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Research Paper

Homology Modelling of *Pseudomonas stutzeri* ZS04 Lipase

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ABSTRACT: *The aim of this study was to predict the 3D structure of the lipase Pseudomonas stutzeri ZS04 and to evaluate the impact of improving protein structure on the structure's modeling. In this research, modeling of the enantioselective protein lipase from Pseudomonas stutzeri ZS04 has been carried out. This research was conducted in silico based on bioinformatics data to determine the 3D structure of the protein.*
KEYWORDS: *Prediction, Lipase, Modelling*

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I. INTRODUCTION

Protein is an essential part of organisms and is involved in almost all processes in cells (1). Some proteins are enzymes that function as catalysts in biochemical reactions and are vital for metabolism. The tertiary structure of a protein is a 3D structure made up of secondary structures folded together (2). The secondary structure of proteins consists of a series of tens to thousands of amino acids. Proteins can generally undergo reversible structural changes in carrying out their biological functions. Alternative structures of the same protein are known as conformers.

Lipases naturally catalyze the hydrolysis of triacylglycerols, they also exhibit strong catalytic activity and great enantioselectivity for a wide range of synthetic chiral substrates. Lipases are extremely adaptable enzymes that have caught the interest of numerous industrial processes. Sources of lipase such as from animals, vegetables, and microbial (3).

Lipases can separate racemic mixtures that can be used for pharmaceuticals. In the efficacy of many drugs chirality is a key factor. In pharmaceutical chemistry, the production of single enantiomers of drug intermediates has become increasingly (3). Hundreds of secondary alcohols have been successfully reacted under conditions of high enantioselectivity by lipases such as BCL/PS lipase from Amano (4).

Lipase from Pseudomonas stuzeri ZSO4 is a lipase isolated from oil-contaminated soil samples and is tolerant to organic solvents. With high substrate specificity and theoretically high conversion yield, lipase ZS04 demonstrated outstanding enantioselective esterification toward the (R)-1-(4-methoxyphenyl)-ethanol (MOPE), a critical chiral intermediary in medicines as well as in other analogs. Lipase ZS04 was a promising biocatalyst in chemical synthesis and pharmaceutical applications because of its significant advantage over comparable strategies (5). The aim of this study was to predict the 3D structure of the lipase *Pseudomonas stutzeri* ZS04 and to evaluate the impact of improving protein structure on the structure's modeling.

II. METHOD

This study used the homology modeling approach to predict the 3D structural shape of the lipase enzyme *Pseudomonas stutzeri* ZS04. The software used were BioEdit, Chimera 1.16, and Discovery Studio 2020. The web servers used were NCBI, Galaxy web, EMBL-EBI, and Verify3D, sequence of the P*seudomonas stutzeri* ZS04 lipase protein.

Preparation of Lipase Enzyme Sequences

Lipase enzyme sequences were prepared by searching the NCBI database. Furthermore, the lipase enzyme sequences obtained were then prepared using the BioEdit software.

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Homology Modeling of Lipase Protein

The lipase protein model was built using a homology modeling approach on the Swiss-Model web server. A template search using the NCBI web server. The target protein sequence was uploaded to the SWISS-MODEL website (http://swissmodel.expasy.org/). To simulate the model structure, the template with the highest identity value was chosen. The resulting model was then evaluated using Procheck on EMBL-EBI, Molprobity®, and Verify3D®. Furthermore, structural repairs were carried out using the Galaxy web server and then re-evaluated

III. RESULTS AND DISCUSSION

Protein sequences were obtained from NCBI with access code AGL91256.1 *Stutzerimonas stutzeri* lipase (picture 1). The protein lipase sequence showed 311 amino acid residues.

