

Abstract

Equistatin from the sea anemone *Actinia equina* contains a protein domain Eqd2 which inhibits aspartic peptidases and has not been characterized in detail. Recombinant Eqd2 was produced in the yeast expression system, and a protocol for its chromatographic purification was designed. The inhibitory specificity of Eqd2 was determined using a fluorescence inhibition assay, showing that Eqd2 is a highly selective inhibitor of cathepsin D-like and pepsin-like aspartic peptidases of family A1. Furthermore, size exclusion chromatography was used to analyze the Eqd2-peptidase complex and Eqd2 oligomerization in solution. Initial screening of crystallization conditions for Eqd2 was performed towards its structural analysis. This work provides important new information about Eqd2 as a unique type of natural inhibitors of aspartic peptidases. Its interaction mechanism can be exploited in the development of synthetic mimetics for regulation of medically important peptidases.

(In Czech)

Key words: peptidase inhibitors, proteolytic enzymes, activity and inhibition of enzymes, recombinant expression, protein purification, protein crystallization, equistatin