ABSTRACT OF DOCTORAL DISSERTATION

Interaction of the human ribosomal P proteins with the catalytic ricin A subunit

The eukaryotic ribosomal stalk is a protein complex within the ribosomal GTPase center that is responsible for stimulating GTP hydrolysis through translational GTPases, proteins that assist the ribosome in the translation process. The stalk complex is composed of the uL10 ribosomal protein and P1/P2 proteins which are unique for eukaryotes. Two P1-P2 heterodimers together with the uL10 protein form a pentameric complex with the uL10(P1-P2)$_2$ configuration. The pentameric complex is responsible for the interaction with translational GTPases, playing a role in their binding to the ribosome and is involved in the stimulation of GTP hydrolysis mediated by translational GTPases. Consequently, the pentameric complex is responsible for the high translational processivity of the ribosome. The ribosomal proteins included in the pentameric complex contain a highly conserved C-terminal fragment that is critical for interaction with translational GTPases. The C-terminal fragment is believed to be the main binding platform for the family of translational GTPases, providing full functionality for the ribosome. Interestingly, a number of studies have shown that these proteins are also a target for the Ribosomal Inactivating Protein (RIP) family, which are considered to be one of the most toxic proteins in nature. RIP proteins lead to the depurination of rRNA within the GTPase center, which leads to inhibition of ribosome-mediated translation, resulting in cell death.

The aim of the research in this study was to prescribe the role of individual P proteins, and above all to define the role of individual C-terminal fragments in the interaction with the RIP protein - ricin. For this purpose, the interaction of human P protein complexes and their deletion forms with the ricin catalytic subunit (RTA) was investigated. It should be emphasized that despite of many years of research, the interaction of RIP proteins with ribosomal P proteins has not been thoroughly investigated. The participation of P proteins and their C-terminals in the binding of RIP proteins was described using a series of biochemical tests, but the analyzes were carried out only in the so-called isolated experimental systems using various forms of

oligomeric P proteins, such as P2 homodimers or P1 oligomers, or with the use of short peptides. Due to the fact that P proteins preferentially form biologically significant P1-P2 heterodimers, which are considered to be the central functional element of the ribosomal stalk, this study investigated the interaction of the P1-P2 dimer with RIP proteins, using the heterodimer as a so-called the smallest functional structural unit of the stalk. As a part of the research, *de novo* genetic constructs were developed to obtain the so-called deletion forms of human P proteins in which the 16 conserved C-terminal amino acids have been removed (respectively named $P1_{\Delta C}$ for the P1 protein lacking the CTD fragment and $P2_{\Delta C}$ for the P2 protein also lacking the C-terminal fragment). The scope of research included a number of activities in the field of molecular biology and biophysical analyzes, including genetic and protein engineering, Size Exclusion Chromatography (SEC), Circular Dichroism (CD), Nano differential scanning fluorimetry (nanoDSF) and Native Mass Spectrometry (MS). In addition, techniques to analyze protein-protein molecular interactions such as Microscale Thermophoresis (MST) and Bio-layer Interferometry (BLI) were used.

The first task was to obtain recombinant P1, P2 proteins and their deletion forms - $P1_{\Delta C}$ and $P2_{\Delta C}$. For this purpose, heterologous expression and purification of P proteins was performed depending on the biophysical parameters of individual proteins. Obtained individual recombinant proteins were used to form protein complexes: P1-P2 heterodimer, heterodimeric deletion forms - P1-$P2_{\Delta C}$, $P1_{\Delta C}$-P2, $P1_{\Delta C}$-$P2_{\Delta C}$ and P2-P2 and $P2_{\Delta C}$-$P2_{\Delta C}$ homodimers. In the next step, the structural characterization of the obtained P protein complexes was carried out using biophysical methods such as SEC, CD, nanoDSF and "native"-MS. In parallel, the heterologous expression of the ricin catalytic subunit (RTA) was performed. The obtained recombinant protein was purified by affinity chromatography. Parallel to the dimer analyzes the biophysical characterization of RTA was performed. The protein:protein interaction study was performed using two methods: MST and BLI. As a results of the research, the values of dissociation constants were determined from which the level of affinity of individual complexes to RTA could be specified. The obtained results indicated that the C-terminal region of P proteins is responsible for the interaction with RIP proteins. Moreover, these studies allowed to precisely define the role of individual C-terminal elements, indicating the unequal role of these polypeptide fragments within the P1 and P2 proteins - it was observed that the C-terminal fragment derived from the P1 protein plays a dominant role in the interactions with RTA. The results of the analyzes suggest that not only the C-terminal fragment, but also the spatial organization of the P1-P2 dimer is important for the interaction with RTA. On the basis of the

obtained results, a model of interaction of the P1-P2 heterodimer was proposed, taking into account the spatial organization of the complex, indicating that the system of two P1-P2 proteins ensures the proper spatial orientation of the C-terminal fragment of the P1 protein, which promotes interaction with RIP proteins.

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