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>AGL91256.1 lipase [Stutzerimonas stutzeri]
MNKNKTLLALCLGSALALSGQAFAATGSGYTATKYPIVLTHGMLGFDSLLGIDYWYGIPSALRRDGAQVY
ITEVSQLNTSELRGEELLAQVEEIVAISGKPKVNLIGHSQGGPDIRYVAGVRPDLIASVTSVGAPHKGSD
VADLIRKVPEGSSGEAIIAGLVNAMGALINFLSGSNSSAPQNALGALESLNSEGAARFNAKYPQGIPTSA
CGEGAYVVKGVRYYSWSGTSPLTNPLDVSDAVMVAGSLAFDEANDGLVGRCSSHLGMVIRDNYRMNHLDE
VNQVLGLTSLFETDPVSVYRQHANRLKNAGL
```

Figure 1. Sequences of lipase protein

Amino acid sequences obtained from NCBI were prepared using BioEdit® software and the format used was fasta file. The amino acid sequence of the *Pseudomonas stutzeri* ZS04 lipase is used in determining the template to be used in the construction of the lipase enzyme model.

Homology Modelling

3D structure prediction of lipase usied web server of Swiss-Model®. The protein being modeled is a protein that does not yet have a structure in the RCSB PDB database. The use of a web server in model construction is based on the ease of use and accuracy of the resulting model. The 1ex9.1.A Lactonizing lipase was used as a template with a sequence identity of 80.14%, monomer oligo state, QMEAN 0,88 and GMean z score -0.89. The created model will be closer to the template structure if the percent identity value is greater (figure 2). The percent identity of target and template sequences is the initial assessment of model quality. The results of sequence alignment can be seen in figure 3. Protein structure prediction relies on the quality evaluation of protein structures, which is a big aspect of experimental structure validation.

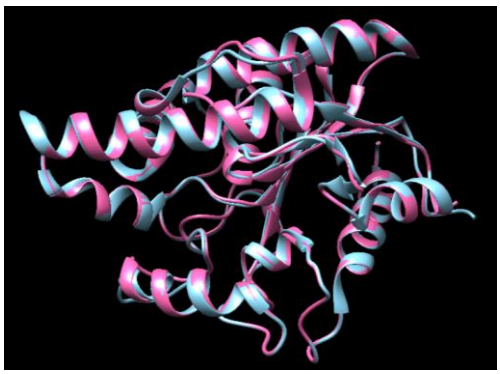

Figure 2. Superimpose Protein target (pink) and 1ex9.1.A (light blue)

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Figure 3. Sequens alignment

