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Dr. Mark A. Gilbertson, EM-52
U.S. DOE
Director, Office of Science and Risk Policy
Room 5A-031
1000 IndependenceAvenueSW
Washington, DC 20585-0002

Re: DE-FG07-97ER62314

Dear Dr. Gilbertson,

This letter constitutes the annual report for the above listed grant entitled "Structural biology of the sequestration and transport of heavy metal toxins: NMR structure determination of proteins containing the –Cys-X-Y-Cys- metal binding motif '. This letter summarizes the major accomplishments of the past year.

As described in the original proposal, the research is aimed at using the methods of structural biology to describe proteins containing the –Cys-X-Y-Cys metal binding motif. Several different types of proteins are being investigated because this metal binding motif is involved in both the sequestration and transport of the heavy metals throughout bacterial and mammalian cells. In addition, this same metal binding motif has been shown essential in the binding and transport of a variety of metals. Our current research is focussed on mercury and copper, important as both components of biological systems and as toxins.

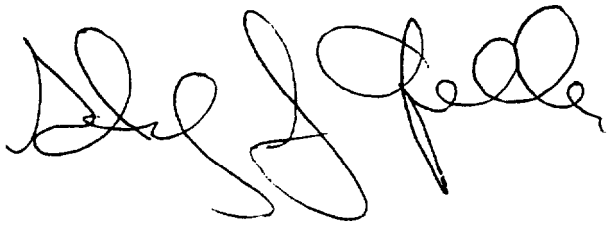
During the past year we completed the structure determination, including a description of the backbone dynamics of merP, the prototype of the soluble metal binding proteins with the –Cys-X-Y-Cys- motif. This was published: R. A Steele and S. J. Opella, Biochemistry 36, 688-6895 (1997) "Structures of the Reduced and Mercury-Bound Forms of MerP, the Periplasmic Protein from the Bacterial Mercury Detoxification System". The structures of the reduced and mercury-bound forms of merP, the periplasmic protein, which binds Hg(II) and transfers it to the membrane transport protein merT, were determined in aqueous solution by multidimensional NMR spectroscopy. The 72-residue merP protein has a βαββαβ fold with the two helices overlaying a four-strand antiparallel b sheet. Structural differences between the reduced and mercury-bound forms of merP are localized to the metal binding loop containing the consensus sequence -Gly-Met-Thr-Cys-X-Y-Cys-. The structure of the mercury-bound form of merP shows that Hg(II) is bicoordinate with the Cys side chain ligands, and this confirmed by the chemical shift frequency of the ^{199}Hg resonance.

We have made substantial progress in determining a second member of this family of protein, the first heavy metal binding domain of the P-type ATPase associated with Menkes disease. All of the the heavy-metal-transporting ATPases have a module with about 70 residues, highly homologous to merP, repeated up to six times near the N-terminus. The ATPase associated with Menkes disease has six repeats containing the characteristic –Cys-X-Y-Cys- metal binding

motif. We have cloned and expressed the first of these domains using very similar procedures to those used initially with merP, and we have been able to obtain samples uniformly ^{15}N labeled and uniformly ^{13}C and ^{15}N labeled for the full range of two- and three-dimensional solution NMR experiments used to determine the three-dimensional protein structure. The assignment process is about 90 % complete, and we expect to obtain preliminary folded structures in the near future.

We have also made substantial progress on determining the structures of membrane proteins bound transport proteins that contain the –Cys-X-Y-Cys- metal binding motif. Two different approaches are being used to determine the structure of these membrane proteins. Samples of the protein in micelles have been optimized for multidimensional solution NMR spectroscopy. The assignment of the protein spectrum is approaching completion. This relies on the extensive use of uniformly ^{2}H, ^{13}C, and ^{15}N labeled samples. In addition, the study of merT is part of our program to develop solid-state NMR as a method for protein structure determination. It is particularly applicable to membrane proteins because they can be prepared in highly oriented samples for the most powerful experiments. Now, merT is used along with other proteins, including fd coat protein, vpu from HIV, and channel-forming peptides from ligand gated ion channel receptor proteins to develop these methods. It is now feasible-to obtain well-resolved spectra of uniformly labeled proteins, and to measure multiple orientationally dependent spectral parameter for each residue for use in structure determination. The chief. problem to overcome is the assignment of the resolved resonances to specific sites in the proteins. Several lines of spectroscopy are being developed for this purpose, including abundant and dilute spin-exchange, which is particularly well suited for highly helical proteins, and triple resonance ^{1}H, ^{13}C, ^{15}N experiments on uniformly labeled proteins. Thus, both short-range through-space and separately, through-bond approaches are being developed. Very good results have been obtained on model peptides and these experiments are in the process of being applied to proteins. This is the essential step for moving forward with the structure determination of merT in membrane bilayers.

Sincerely,



Cc: R. Hersch