Crystal structure of the aconitase form of human iron regulatory protein 1.

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In animals, two highly homologous cytosolic Iron Regulatory Proteins, IRP1 and IRP2, are involved in the metabolism of this biologically essential metal. Both proteins can bind to specific mRNA regions called Iron Responsive Elements and thereby control the expression of proteins involved in the uptake, storage and utilization of iron. IRP2 is now known to be critically involved in Fe homeostasis. IRP1, however, loses its RNA-binding ability in its major form found in most tissues and normal cell cultures, wherein it contains a [4Fe-4S] cluster and becomes a cytosolic aconitase, catalyzing the conversion of citrate to isocitrate. The exact physiological role of IRP1 is still not entirely clear, but there are indications that it may be involved in oxidative stress response.
Human IRP1 has a molecular mass of 98 kDa. Starting from the recombinant enzyme produced in bacteria as aconitase, we have been able to crystallize this [4Fe-4S]-containing form under anaerobic conditions and collected X-ray diffraction data up to a maximum resolution of 1.85 Å. Attempts to solve the structure by molecular replacement, using the structure of the homologous bovine mitochondrial aconitase which shows about 22% sequence identity with IRP1, did not work. Subsequently, a second slightly different crystal form diffracting to 2.5 Å resolution was obtained [1]. Anomalous signals of the iron-sulfur cluster and a fortuitous zinc site in the native enzyme and data from an isomorphous gold derivative of this crystal form have been successfully used for phasing. Currently the structure is being refined and analyzed with respect to its aconitase and potential RNA-binding functions, using the results from numerous site-directed mutagenesis and other studies that are available in the literature.

1. J Dupuy, C Darnault, X Brazzolotto, LC Kuhn, JM Moulis, A Volbeda & JC Fontecilla-Camps (2005) Crystallization and preliminary X-ray diffraction data for the aconitase form of human iron regulatory protein 1. *submitted*