Mapping of the interaction between STAT1 and flavonoids

Abstract

An experimental approach is described, in the first part of this Ph.D. work, for determining protein-small molecule non-covalent ligand binding sites and protein conformational changes induced by ligand binding. The methodology utilizes a combination of multiple technical approaches: limited proteolysis, MALDI TOF MS, circular dichroism and Surface Plasmon Resonance (SPR) to determine the binding sites in signal transducer and activator of transcription 1, STAT1 (87kDa)-flavonoid (Epigallocatechin-3-gallate, Myricetin and Delphinidin, about 500 Da) non-covalent complex. Comparing relative ion abundances of peptides released from the limited proteolysis of STAT1 and the STAT1-flavonoid complex after 0, 5, 15 and 30 minutes of digestion revealed that the binding of flavonoid induced a significant change in surface topology of STAT1. An increase in ion abundance and a different peptide profile suggest that the flavonoids obstruct the access of the proteases to one or both termini of specific peptides, identifying flavonoids binding region. Taken together, MALDI MS and SPR data led us to assume that the binding sites are close to Tyrosine 701 and that the flavonoids probably act disturbing the phosphorylation of TYR701 and the following dimerization and activation of STAT1.

PDIA6-BiP complex: role in the regulation of the unfolded protein response

Abstract

The unfolded proteins response (UPR) induced in many experimental settings is an extremely strong response that usually leads to cell death rather than to restoration of the ER homeostasis. Because the outcome of UPR signaling determines cell fate, a key unresolved molecular question is how UPR signaling is attenuated. Indeed, it is often under-appreciated that UPR signaling in response to stress is transient and is attenuated. Recently it has been proved that yeast UPR matches its output to the magnitude of the stress by regulating the duration of IRE1 signaling. An ER protein, known as binding immunoglobulin protein (BiP), binding to UPR sensors regulates their deactivation. Our idea, described in the second part of this Ph.D. work, is that there is another luminal ER factor, which interacts with the UPR sensors and is involved in attenuation of their activities. This factor is protein disulphide isomerase 6, PDIA6 (also known as P5), a poorly understood member of the protein disulfide isomerase (PDI) family, whose absence, according to our data, confers hypersensitivity to ER stress because one of its main action is tied to the sensing of UPR, rather than to the consequences of UPR signaling. We thought that PDIA6 uses its protein disulfide isomerase activity to interact specifically with UPR sensors in the ER lumen and attenuate their activities, thus regulating the duration of ER stress signaling.