Studies on processing and transport mechanism of bifunctional ABC transporter NukT for lantibiotic nukacin ISK-1

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Thesis Summary

With development in molecular biology and bioinformation techniques, a new horizon has appeared for designing novel bioactive peptides and enzyme engineering.

Among these nature resources, lantibiotics and other antimicrobial peptides have a long history to influence our life. Lantibiotics, the lanthionine-containing peptide, are named after their unique intramolecular structure formed between unusual amino acid residues. The structure of thioether-bridged amino acids makes lantibiotics more tolerant towards hash environment conditions and active across a wide range of pH and temperature. To produce lantibiotics extracellularly, prepeptides composed of N-terminal leader peptide and C-terminal propeptide undergo several post-translational modification processes, including directional dehydration of specific sites, cyclization to form thioether bridge, cleavage of leader sequence and transportation. Therefore, investigation of the enzymes related with lantibiotic biosynthesis might lead us to an approach for engineering of novel antimicrobial peptides.

In this thesis, we focused on the ABC transporter maturation and secretion (AMS) protein, NukT, for the lantibiotic nukacin ISK-1 produced by *Staphylococcus warneri* ISK-1. NukT cleaves off the N-terminal leader peptide of modified NukA which is formed by dehydration and cyclization of prepeptide NukA. NukT also secretes mature nukacin ISK-1. So far, only pieces of information have been reported on this kind of bifunctional transporter. Thus, the work lies with us to investigate the secrets behind nukacin ISK-1 transporter and hopefully to develop new system based on understanding the biosynthesis mechanism.

Firstly, a reconstitution procedure to gain complete NukT and its mutant with maintained bioactivity after purification is searched. Full-length NukT was first time to be expressed, purified and reconstituted into liposomes with recovered both peptidase and ATPase activities. For optimization, some important factors

during reconstitution and methodology are also discussed. At last, an optimized procedure is found to reconstruct NukT in both natural lipids (*E. coli*/PC) and artificial lipids (POPE/POPG) with rescued functions.

Secondly, we focused on evaluating these essential issues for peptidase reaction of NukT proteoliposomes. As a member of AMS proteins, NukT consists of three regions: an N-terminal peptidase domain (PEP), a C-terminal ATP-binding domain (ABD) and a transmembrane domain (TMD). Previous researches have determined that NukT is mainly responsibe for cleavage of the leader peptide of modified NukA and transport nukacin ISK-1 through a potential PEP-ABD cooperation. As highlights in results, it has been unveiled that the hydrolysis of ATP at C-terminal ABD has significant effects on the peptidase activity of N-terminal PEP for production of nukacin ISK-1.

Finally, the domain-to-domain cooperation of NukT has been further discussed. By evaluating the influence of peptidase substrates and products on ATPase activity, it is found that peptidase substrates lead to more significant stimulation of ATP hydrolysis than leaderless products. It is suggested that the leader sequence plays an important role for directing biosynthesis and final transportation. In addition, it should be reconsidered that the domain-to-domain cooperation is perhaps more intimate than previous expectation.

Based on these results, a novel transport cycle mechanism is given for NukT and other bifunctional transporters (Fig. 1). Combining the general alternating access model of ABC transporters and our findings, here we propose a putative transport mechanism for such bifunctional transporter.

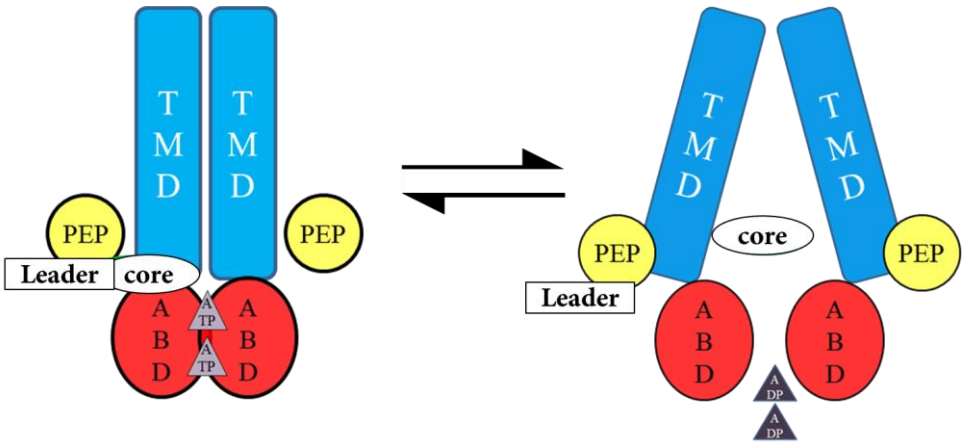

Figure 1 Transport mechanism model for bifunctional transporter.
In the ATP binding state, ATP is bound within ABD and ready for hydrolysis, providing transformation energy. Once leader peptide cleavage is accomplished, the product is transported through the TMD and ATPase activity is stimulated to reset the entire structure, preparing for the next transport cycle. *Leader-core*, modified NukA; *Leader*, leader peptide; *core*, mature nukacin ISK-1.