Cofilin Oxidation Induces Conformational Changes and Decreases Actin Polymerization *in Vitro*

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Cofilin is an actin-binding protein that has a fundamental role in the regulation of actin dynamics. Although cofilin is regulated by factors such as phosphorylation, pH, binding of phosphoinositides, and subcellular compartmentalization, little is known about the effect of oxidation on its biological activity. Here we evaluate the role of cofilin oxidation on its ability to regulate actin polymerization. Recombinant cofilin (Cytoskeleton) was incubated with taurine chloramine (TnCl). Cofilin oxidation was confirmed by Cys-5-iodoacetamine fluoresceine (5-IAF) derivatization and change in protein's pI. Fluorescence assay of actin polymerization carried on with different cofilin (or oxicofilin): actin ratios (1:4, 1:8, 1:16 and 1:32) and Western Blot analysis demonstrated that oxidized cofilin loses its influence on actin polymerization. Circular dichroism analysis showed a change in cofilin structure upon oxidation. Mass spectrometry showed that cofilin is preferentially oxidized in Cys residues, and Cys39-80/Cys139-147 disulfides are the main products. Additionally, in order to obtain further structural insights into the process molecular dynamics simulations employing the GROMACS package and GROMOS96 force field were performed. These insights suggested that, while cofilin oxidation had no major influence on actin structure, the disulfide formation decreases its flexibility, causing a mild change on its conformation and so increasing the distance between actin and cofilin molecules. Taken into account, our results demonstrate that cofilin oxidation causes Cys disulfides formation and conformational changes, leading to a lost in actin-binding properties. Therefore oxidation demonstrates to be an efficient way to regulate cofilin biological activity over the dynamics of actin cytoskeleton. Supported by CNPq Universal (funds 479860/2006-8 and 472174/2007-0).