A B S T R A C T

The majority of the mitochondrial proteins is synthetized on the cytosolic ribosomes in the form of the protein precursors bearing mitochondrion-targeting signal presequences. Once the protein precursor has reached the mitochondrial matrix the signal presequence is no longer necessary and is cleaved off by heterodimeric mitochondrial processing peptidase (MPP; α/β). Although the crystal structure of MPP is available, the MPP mechanism of function is still matter of discussion.

An all atomic, non-restrained molecular dynamics (MD) simulation in explicit water was used to study in detail the structural features of the highly conserved glycine-rich loop (GRL) of the regulatory α-subunit of the yeast MPP. Wild-type and GRL-deleted MPP structures were studied both in the presence and absence of a substrate in the peptidase active site. Targeted MD simulations were employed to study the mechanism of substrate translocation from the GRL to the peptidase active site. We demonstrate that the natural conformational flexibility of the GRL is crucial for the substrate translocation process from outside the enzyme towards the MPP active site. We show that the α-helical conformation of the substrate is important not only during its initial interaction with MPP (i.e. substrate recognition), but also later, at least during the first third of the substrate translocation trajectory. Further, we show that the substrate remains in contact with the GRL during the whole first half of the translocation trajectory where hydrophobic interactions play a major role. Finally, we conclude that the GRL acts as a precisely balanced structural element, holding the MPP subunits in a partially closed conformation regardless the presence of a substrate in the active site.

Hydrogenosomes are evolutionary related reduced versions of mitochondria that possess MPP-like peptidase – hydrogenosomal processing peptidase (HPP; αβ). We show that HPP is functional as a heterodimer consisting of a regulatory α-subunit and catalytic β-subunit and processes the same set of signal presequences as MPP. On contrary to the MPP, the crystal structure of HPP has not yet been solved. Beside of the

crystallization attempts we employed advanced methods of structural biology to study the structural features of HPP. Specifically, using biological small-angle X-ray scattering and hydrogen-deuterium exchange methods we show that HPP does not undergo conformation changes depending on whether the peptide substrate is or is not present in the peptidase active site. We show that while the wild-type HPP resembles very likely the same quaternary organization as MPP, the structure of the proteolytically inactive E56Q mutant differs from the wild-type one. Chemical cross-linking confirms the low validity of HPP homologous model and suggests that β-HPP is capable of forming homodimers.

With regard to the idea that mitochondria are of the α-proteobacterial origin and to the fact that MPP-like peptidases have been found also in bacteria we worked out an evolutionary scenario for MPP. While MPP and very likely also HPP contain the "full length" GRL, the bacterial MPP-like peptidases contain only the "embryonal" GRL, if any, which is consistent with their biological functions. We conclude that the presence or absence of the "full length" GRL can be considered as evolutionary marker of the physiological function of the given MPP-like peptidase. Furthermore, we hypothesize that the ancestral MPP was attached to mitochondrial inner membrane in the form similar to the *Sphingomonas sp*. heterodimeric peptidase where the prolongation of GRL from the "embryonal" to the "full length" form occurred and, thus, the MPP physiological function of processing peptidase was established. In this context, we also conclude that MPP is an illustrative example of the organelle-driven evolution of the eukaryotic cell.