Physica B 158 (1989) 117–118
North-Holland, Amsterdam

XAS of the MerR Metalloregulatory Protein

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The central component of a metal-responsive genetic switch, the MerR metalloregulatory protein, is one of the first examples of an intracellular heavy metal receptor [1]. The *merR* gene product mediates the induction of the mercury resistance phenotype in bacteria [2], and resistant cells respond to subtoxic Hg(II) levels (10^{-6} -10^{-8} M) with transcriptional activation of the *mer* operon [3]. Genetic evidence indicates that the *merR* gene product, MerR, exerts negative control of the structural genes in the absence of Hg(II), and positive control in the presence of Hg(II) [3].

At present very little is known about the physical or biological properties of this protein, however it has been shown to have a very high specificity for mercuric ion. Recent work has demonstrated that mercuric ion converts the purified MerR protein from a repressor to an activator of the bacterial mercury resistance genes. In order to address, from a structural perspective, the mechanisms responsible for positive and negative regulation of the structural *mer* genes, we have measured x-ray absorption spectra for Hg + MerR.

Our goal is to determine the nature and the number of critical protein-mercuric ion interactions. Crystal structures of mercuric-thiol complexes typically show two very short mercury sulfur bond distances, but sometimes also show additional interactions with sulfur, nitrogen and oxygen donors at distances which exceed typical bonding interactions. The weak interactions are, in some cases, the result of crystal packing forces. However, metal ligand distances in an intermediate range (2.5-3.5 Å) are often the result of secondary bonding interactions between the metal and the donor ligand. These interactions can potentially play an important role in providing additional thermodynamic stability and metal ion specificity for the metal protein complexes.

The Fourier transform of the MerR EXAFS data are shown in Fig. 1. These data show a single large peak, indicative of a metal-ligand distance of ca. 2.4 Å. This peak can be simulated using Hg-S parameters but not using Hg-N parameters, suggesting predominantly sulfur ligation. This result, however, is inconsistent with known Hg coordination chemistry, since the average bond length (2.42 Å) suggests a first shell coordination number of 2, while the apparent coordination number is 4. In addition, the Debye-Waller factor increases between the model compound ($Hg(SCN)_2$) and MerR, although a decrease is expected (MerR was measured at 10K while the models were measured at room temperature).

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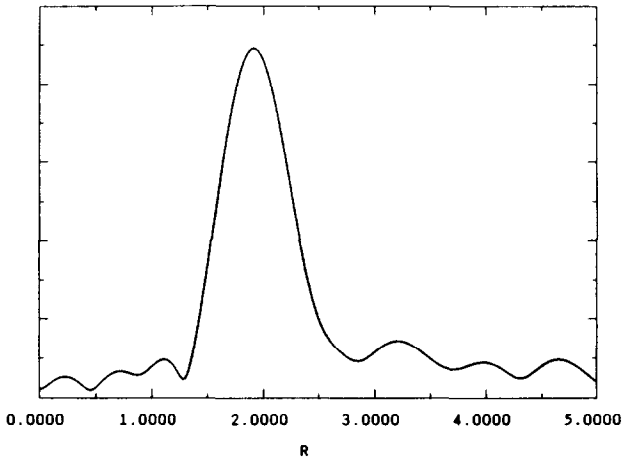

Figure 1. Fourier transform of k^3 weighted EXAFS data for MerR.

A two shell model (sulfurs at 2.35 and 2.51 Å) gives a dramatically better fit, and accounts for the inconsistencies discussed above. Specifically, the increase in Debye-Waller factor (between model and MerR) observed for the 1 shell fit is now interpreted as arising from static disorder. Assuming a Hg-S stretching frequency of 150 cm^{-1}, one predicts that $\Delta\sigma^2$ should be -2.6×10^{-3}. The observed increase in $\Delta\sigma^2$ for the 1 shell model would then indicate a static contribution of 5.6×10^{-3}. The latter is indicative of a mean spread in distances of ca. 0.15 Å, which is precisely the spread observed in the two shell fits. A cumulant analysis of $\ln[\chi(\text{MerR})/\chi(\text{model})]$ confirms that the disorder term (C4) is statistically significant. Using a 2-delta-function distribution, the cumulant analysis indicates a spread in distance of 0.16 Å.

Additional data are required to obtain a complete understanding of the structural properties of MerR. These preliminary results suggest, however, that a novel HgS_2S_2' site may be responsible for the unique Hg binding properties of MerR.

Acknowledgements. This work was supported in part by the NIH (GM-38047). JEPH is the recipient of a Camille and Henry Dreyfus Distinguished Young Faculty Award.

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