Abstract

The speed and accuracy of protein synthesis depends on the interaction of the ribosome with protein factors with GTPase activity, the so-called trGTPases. Binding of trGTPases to the ribosome occurs within the GTPase-associated center, located on the large ribosomal subunit. The eukaryotic GTPase-associated center consists of a conserved fragment of rRNA called the sarcin-ricin loop (SRL), and the protein part, which is a complex of P-proteins forming a characteristic lateral protuberance on the ribosome. In humans, the P-protein complex is composed of two P1-P2 heterodimers attached to the ribosome *via* the uL10 protein, forming the pentameric uL10(P1-P2)$_2$ structure. The element directly involved in interaction with trGTPases is a conserved C-terminal fragment of P proteins, which is also an interaction site for a group of toxins known as ribosome-inactivating proteins (RIPs), such as ricin or trichosanthin. The eukaryotic P-protein complex has five identical C-terminal fragments, but the function of their multiplication is not fully understood. The role of post-translational modification of these proteins in the form of phosphorylation of conserved serine residues located within the C-terminal fragments is also unclear.

The aim of the study was the functional characterization of C-terminal fragments of ribosomal P-proteins in their interaction with RIP toxins. The most important aspect of the research was to elucidate the molecular mechanism of interaction between P-protein complexes and RIPs, with particular emphasis on the function of individual C-termini of P1 and P2 proteins in RTA and TCS binding and the role of phosphorylation of conserved serine residues in the P-proteins C-terminal sequence in interaction with RIPs. As part of the dissertation, a review was also prepared, summarizing the research on the interaction of ricin with the ribosome.

The experimental model comprised of complexes of human P-proteins in the form of P1-P2 protein dimers and uL10 (P1-P2)$_2$ pentamers, and recombinant RTA and TCS. To determine the role of the individual C-terminal fragments of the P1/P2 proteins, a genetic system has been developed that allows for the efficient expression and purification of the P-protein pentamer with truncated C-terminal fragments (ΔC) within the P1 or P2 protein. Genetic constructs for the expression of P1 and P2 proteins with S/A and S/E mutations in the C-terminal fragment were prepared as well, allowing to study the role of C-terminal phosphorylation of P proteins. All used proteins were obtained by heterologous overexpression in the bacterial system and multistep purification, and then their biophysical

characterization was performed. The analysis of the interaction of P-protein complexes with RIP toxins was performed using microscale thermophoresis and biolayer interferometry.

It was shown that the interaction of RTA with P-proteins display high affinity, and the kinetic profile indicates a rapid association and dissociation of the resulting complex. Based on kinetic analyzes, it was observed that the stoichiometry of the interaction is 1: 1, i.e. one RTA molecule interacts with P-protein complex, regardless of the number of C-terminal fragments. The results indicate that the presence of additional copies of the C-terminal fragments of the P-proteins in the pentameric structure contributes to the increased association rate and stabilizes the nascent complex. The pivotal role of the P1 protein in the interaction of the P-protein complex with RTA was also indicated. Based on the results of the analyzes, a mechanistic model of the interaction of P-proteins with RTA has been proposed, showing that delivery of the toxin to SRL does not depend solely on interaction with the C-terminal fragment of the P1 protein; the structural context within the full pentamer, constituting the native form of ribosomal stalk complex, plays also an important role in anchoring the toxin to the ribosome.

The analysis of the interaction of RTA and TCS with P1-P2 protein dimers with the S/E mutation, structurally mimicking the phosphorylated state, indicated that phosphorylation of serine residues in the C-terminal fragment of P proteins leads to a decrease in the affinity to RIP toxins, mainly due to the increased dissociation rate of the resulting complex. Based on the modeling of the structure of the C-terminal fragment of P proteins, it has been proposed that the introduction of an additional negative charge through phosphorylation of serine residues induces the formation of α-helix, thereby destabilizing the RIP-P-protein complex and leading to its rapid degradation.

As part of this dissertation, a review was also prepared, which summarizes the current knowledge regarding the interaction of ricin with the ribosome at the molecular level, taking into account the role of the P protein complex. The mechanism of sarcin-ricin loop depurination by RTA in the context of the functioning of the eukaryotic GTPase-associated center was also described, taking into account the structural features of SRL and its role in the stimulation of GTP hydrolysis by trGTPases, as well as the consequences of depurination on the protein synthesis.

Keywords: P-protein complex, GTPase-associated center, ribosome-inactivating proteins

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