Structural and bioinformatic characterization of an arsenate reductase from Leishmania major

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Motivation

Arsenic and antimony are soft metals present in the biosphere due to either the use of pesticides and herbicides in agricultural and industrial activities or the leaching from geological formations. The physiological effects of these metalloids are still poorly understood, but they are known to interfere with biological processes, given their similarity to phosphorous and sulfur. As a result, living cells have evolved scavenging systems to sequester and pump arsenic and antimony outside the cell. Arsenic detoxification involves the reduction of As(V) to As(III), which is then selectively captured and extruded by specific membrane channels. Antimony should be extruded with similar mechanisms. Antimony has also a relevant role in the treatment of leishmaniasis, a parasitic disease caused by the protozoan parasite Leismania sp. The drug Pentostan (sodium stibogluconate) contains the inactive pentavalent antimony Sb(V), which is concerted to the active trivalent form Sb(III)by a recently identified Leishmania enzyme, LmACR2

Methods

LmACR2 has been cloned, overexpressed and crystallized. Its three-dimensional structure has been elucidated with X-ray diffraction methods, and refined at 2.15 Å resolution. The crystal structure of the protein was solved using the SIRAS method (Single Isomorphous Replacement with Anomalous Scattering).

Results

LmACR2 is comprised of 127 amino acids (14.5 kDa). The protein displays significant sequence homology and structural similarity with members of the molecular family of Cdc25 phosphatase (26 % identity spanning the whole catalytic domain of the human Cdc25A phosphatase). In particular, the conformation of the active site loop, hosting the invariant catalytic Cys residue, displays strong structural conservation in both enzyme families. The distinct substrate affinity and catalytic activities are therefore to be ascribed to the distinct amino acid composition of the active site loop and the neighborhood of the catalytic Cys. The combined sequence and structure comparison among members of the two enzyme families, together with the available biological information allows to define sequence / function relationship. The neighbor-joining three obtained for these proteins shows that several active site loop motifs can be assigned to phosphatase and As/Sb reductase activities, respectively. The subtle dependence of substrate specificity on the amino acid composition of the active site loop display the versatility of this ubiquitous protein family. In some cases a bifunctional activity can also be suggested.

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