Intrinsic mechanisms in Hsp100 proteins.

Agnieszka Jurczyk

supervisor: Prof. Krzysztof Liberek
Department of Molecular and Cellular Biology, Intercollegiate Faculty of Biotechnology,
University of Gdańsk, 80-822 Gdańsk, Kładki 24, Poland

Hsp104 from *Saccharomyces cerevisiae* is a molecular chaperone which rescues proteins from the insoluble aggregates formed in the cell under stress conditions. Hsp104 consists of the N-domain, two AAA^{+}-type nucleotide binding domains: NBD1 and NBD2 and a helical, mobile M-domain protruding from NBD1. For its protein-reactivation activity, Hsp104 requires the presence of the Hsp70/40 chaperone system, which interacts with the M-domain of the disaggregasse. The change of position of the M-domain against the NBD1 regulates Hsp104.

In the recent studies we have identified the network of the ionic interactions at the interface between NBD1 and M-domain of Hsp104, formed by the triad of conserved charged amino acids, namely D184, K358 and D484. We have shown them to be situated close to each other, at the distance allowing the significantly strong ionic interactions to be formed between the pairs:184-358 and 358-484. The different rearrangements of the interactions, studied by the charge-reversing substitutions, led to the drastic, either stimulatory or inhibitory effect on Hsp104 activity as well as on the viability of the cells carrying the mutated *HSP104* gene. Also, we made an inquiry into the underlying mechanism of the Hsp104 deregulation caused by the artificially generated repulsion between the central residue at position 358 and its partner on the M-domain, D484. All the performed substitutions of the lysine 358: to serine, alanine or glutamic acid, caused the abnormal activity of Hsp104, suggesting that the original positive charge of this residue is necessary for the regular behaviour of this protein. The covalent linkage of the M-domain to NBD1 in a disulphide-crosslinked cysteine Hsp104 variant K358C_D484C completely inactivated the protein. Additionally, the charge-reversing substitutions were combined with the Walker B E687Q substitution to measure the effect of the Hsp104-stimulating mutations on the NBD1-only ATPase activity. I performed a detailed analysis of the influence of the substrate, casein, in ATPase activity of these protein variants. The preliminary results show that the hyperactive variants display higher affinity towards ATP which is not further increased in the presence of the substrate, which is a characteristic of the wild type protein. To further test the binding and dissociation of substrates by different Hsp104 variants, I generated another artificial unfolded substrate: fluorescently labeled, reduced, carboxymetylated lactalbumine for the anisotropy fluorescence measurements, which are now in progress.

Additionally I showed that the investigated regulatory mechanism is restricted only to the disaggregating Hsp100-family members, possessing the M-domain, like Hsp104 and bacterial ClpB, while no stimulatory effect is observed for the 358-homologous substitution in the ClpA protein, a subunit of ClpAP protease. Other Hsp100 family members, including ClpC, ClpV, ClpD, ClpL and ClpE exhibited little or no charge conservation in 358 and 484-homologous positions. On the contrary, 184-homologous position is in almost all of 3500 amino acid sequences analyzed occupied by acidic residue. It may indicate that this amino acid may play important role in activity of Hsp100 proteins, the details of which remain to be elucidated.

The described system of ionic interactions involving the switching between the two or more alternative ionic bonds may constitute a link between the previously shown motions of the M-domain against NBD1 and Hsp104 activation associated with these motions.