

Molecular mechanism of 14-3-3 protein dependent regulation of caspase-2

Abstract

Caspase-2 is a protease standing apically in the cascade of reactions leading to apoptosis. Properly functional apoptosis eliminates damaged cells, autoreactive lymphocytes or redundant groups of cells in ontogeny. The process of caspase-2 activation must be precisely regulated. One of the described ways of caspase-2 regulation causing its inhibition is posttranslational modification phosphorylation with subsequent binding of the regulatory scaffold protein 14-3-3. The aim of this dissertation is to explain the molecular mechanism of this regulation. To understand the interaction between the proteins, it was necessary to first identify the phosphorylation sites in the caspase-2 molecule recognized by the 14-3-3 protein and then describe the detailed structure of the binding complex. The structure was characterized by a number of biochemical and biophysical methods, such as analytical ultracentrifugation, native electrophoresis in TBE buffer, polarization-fluorescence assay, hydrogen/deuterium exchange coupled to mass spectrometry, or crystallization; and the results led to stimulating conclusions. Activation of caspase-2 begins with its binding to adaptor proteins, cleavage and dimerization of the catalytic subunits. The results showed that the 14-3-3 protein can inhibit caspase-2 activation by sterically occluding of sequences necessary for dimerization and/or binding of adaptor proteins. A second possible mode of inhibition is the overlapping of the nuclear localization sequence of caspase-2 and, as a consequence, the effect on the translocation of caspase-2 into the cell nucleus, where it is the only caspase to be activated under certain conditions.

Keywords

caspase-2, procaspase-2, 14-3-3, protein-protein interaction, phosphorylation, nuclear localization sequence, apoptosis