DISRUPTION OF IRON-BINDING FROM ITS COORDINATION SITES AFFECTS Lia1 TERTIARY STRUCTURE: A MULTI-APPROACH ANALYSIS

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The exquisite aminoacid hypusine occurs exclusively in one major cellular protein, eIF5A. While deoxyhypusine synthase catalyzes the formation of deoxyhypusine, further hydroxylation of this intermediate generates the mature hypusine form. Although different biological functions have been attributed to eIF5A, its precise role remains unclear. In an attempt to identify eIF5A-interacting partners, the protein Lia1 was identified. Recently, *LIA1* has been demonstrated to encode the metalloenzyme responsible for the final step of hypusination. Lack of structural information on Lia1 and difficulties in crystallizing this protein led us to this multi-approach spectroscopic study. Combined techniques were used to investigate in which extent the presence of chelating compounds and pH variation affects Lia1 thermal stability, metal binding and activity. Evaluation of chemical-induced unfolding suggests the presence of two domains in this monomeric protein. Fluorescence quenching by KI and CsCl revealed negatively charged residues surrounding its single tryptophan residue. XRF confirmed that the yeast protein is indeed a metalloenzyme constituted of iron. Finally, the conformational changes induced by removal of iron from its coordination sites were analyzed by SAXS. Our results suggest that although the loss of iron does not affect Lia1 secondary structure, it exerts a significant effect on its tertiary structure, leading to a less compact and elongated structure than the iron-containing enzyme.

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