Cytochrome c-based films and fibril superstructures as protein biomaterials: a SEM and IR spectroscopy investigation

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Cytochrome c (cyt c) is an evolutionarily conserved heme protein of ∼12 kD and 104 amino acids residues, with a primary function in electron transfer between complex III (ubiquinol:cyt c reductase) and complex IV (cyt c oxidase) in the respiratory chain in mitochondria. In addition, cyt c is a key component in activation of apoptotic cell death signals, as well as a ROS scavenger in healthy cells [1]. Native structure of cyt c consists of four α-helices forming a compact core around the covalently attached heme moiety. Despite its stable structure, dysfunction of cyt c may be involved in oxidative stress in mitochondria and trigger mechanisms linking apoptosis to amyloidogenesis. Amyloidogenic proteins have the capability of creating highly specific non-covalent contacts, leading to self-assembling into β-sheet linear aggregates [2]. Besides to be associated to a wide range of degenerative diseases (Alzheimer's and Parkinson's diseases) [3], amyloid fibrils can be useful as building blocks for protein-based functional materials, with applications in optoelctronics, gas sensing and edible coatings. As biomaterial, cyt c was employed to create porous nanostructures for toxic vapor gas sensing and self-assembling polypeptide fusion proteins with cyt c allowed to achieve high densities of metalloporphyrins on amyloid fibrils [Baldwin 2006][3]. Despite its relevance, fibrillation of cyt c has not been deeply addressed and both mechanism of polymerizaton and structural information on oligomers are partially known [4]. Scanning electron microscopy (SEM) and infrared (IR) spectroscopy are powerful tools in the identification of morphological properties and modifications in secondary structure that underly fibril formation either in the development of neurodegenerative disorders or in fibril-based novel biomaterials. In this study, polymerization and fibril formation of cyt c were studied in alkaline conditions at pH values below (Tris-HCl buffer, pH 9) or above (NaOH, pH 13) the isoelectric point of the protein (pH∼10). The effect of base type and protein concentration was analyzed. Characterization by SEM, ThT fluorescence and IR spectroscopy, together with PCA analysis of the IR spectra, was carried out to elucidate morphology and secondary structure of polymers/fibrils from cyt c. The results provided evidence that a one-step kinetic control of the fibril morphology was obtained as a function of pH of the medium. IR spectroscopy indicated that fibril elongation utilized either ordered (Tris-HCl) or disordered (NaOH) structures. Cyt c polymerization could be directed towards the achievement of fibrils superstructures or extended films with amyloid-like nature, prevalence of one or the other final structures being dependent on protein concentration and incubation time.

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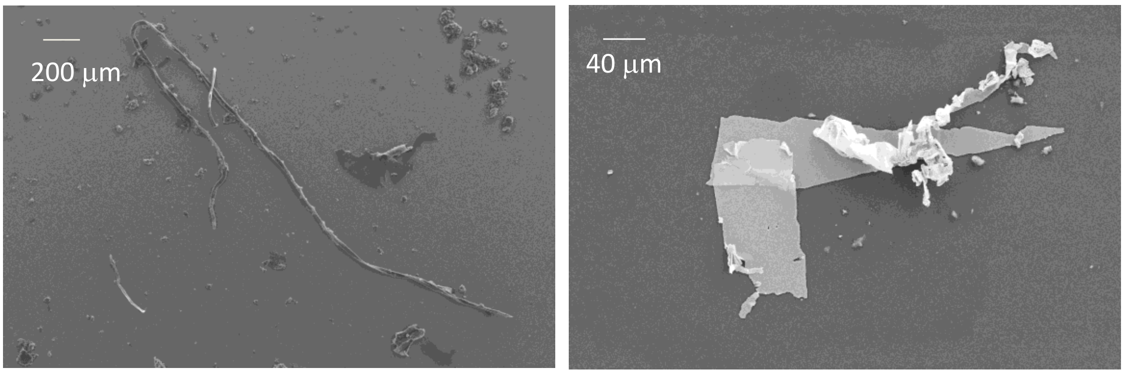

Figure 1 *SEM micrographs of samples of cyt c aggregated via Tris-HCl (left panel) or via NaOH (right panel).*

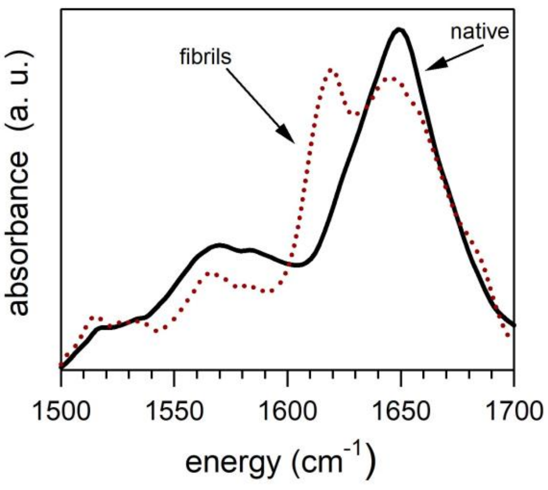

Figure 2. *Infrared absorption spectrum of cyt c (90 μM) in TRIS-HCl buffer (50 mM, pH 9) in the amide I region. Continuous spectrum refers to the native state of the protein, the dashed spectrum to protein after two-hours incubation. Red shift and enhancement of the β-sheet contribution indicates formation of amyloid fibrils.*