Molecular basis for the interaction between cytochrome c and its novel apoptotic target 14-3-3ε

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Cytochrome *c* (C*c*) is a mitochondrial protein which results essential to triggering apoptosis, when it is released into the cytosol. Traditionally, the activation of the apoptosome, and thereby the activation of the caspases, was considered the only cytosolic role of C*c*, through its binding with Apaf-1. However, our group evidenced that C*c* interacts with several nuclear and cytosolic targets upon cell death stimuli. Among these novel apoptotic targets, 14-3-3ε stands out due to its importance in several processes, such as cellular cycle and apoptosis. Thus, the study of the interaction between C*c* and 14-3-3ε rises special relevance for the understanding of the apoptosis signalling.

In this work, we tackle the interaction of C*c* with 14-3-3ε using Isothermal Titration Calorimetry (ITC) and Nuclear Magnetic Resonance (NMR). Calorimetry assays show C*c* binds 14-3-3ε at two different binding sites, with binding affinities in the μM range. The combined use of site directed mutagenesis on 14-3-3ε and ITC assays allows the identification of the concave groove and the convex face of 14-3-3ε as C*c*-binding sites. Furthermore, both ITC and ^{19}F NMR titrations of C*c* with the C-terminal tails of 14-3-3ε expose these tails interact directly with C*c*. In addition, the C*c* NMR perturbation map reveals how the metallo-protein recognizes 14-3-3ε using the rim of the heme group.

Finally, a structural docking for the C*c* / 14-3-3ε complex is presented based on molecular dynamics along with restrain-driven HADDOCK computations. Then, our data provide molecular basis for a novel cytosolic interaction involving C*c*, enhancing its apoptotic function.