QMEAN is a composite scoring function that can estimate the quality of the entire structure and the quality per amino acid residue based on a single model (Pascal Benkert). The QMEAN Z-score provides an estimate of whether the model formed has a quality comparable to the experimental structure (MAHAK TUFCHI). A score of -4.0 or lower is indicative of a very low quality model. Therefore, the model in this study was relatively good because the QMEAN Z-score is -0.89. Figure 3 illustrates the position of the model (red asterisk) on the distribution of Z values. The red asterisk is still in the area 1 < Z-score < 1, meaning that the model is still quite good.

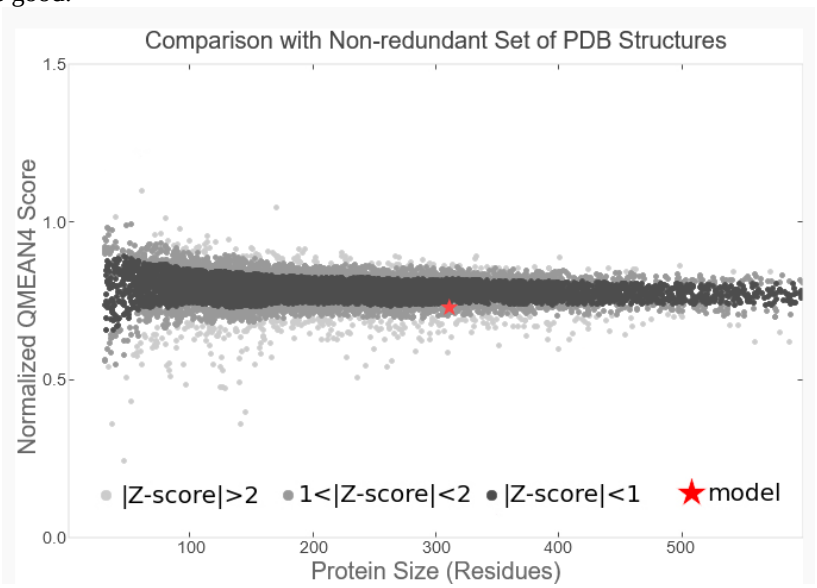

Figure 4. Z score QMEAN of absolute model quality

Based on the parameters of the Ramachandran plot, it shows that the favored area in the model is 89.5%. A good quality model would be expected to have over 90% in the most favored regions. Improvements to the protein structure were made to obtain a better 3D structure. Improvements to the 3D protein structure were carried out using the Galaxy web server.

Improvements to the 3D protein structure were carried out using the https://galaxy.seoklab.org server. The quality of the repaired 3D structure was then checked again using the Ramachandran plot. The results of structural improvements using galaxy web obtained 5 models. The selected model is model 1 with a value of RMSD 0.255, MolProbity 1,566 and Clash score 11,2 (figure 5). The MolProbity score of the protein lipase model is 1.56, meaning that the model is quite good. The MolProbity score indicates one value expected to describe the crystallographic resolution. If the model structure has a lower MolProbity score than the template crystallographic resolution, then the quality of the model is said to be better than the average structure at that resolution. The template crystallographic resolution for this model is 2.5 (vincent B).

Model 1 was chosen because it has a lower RMSD value. This signifies better accuracy. Poor Rotamer is used to see the location of the multidimensional distribution of residues. A good model is a model that has a Poor Rotamer <0.3%. MolProbity Score is combination value of Clash Score, Poor Rotamer, and Ramachandran Favored (Chen et al., 2009).

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Model	GDT-HA	RMSD	MolProbity	Clash score	Poor rotamers	Rama favored
