

JOSEPH F. FOSTER MEMORIAL CHEMICAL BIOLOGY AND BIOCHEMISTRY SEMINAR

Monday, April 1, 2024
3:30 PM, BRWN 4102

“Analysis of the substrate specificity and binding site of the yeast zinc metalloprotease, STE 24.”



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Abstract:

The Yeast Zinc Metalloprotease is a unique and ubiquitous enzyme that has been a subject of study for many years. This enzyme is involved in the maturation of the yeast mating pheromone **a**-factor by performing two distinct cleavages in the same substrate. Firstly, Ste24 cleaves the three terminal residues of **a**-factor which is then followed by an upstream N-terminal cleavage. In humans, defects in this metalloprotease or its substrate, Prelamin A, typically result in a range of progeroid disorders. Furthermore, the severity of these diseases has been directly linked to the catalytic activity of the enzyme. Treatments for these diseases are difficult to develop due to the limited knowledge available on the catalysis, substrate recognition and functions of Ste24 and its homolog. As such, this study aims to expound on the substrate specificity and the binding site of Ste24. Identifying the substrate requirements of Ste24 has been an increasingly interesting topic due to the implication of Ste24 in a variety of unrelated functions. Ste24 and its human homolog, ZMPSTE24, have almost identical crystal structures and are both able to cleave the substrate of the other at both cleavage sites. As a result, studies on either homolog are highly applicable to the other. With that in mind, various studies have implicated both Ste24 and ZMPSTE24 in a wide range of newly discovered functions, such as translocon unclogger, ability to defend against viruses, and influencing the synthesis of chitin (a polysaccharide that forms the cell wall of fungi). To perform these varied functions, Ste24 has to have a broader substrate recognition ability than previously believed. To elucidate the substrate specificity of Ste24, short peptide sequences containing varying CaaX sequences were developed and tested for C-terminal activity through a radioactive methyltransferase-coupled diffusion assay. Secondly, we tested the necessity of carboxymethylation for the upstream N-terminal cleavage using 33-mer analogs of **a**-factor, developed to mimic the C-terminally cleaved peptide. Finally, we interrogated the binding site of Ste24 through the use of a radioactive methyltransferase-coupled diffusion assay (C-terminal cleavage), a FRET-based assay (N-terminal cleavage), and photocrosslinking assays (binding). Together, these data presented a clear image of residues necessary for the cleavage and binding of substrates within Ste24.



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“Unveiling Crucial Substrate Binding Residues in STE14, The Yeast Isoprenylcysteine Carboxylmethyltransferase.”



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Abstract:

CaaX proteins, comprising approximately 300 members, represent a diverse group implicated in fundamental cellular processes, such as cellular growth. In order to carry out such vital cellular functions, CaaX proteins need to undergo three sequential post-translational modifications (PTM) through the CaaX pathway which consists of isoprenylation (farnesylated or geranylgeranylated), endoproteolysis, and methylation. Among the CaaX family of protein, the Ras superfamily, plays a pivotal role in cell growth and survival. Mutations in *RAS* are associated with a spectrum of cancers, presenting a significant challenge for therapeutic intervention. Our research focuses on methylation, the third PTM, which is carried out by ER localized membrane protein Isoprenylcysteine carboxyl methyltransferase (Icmt), and its potential as a therapeutic target, particularly for Ras-driven cancers.

Icmt is unique as it is the sole methyltransferase which carries out the third PTM of methyl esterification of CaaX proteins with the aid of cofactor SAM which serves as the methyl donor. Additionally, how Icmt brings close these two chemically diverse molecules for catalysis, is very intriguing and yet to be fully understood. Our research focuses on studying the structural and functional properties of Ste14, the yeast analog of Icmt, in order to better understand the Icmt family of proteins. Ste14 is much more economical and is 41% identical and 62% similar to human Icmt, making it a great system for experimental use.

One of our projects dives into Ste14 and substrate binding, focusing on geranylgeranylated substrate specificity. Conserved residues found in Loop 2 and Transmembrane 3 of Ste14 were assessed for their activity and specificity with AGGC, the minimal geranylgeranylated CaaX substrate, upon mutation to alanine. Mutants which showed nearly no activity with AGGC were further analyzed to understand if this loss of activity was due to the mutant's inability to bind with AGGC. A photoreactive AGGC analog was used to carry out the photolabeling experiments and residues were analyzed for their binding ability with geranylgeranylated substrate. Mutants were further analyzed to understand the effect of mutation on structural integrity, to gauge which residues are essential for catalysis and for maintaining structural integrity of Ste14. This project would overall help better understand the lesser studied Ste14-substrate binding.



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