The Influence of the Conformation and Hydrophobicity Change of the Ricin A-chain on the Intracellular Transport of this Toxin

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Ricin is a natural, extremely potent protein toxin isolated from castor beans. A scientific interest connected with this toxin is associated with the possibility of using ricin as a model protein in the study of intracellular transport of proteins, including recognition of misfolded glycoproteins in the endoplasmic reticulum (ER) and their retrotranslocation from the ER to the cytosol. This knowledge might contribute to the general understanding of proper function of all eukaryotic cells. Moreover, cell trafficking of ricin has been extensively studied due to the variety of medical applications in which it can be used. A detailed knowledge of the mechanisms of intracellular transport of ricin gives the possibility of using this protein as a component of modern immunotoxins and vaccines. In addition to medical applications, due to its high toxicity, ricin is considered as a biological weapon. For many years, a study to develop a specific vaccine against ricin have been conducted.

The ricin holotoxin is a heterodimeric protein consisting of the A-chain (RTA) with enzymatic activity and the cell surface-binding B-chain (RTB), linked together by a single disulfide bond. The A-chain inhibits protein synthesis by irreversibly inactivating eukaryotic ribosomes. RTB recognizes cell surface glycolipids or glycoproteins with β-1,4-linked galactose residues. Cell-bound ricin is taken in by endocytosis. After endocytosis, the toxin is initially delivered to early endosomes, from where the majority of the endocytosed toxin recycles back to the cell surface, starts to be degraded and proceeds to late endosomes/lysosomes where further degradation is conducted. A minor fraction (~ 5%) of ricin is transported from early endosomes to the trans-Golgi network (TGN) and further to the endoplasmic reticulum. In ER, ricin holotoxin is reduced, and enzymaticaly active A chain (RTA) is translocated to the cytosol using the pathway which is normally followed by misfolded ER proteins for targeting to the ER-associated degradation (ERAD) machinery.

Ricin A-chain contains a 12-residue (Val245 to Val256) hydrophobic C-terminal region which plays a crucial role in its cytotoxicity. In the present study, I introduced a point mutation changing Pro250 into Ala in the hydrophobic region of RTA (RTA_{P250A}), in order to investigate the effect of this mutation on vesicular transport of ricin, its retrotranslocation from the ER to the cytosol, and in order to study the interactions between ER chaperone proteins, EDEM1 and EDEM2, and RTA_{P250A}. In my work, I have demonstrated that modified

ricin (P250A) is more extensively degraded in endosomes/lysosomes than the wild-type protein; moreover, I showed here that a smaller fraction of P250A ricin is retrotranslocated from ER to the cytosol when compared to its wild-type counterpart. This affects the cytotoxicity of P250A ricin, which is considerably reduced relative to the wild type ricin. This effect is not limited to only one cell type.

EDEM1 and EDEM2 recognize misfolded glycoproteins in ER and accelerate their ERAD. These proteins also facilitate ricin A-chain transport from ER to the cytosol. My study revealed that in contrast to wild-type ricin, retrotranslocation of the ricin P250A variant to the cytosol is EDEM1- and EDEM2-independent. Pull-down and coimmunoprecipitation experiments showed that efficiencies of the interactions between EDEM1 and RTA_{P250A} and between EDEM2 and RTA_{P250A} were significantly decreased. The P250A mutation alters the secondary structure of the toxin which results in a more helical structure of the protein. I have concluded that recognition of proteins by EDEM1 and EDEM2 may be determined by the structure of the ERAD substrate.

Furthermore, the results of my experiments, in which I studied the interactions between EDEM1 and EDEM2 and the ricin A-chain with reduced and increased hydrophobicity showed clearly that an appropriate high degree of hydrophobicity of recognized protein's determinants influence their interactions with EDEM family proteins. Moreover, this relationship applies not only to ricin. In my work I used also another model protein, misfolded pancreatic β-secretase (BACE456), which contains a highly hydrophobic C-terminal region. Reduced hydrophobicity of BACE456 was also associated with a decreased level of interactions with EDEM1 and EDEM2 proteins. These results provide an important information, which might be necessary for understanding of how the EDEM family proteins recognize substrates directed for the proteosomal degradation.

In conclusion, the results presented in my study contribute to the general understanding of ricin intracellular transport, the role of the hydrophobic region of RTA in ricin cytotoxicity and the mechanisms of the recognition of misfolded proteins in ER. This knowledge might contribute to better understanding of biology of eukaryotic cells as well as bring new solutions into ricin medical applications.