Área: INO

Spectroscopic investigation of the recombinant human cofilin-1: a bioinorganic study

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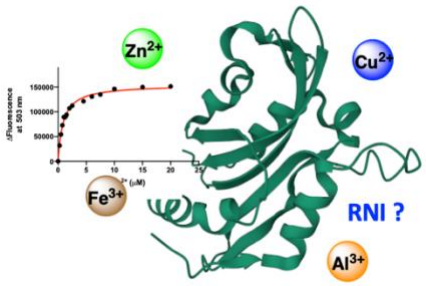
Highlights

Cofilin-1 is implicated in Parkinson's disease

Recombinant production of cofilin-1 and spectroscopic investigations

Interaction and structural modifications promoted by metal ions and RNI

Resumo/Abstract



Complex and incurable neurodegenerative diseases such as Alzheimer's and Parkinson's disease affect millions of people around the world and have raised great interest and concern of the scientific community.[1] The formation of protein aggregates has been a hallmark of neurodegenerative diseases including Parkinson's Disease (PD). Some proteins expressed in the brain are susceptible to misfolding and aggregation processes such as α-synuclein, which has been implicated along with others in Parkinson's disease[2]. The role of transition metals or other agents in the protein aggregation process, particularly α-synuclein, has been explored by bioinorganic chemistry community. In this context, human cofilin-1, a protein with several biological roles, including potentially involved in PD, has likely an important role in the early stages of neurodegenerative process[3]. Aiming to shed light on species that could interact with cofilin-1 and eventually lead to misfolding and or aggregation, recombinant production and structural investigation of this protein was carried out. By employing fluorescence, circular dicroism and thiol reactive reagent, metal ions were investigated as well as RNI (reactive nitrogen species, e.g., NO and HNO). Initially, optimization of the routine of fluorescence assay involving the human Cofilin-1 in the reduced and oxidized forms (free thiols or disulfide bridges) was carried out along with the investigation of its possible interaction with ions (Fe^{3+}, Zn^{2+}, Cu^{2+} and Al^{3+}). Intrinsic and extrinsic fluorescence were explored either using excitation of the tryptophan residue or bis-ANS. The results suggested an apparent interaction of the ions at least with native cofilin-1. There are changes in the maximum of the protein emission indicating conformational changes along with an increase in the light scattering using Zn^{2+} even at below 15 μmol L^{-1}, which was also noticed for Al^{3+}. This observation is encouraging and may, eventually, assist on further investigation of the possible mechanisms of cofilin-1 in the neurodegenerative diseases, especially in PD. Extrinsic fluorescence using bis-ANS showed a K_d for this probe at 3.9 μmol L^{-1}, whose studies indicated disturbance of profile upon use of metal ions. These measurements provided an apparent K_d for some of the metal ions. Zinc ions showed binding without much change in the bis-ANS emission profile, while iron(III) and copper(II) ions caused major changes. Circular dichroism has been done to evaluate conformational changes as well oxidation of the thiols of cofilin-1, which has also been explored upon treatment with NO and HNO donors. These studies opened exciting opportunities to shed some light on the possible biological role of these metal ions and reactive nitrogen species with human cofilin-1, considering these species might be detected in the brain.

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