13. ABSTRACT (Maximum 200 words)

The objective of this research is to develop designed metal-binding proteins to report upon the presence of metal ions in solution. We use small, robust protein frameworks as scaffolds on which to design novel metal-ion binding sites. The metal binding site design is computational, to allow an exhaustive sampling of all possible sites. The sites are designed with defined geometries and a variety of primary ligands to metal, which allow a range of different metal-ion binding specificities and affinities to be arrayed. The aim is to couple the metal-binding event to a change in fluorescence of an appropriate probe. For practical applications, reagent-less systems are preferred, but in the development stages, extrinsic reporter probes are also investigated.
FINAL REPORT

Grant #: N00014-95-1-0913

PRINCIPAL INVESTIGATOR: Prof. Lynne Regan

INSTITUTION: Yale University

GRANT TITLE: Signal Transduction by Designed Metal-Binding Proteins

AWARD PERIOD: 1 May 1996 - 30 Apr 1999

OBJECTIVE: To develop designed metal-binding proteins to report upon the presence of metal ions in solution

APPROACH: We use small, robust protein frameworks as scaffolds on which to design novel metal-ion binding sites. The metal binding site design is computational, to allow an exhaustive sampling of all possible sites. The sites are designed with defined geometries and a variety of primary ligands to metal, which allow a range of different metal-ion binding specificities and affinities to be arrayed. The aim is to couple the metal-binding event to a change in fluorescence of an appropriate probe. For practical applications, reagent-less systems are preferred, but in the development stages, extrinsic reporter probes are also investigated.

ACCOMPLISHMENTS: We have designed a number of variants of the B1 domain of IgG-binding protein G that bind metal ions. The sites are at different positions in the protein and are formed by different combinations of primary ligands to metal (His and Cys combinations). The designed sites bind metals with a range of affinities, from sub-nanomolar to micromolar. They show a range of metal-ion binding specificities, with 1000-fold higher affinity for Zn versus Co at tetrahedral sites. We have shown that for a number of these sites there are changes in intrinsic Trp fluorescence upon metal-ion binding. The wavelength of Trp fluorescence emission is not ideal for practical sensing purposes (too short). We have therefore also investigated the effect of metal-ion binding upon the fluorescence of a hydrophobic dye, ANS. ANS is thought to interact with exposed hydrophobic patches on a protein. Several of our designed metal-ion binding proteins show differences in both the intensity and wavelength maximum of ANS fluorescence upon interaction with metal ions. Presumably, metal-ion binding induces conformational changes in the proteins, which can be detected by changes in their interaction with ANS. The observation of changes in wavelength maximum of emission in response to metal ion binding is particularly exciting, because it allows for ratiometric sensing, with its many attendant advantages.
In collaboration with Prof. David Walt, Tufts University, also supported by the ONR, we have immobilized these proteins in gels at the ends of fiber-optic cables, in the system that the Walt group has developed. The immobilized proteins plus ANS are able to detect the presence of low concentrations of Zn ions in beakers of test solution; there is no such response from control solutions.

CONCLUSIONS: The strategy holds great potential for the development of sensors for metal ions in the range of concentrations that is of interest to the ONR.

SIGNIFICANCE: Demonstration of how rational design of metal-ion binding sites can be linked with signal transduction system.

PATENT INFORMATION: None

AWARD INFORMATION: Herbert Dickerman Award, Wadsworth Center, New York, for "Exceptional Creativity in Research"

PUBLICATIONS AND ABSTRACTS:

