

Abstract

The research described in the PhD thesis is focused on a protein that degrades messenger ribonucleic acid (mRNA) – the Decapping Scavenger (DcpS). The protein's cellular role is to hydrolyze the mRNA 5' end during the last stages of degradation of short oligonucleotide fragments. The known crystallographic structure of the protein dimer (molecular mass of a monomer is $40 \cdot 10^3$ u) did not answer the questions on the molecular mechanism of action.

In the presented research, the mechanism of mRNA 5' end binding was determined, as well as specificity of enzymatic hydrolysis; also, affinity towards a wide class of synthetic cap analogs with potential therapeutic application was found. Different chemical modifications within the structure of the studied compounds allowed a precise image of the molecular interactions and specificity of the DcpS active site to be determined. Thermodynamic parameters of cap binding by DcpS were obtained, also is comparison to the best-known cap-binding protein, translation initiating factor eIF4E.

A range of complementary biophysical methods was used: calorimetry, absorption spectroscopy, fluorescence spectroscopy (both, steady-state and time-resolved) and circular dichroism spectroscopy. A large part of the experimental studies is innovative. For the first time, isothermal titration calorimetry was used to determine the stoichiometry of the protein-cap system, and circular dichroism in near-UV range was used to study structural changes within the protein. The experiments with use of fluorescently labeled mRNA 5' end analogs opened the possibility to use new techniques for studying interaction between cap and proteins.