Aleksandra Ferenc-Mrozek

„ Zastosowanie wybranych metod fizyki i biologii molekularnej w badaniach struktury i oddziaływań białek z rodziny DcpS”

Abstract:

The object of my research described in the doctoral thesis is the DcpS protein (Decapping Scavenger), which is involved in the degradation of the structure (the so-called cap) located at the 5 'end of the messenger ribonucleic acid (mRNA). Hydrolysis of the 5 'end of mRNA by the DcpS enzyme within short oligonucleotides protects the cell from the accumulation of cap molecules, which can result in inhibition of processes such as protein biosynthesis on the mRNA template or pre-mRNA maturation. Disorders associated with dysfunction of components involved in mRNA metabolism (including the DcpS enzyme) in humans can cause, among others, diseases of the nervous system, cardiovascular system and even cancer. The experiments presented in the these were performed on DcpS proteins derived from *H.sapiens* (human), *Caenorhabditis elegans* (free-living nematode) and *Ascaris suum* (parasitic nematode - pig roundworm). Knowledge of the mechanisms that ensure proper mRNA life cycle in selected organisms is very important because:

- *C.elegans* is a model organism used in molecular biology, used to understand key processes in eukaryotic cells,
- *A.suum* is a cosmopolitan parasite probably capable of living in humans,
- for almost a decade, there have been new reports on the association of human DcpS enzyme dysfunction with mental and physical impairment, which has been registered as a new disease entity in medicine.

The experimental results described in the dissertation were divided into two parts. The first concerned the preparation of a suitable bacterial construct for the “production” of DcpS proteins in bacterial cultures. In this part, I analyzed the effect of the localization of a label consisting of a sequence of 6-10 histidines (used to purify the protein by metal-affinity chromatography) at the N- or C-end of the DcpS protein from *H.sapiens, C.elegans* and *A.suum* on the structure, stability and hydrolytic properties. My results confirmed that the polyhistidine sequence does not significantly affect the activity, thermostability and secondary structure of DcpS proteins. The obtained differences, e.g. a twofold increase in enzyme activity or a shift of 1°C in the melting temperature compared to the native protein (no histidine label), can rather be considered in terms of tendencies to change in certain directions. I also explained the doubt regarding the substrate specificity of the DcpS enzyme for the m^7GDP molecule in the context of the label localization in the protein, which was not described unambiguously in the literature. The experiment that paved the way for further research, which was presented in the second part of the results, was the observation of changes in secondary structures during thermal denaturation of DcpS proteins by circular dichroism spectroscopy (CD). CD is a technique often used to control the process of thermal and chemical unfolding of proteins. DcpS proteins from *H.sapiens* and nematodes showed an increase in β-sheet populations with increasing temperature. Typical denaturation processes lead to the loss of protein structure elements such as α-helices or β-sheets. Therefore, situations where an increase in the level of β-structures is observed during denaturation are an interesting phenomenon that requires detailed analysis and explanation.

There is no information in the literature about thermal stability of DcpS proteins and the mechanisms of their aggregation. I decided to explore this gap and characterize DcpS enzymes. Scientists are studying the effects of stress factors like temperature or the presence of a denaturant, which in my study was guanidine hydrochloride, on the stability of protein structures. Paradoxically, following the process of denaturation, i.e. the destruction of the protein structure, allows us to better know and understand not only the mechanisms of eventual aggregation, but also the interactions that are crucial in the process of folding the protein to its specific conformation and important for the stabilization of this structure.

My experiments showed that DcpS proteins from humans and nematodes incubated at a constant temperature of 37°C form aggregates with morphology and secondary structure characteristic of amyloids. An important step for my work was to include human DcpS protein with a mutation in the study, which showed a stronger tendency to aggregate compared to human protein in its native form. In the context of the obtained results regarding the formation of amyloid fibers, it was necessary to try to explain this process. The mechanism of aggregation of DcpS proteins turned out to be a extremely challenging topic to determine. Thermal and chemical denaturation indicated its probable paths, where one of the stages is probably phase separation and formation of protein condensates.

All obtained results are the first reported studied on aggregation of DcpS proteins. It is difficult to determine at the moment whether the fibers formed by the DcpS enzyme have a functional or pathological character and whether their presence in *in vitro* experiments will be confirmed *in vivo* in cells. The results obtained are a solid starting point for further research and considerations on this topic.

In the work, a number of methods were used, including fluorescence spectroscopy, circular dichroism spectroscopy, dynamic light scattering, differential scanning calorimetry, differential scanning fluorimetry, confocal and electron microscopy. Some bacterial vectors encoding DcpS proteins, used for its production in bacterial cultures and purification, were designed and performed by me using molecular biology methods. I used also the services of commercial laboratories and those belonging to the university Core Facility.

In the introduction of my thesis I presented the current state of knowledge on DcpS proteins from humans and selected nematodes. In order for the role of DcpS protein to be understood in a biological context, in the literature part I, I described the processes and issues related to mRNA metabolism in the cell. In light of the obtained results, an important subchapter of the introduction is also: “Physicochemistry of protein aggregation”, describing the mechanisms of aggregation and its medical aspect. In the following chapters, I presented the research objectives and characteristics of the research methods used. The results obtained along with the analysis of the experiments in the context of literature data are presented in the chapter: Results, Conclusions and Discussion, which was divided into two parts. The last chapter: Summary contains the most important conclusions from the conducted research.