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Type	Oral communication
Session	Molecular Dynamics Simulations
Title	Molecular dynamics analysis of MTGase and KalbTGase enzymes
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Motivation

Microbial transglutaminases (mTGase) are enzymes widely used in the pharmacological and biotechnology field, in the textile industry and in the food industry. Its main feature exploited is the ability to catalyze cross-links formation between γ-carboxyamide group of glutamine residues and Ɛ-amino group of lysine residues by acyl-transfer reaction. This reaction induces significant changes into the functional property of the food matrix as gelation, emulsification, foam formation, viscosity and water retention capacity; moreover, allows the formation of Protein-DNA/Protein/Polymer conjugates for research and biotechnological purposes. So far, because of its facility of expression and purification, the only TGase enzyme widely used for industrial applications is the microbial TGase extracted from Streptomyces mobaraensis (MTGase). We recently reported a classification of microbial transglutaminases, aimed to help the finding of novel forms with potential applications [Giordano and Facchiano 2019]. Among the novel forms recently decribed, a novel mTGase extracted from the organism Kutzneria Albida (KalbTGase), more selective than MTGase, has been described and proposed for applications in drug delivery [Steffen et al, 2017].
The present study purposes a comparison between KalbTGase and MTGase structures by means of molecular dynamic simulations, in order to analyze differences and similarity between these two enzymes at different conditions and investigate the differences related to the increased substrate specificity of KalbTGase.

Methods

Molecular dynamic (MD) simulations have been performed at the temperature of 300K, 335K and 355K in NPT for both KalbTGase (PDB:5M6Q) and MTGase (PDB:3IU0) structures. Simulations at 300K have been performed in five repeats each one lasting 350ns, simulations at 335K and 355K have been performed only once and have the duration of 300ns. All the MD simulations were performed using GROMACS 5.0 on Piz Daint supercomputer. PCA, RMSD, RMSF and the Sapphire Plot have been employed for the analyses of the trajectories obtained. The analyses of the active site pocket volume during all the MD simulations have been done by MDpocket server.

Results

All the MD simulations performed on the two molecules at 300K show preserved conformation of the catalytic site and its closest areas, and flexibility of the peripherical loops, wherestructure fluctuation values are higher in MTGase due to the presence of longer loops than KalbTGase. Thus, from these results appear that KalbTGase at room temperature is a more rigid protein than MTGase. The MD simulations at higher temperature give a preliminary result of a possible stability of the molecules also at high temperatures. Little conformational changes especially in the biggest loops happen, as also little rearrangements of secondary structure in areas far from the active site. In general, MTGase, because of the presence of long loops, shows huge flexibility in these areas, but also lower conformational rearrangement when the temperature rises, above all at 335K, suggesting a major preservation of its structure. KalbTGase is a more compact molecule, therefore it presents few long loops that show as well high flexibility, but despite of MTGase it seems to be more adaptable, actually conformational rearrangement far away from the active site domain seems be correlate to the rising of the temperature.
All the volume tests carried out on the MD simulations performed at 300K demonstrate that, although strong similarity between the active site pockets of the two enzymes, the active site volume is smaller in KalbTGase at least of 50 Å3. Volume tests carried out on the MD simulations performed at higher temperature demonstrate that KalbTGase reduces the pocket size at 335K, and preserves the same reduced volume at 355K, instead, MTGase reduces its pocket volume at 335K, and an additional reduction is observed at 355K.
Actually, from the MD simulations performed at 300K, KalbTGase results less flexible than MTGase and

from the active site volume analysis it is possible to see that also its catalytic pocket, during the simulations, is more closed than the one of MTGase.
Moreover, the structural rearrangements at higher temperature that are more favorite in KalbTGase than in MTGase could suggest less adaptability of MTGase, in agreement with the observed reduction of the catalytic pocket, and be related to the higher specificity. On the other hand, KalbTGase structural rearrangements at higher temperatures could suggest operative conditions to obtain major adaptability to different substrates.

References

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Steffen W. et al. J Biol Chem. 2017 Sep 22;292(38):15622-15635. doi: 10.1074/jbc.M117.797811.

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Availability	-

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