produced a sophisticated computer model of the scFv binding site to act as a guide for antibody engineering studies.
- 8) We have isolated Cu(II) and Ni(II) specific peptides using phage display technology. The isolated sequences contain histidine in the context of usually hydrophobic residues. These isolated sequences have improved physical properties compared to a simple polyhistidine peptide sequence.
- 9) We have initiated pilot studies to optimize the capture of metal complexes using the E. coli surface expressed scFv. The scFv reagent is prepared in "ready to use" form by simply growing

a culture of cells. We are currently carrying out a systematic series of studies to find the best format for isolating metal complexes in a highly selective and efficient manner.

- 10) In advances related to the metal-binding work, we have been able to optimize further the efficiency of our bacterial surface expression/FACS selection system for isolating interesting new antibodies. Using the optimized protocols on an scFv that binds the heart glycoside digoxin, we were able to produce an improved scFv with subnanomolar affinity after a single round of selection (Daugherty, P., Chen, G., Iverson B. and Georgiou, G. *Protein Eng.* (1998) in press)! In addition, we have been able to combine multiple mutations isolated in different library screening studies. This new “supermutant” has an extremely high affinity, as the combined mutations conferred an additive increase in binding. These powerful new procedures will be employed in the upcoming metal-complex binding antibody evolution studies.
- 11) In another advance related to the metal-binding work, we have recently completed the saturation mutagenesis studies of an entire antibody binding pocket (Chen G., Mendez, P., Dubrawsky, I., Georgiou, G. and Iverson, B., manuscript submitted). In other words, all of the amino acid residues that contact the bound antigen were changed one at a time to every other possible amino acid. Each mutant, 190 in all, was analyzed quantitatively for binding activity. The extremely high throughput of mutants required by this study was accomplished through optimized techniques we have developed for the in vitro production and analysis of antibody mutants (Burks, E.A., Chen, G., Georgiou, G. and Iverson, B.L. *Proc. Nat. Acad. Sci., USA*, 1997, 94, 412-416). Analysis of the data produced the first comprehensive model of the functional roles played by each amino acid in the binding pocket. This model will be used to steer future antibody engineering efforts.

Planned Activities

The stage is now set to begin the final phase of our work.

- 1) We will begin the AC1106 scFv antibody optimization studies in which the affinity will be improved and the metal specificity will be modulated in different optimized scFv mutants. For these tasks we will utilize our *E. coli* surface display / FACS selection strategy (please see 10 above) as well as our systematic in vitro mutagenesis approach (please see 11 above). The binding properties of all the optimized antibodies will be characterized in detail. These studies will establish how well a generic metal complex specific antibody binding site can be optimized to bind a variety of different metal species with a high level of specificity.
- 2) We will continue to adjust the parameters of the pilot studies to use the metal specific, surface-expressed antibodies and peptides for purification. In particular, we will be analyzing various methods of attaching whole cells as well as isolated membranes to various solid supports. In this way, the loading capacity versus cost will be maximized.