Environmental Management Science Program

Project ID Number 54856

Structural Biology of the Sequestration & Transport of Heavy Metal Toxins: NMR Structure Determination of Proteins Containing the -Cys-X-Y-Cys-metal Binding Motifs

Stanley J. Opella
University of Pennsylvania
231 South 34th Street
Philadelphia, Pennsylvania 19104
Phone: 215-898-6459
E-mail: opella@chestnut.chem.upenn.edu

June 1, 1998

Structural Biology of the Sequestration & Transport of Heavy Metal Toxins: NMR Structure Determination of Proteins Containing the -Cys-X-Y-Cys-metal Binding Motifs

Stanley J. Opella, University of Pennsylvania

Research Objective

The overall goal of our research is to apply the methods of structural biology, which have been previously used primarily in biomedical applications, to bioremediation. We are doing this by using NMR spectroscopy to determine the structures of proteins involved in the bacterial mercury detoxification system. The research is based on the premise that the proteins encoded in the genes of the bacterial detoxification system are an untapped source of reagents and, more fundamentally, chemical strategies that can be used to remove heavy metal toxins from the environment. The initial goals are to determine the structures of the proteins of the bacterial mercury detoxification systems responsible for the sequestration and transport of the Hg(II) ions in to the cell where reduction to Hg(O) occurs. These proteins are merP, which is water soluble and can be investigated with multidimensional solution NMR methods, and merT, the transport protein in the membrane that requires solid-state NMR methods.

Research Progress and Implications

As of June 1998, this report summarizes work after about one and half years of the three-year award. We have made significant accomplishments in three aspects of the NMR studies of the proteins of the bacterial mercury detoxification system.

NMR spectroscopy is well suited for determining the coordination geometry of mercury bound to proteins because ^{199}Hg is a commercially available stable isotope that has spin $S=1/2$ and a very large chemical shift range (more than 3000 ppm) that is highly sensitive to the number and type of ligands. This is particularly true for proteins like merP and merT where Hg(II) is the natural metal ligand. The one-dimensional ^{199}Hg NMR spectra of merP and merT in solution were obtained. There is a single resonance in each spectrum, indicating that there is only one unique Hg(II) bound to each protein. This is the expected result for merP, which has only two Cys residues, but is somewhat surprising for merT where two similar, but distinct metal binding sites are anticipated for the presence of four Cys residues. The chemical shift values observed for Hg (II) bound to both merP and merT are well within the range expected of linear bicoordinate aliphatic thiolate model compounds. Thus, the Hg NMR data demonstrate that the bound Hg (II) has two Cys ligands in both merP and merT.

We determined the three-dimensional structure of merP in solution by multidimensional NMR spectroscopy. This required the preparation of an expression system so that large amounts of isotopically labeled protein could be made and purified. MerP is water soluble, but thought to be weakly associated with the membrane surface. More than 1000 restraints were used in the calculations of the structures of this small 71 residue protein. Both the reduced and metal bound forms of the protein were investigated. Nearly all of the polypeptide is well structured in both forms. Residues-10 – 18, which form the metal binding loop, are the least well-defined portions of the structure. These are also the same residues that have their chemical shifts most strongly perturbed by metal binding; this may have functional significance for the proteins. The dynamics of both forms of the proteins were thoroughly investigated with relaxation measurements at multiple magnetic field strengths. Interestingly, there is a difference in the T2 values, but not other relaxation parameters, for residues 11 – 18 in the metal binding loop, between the two forms of the protein. This can be explained by the residues in the metal binding loop undergoing exchange among multiple

conformations, also pointing to a functional significance to the spectroscopic properties of these residues.

Both the reduced and mercury bound forms of the protein have essentially the same global fold, which consists of two antiparallel alpha helices overlaying a four strand beta sheet. The BaBBaB fold defines a class of aB proteins with antiparallel beta sheets and segregated alpha and beta sections. Since this class of proteins includes RNA binding domains, DNA binding domains, acylphosphatases, and phosphoglycerate dehydrogenase regulatory domain, there does not appear to be common functional theme among them. Thus, the protein fold may serve as scaffolding for holding the metal binding loop in a favorable position for binding Hg(II) and then passing it on to different metal binding site in merT for transport across the cell membrane.

All significant differences between the reduced and metal bound forms of merP are in the region of the metal binding loop. This region contains the highly conserved residues GMTCAAC, which includes the two Cys residues that contribute the sidechain –SH groups for metal ligation. Binding of the mercury causes a sleight unwinding of the helix as the two Cys residues become closer. Another difference is in the position of the aromatic residue Phe38 that lies below the metal binding loop. In the reduced form, this sidechain is oriented towards the binding loop; in contrast, the aromatic ring moves closer to the surface of the protein in the mercury-bound form. The movement of this aromatic ring may explain the large chemical shifts observed in the presence of minor structural changes between the two forms of the protein.

MerP is a water soluble, globular protein. However, merT is a highly hydrophobic membrane protein and is completely insoluble in water. Thus, its study necessitates the use of model membrane environments. The availability of two different well-characterized model membrane systems, micelles and bilayers, enables both multidimensional solution NMR and solid-state NMR experimental methods to be applied. The NMR studies require the use of uniformly ^{15}N and $^{13}\text{C}/^{15}\text{N}$ isotopically labeled samples. We developed a fusion protein expression system for merT that gave the relatively large amounts needed for NMR spectroscopy.

It is possible to prepare samples of merT in SDS micelles that yield two-dimensional HSQC spectra with excellent resolution, especially considering that it is a highly helical protein with limited chemical shift dispersion. The ^{13}C and ^{15}N resonances in double labeled protein samples are relatively broad because the protein lipid complex reorients in solution relatively slowly. As a result the assignment process, which is a necessary prelude to structure determination, has been proceeding slowly. Nonetheless, we are optimistic that the protein will be assigned in the coming months.

It is also possible to reconstitute purified merT into phospholipid bilayers, the definitive membrane environment for a protein with multiple trans-membrane helices. We are developing a general method for determining the structures of membrane proteins by solid-state NMR spectroscopy. It has two sample requirements. The protein has to be immobilized on NMR timescales by its environment, which occurs in lipid bilayers, and it has to be uniaxially oriented with respect to the direction of the applied magnetic field. This can be accomplished with multibilayers oriented between glass plates. We have obtained solid-state NMR spectra of merT oriented between glass plates, as well as magnetically oriented in bicelles, that demonstrate the presence of trans-membrane helices. The initial two-dimensional solid-state NMR spectra of proteins in this family are promising. The next step is to improve the samples and sensitivity so that three-dimensional solid-state NMR experiments can be applied for resolution among all sites and structure determination of the bilayer samples.

Planned Activities

The immediate goal is to optimize the conditions for solid-state NMR structure determination of merT and its variant protein merF, both of which transport Hg(II) across membranes. Then we will apply the multidimensional solid-state NMR experiments needed to obtain complete resolution of backbone sites and to assign them to specific residues in the protein.

Once the structures of both merP and merT are available, we hope to be able to reconstitute them in a model membrane system and be able to study the transfer of metal ions between them.