Initial	1.0000	0.000	1.690	5.5	1.3	95.7
MODEL 1	0.9920	0.255	1.566	11.2	0.0	98.2
MODEL 2	0.9912	0.257	1.697	12.2	0.0	97.5
MODEL 3	0.9912	0.263	1.839	15.2	0.4	97.2
MODEL 4	0.9885	0.269	1.719	12.8	0.4	97.5
MODEL 5	0.9920	0.253	1.733	13.3	0.0	97.5

Figure 5. Refinement model

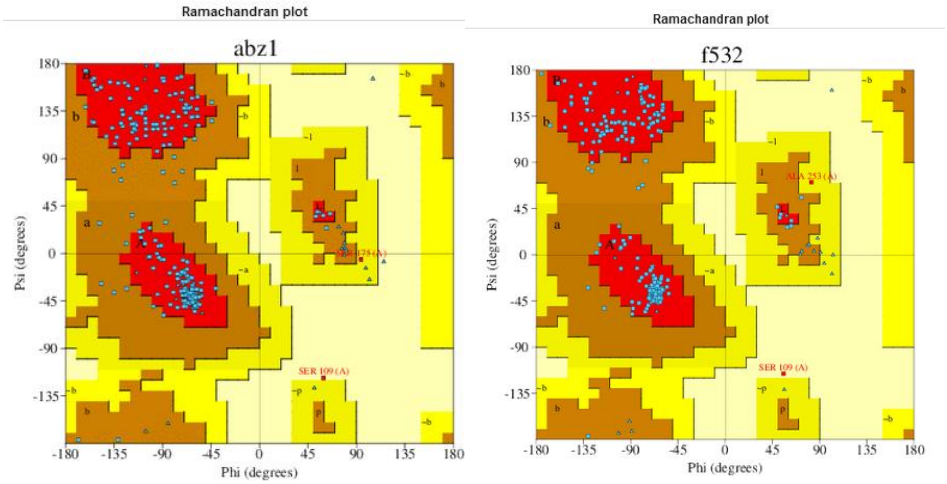

Figure 6. Ramachandran plot before (left) and after repair (right)

The value of favored regions is 92.8%. The repair results show good structural quality. The protein's secondary structure was modified during the structural repair. This can be seen in table 1 and figure 7. During the folding process, a protein adopts a number of conformations before settling into its final, stable, and characteristic form. A protein's final shape is the most energetically advantageous one.

Table 1 Secondary structure before and after repair

	Strand	Alpha helix	3-10 helix	Other	Total residue
Before	42 (14.8%)	106 (37.5%)	3 (1.1%)	132 (46.6%)	283
After	42 (14,8%)	110 (38.9%)	3 (1.1%)	128 (46.2%)	283

Secondary structure	Refinement	Strand 1			Strand 2			No. of helices	No. of residues	
		Start	End	Length	Start	End	Length		Loop	Helix
Beta-alpha-beta-motifs	Before	Val221	**Gly228**	**8**	**Met267**	**Tyr273**	7	1	**38**	**11**
	After	Val221	**Gly228**	**8**	**Met267**	**Tyr273**	7	2	**38**	**17**

Secondary structure	Refinement	Start	End	No resid
Helices	Before	-	-	
		-	-	
	After	Pro123	**Leu125**	**3**
		Arg260	**Ser263**	4
Disulphides	Before	**A 211**	**A 261**	
	After	-	-	

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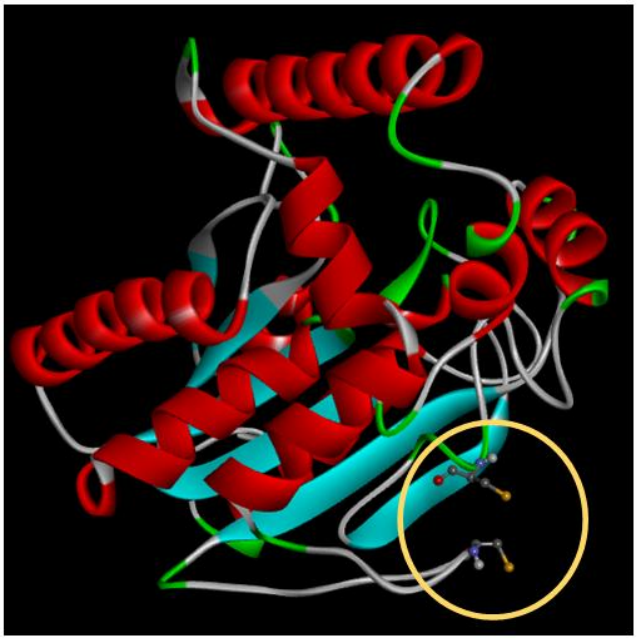

Figure 7. BetaDisulphides

33 beta turns

Turn	Sequence	Turn type	H-bond
Gly29-Ala32	GYTA	I	
Thr33-Pro36	TKYP	VIII	
Gly42-Gly45	GMLG	II	Yes
Ser48-Gly51	SLLG	IV	
Leu49-Ile52	LLGI	I'	Yes
Asp53-Tyr56	DYWY	IV	
Trp55-Ile58	WYGI	II	Yes
Ser75-Asn78	SQLN	VIII	
Arg122-Leu125	RPDL	I	Yes
Pro123-Ile126	PDLI	I	
Ser131-Ala134	SVGA	IV	
Pro135-Gly138	PHKG	I	Yes
Pro149-Ser152	PEGS	II	Yes
Asn176-Ala179	NSSA	I	Yes
Pro203-Ile206	PQGI	II	Yes
Ser209-Gly212	SACG	IV	
Ala215-Val218	AYVV	VIII	
Val217-Gly220	VVKG	IV	
Val218-Val221	VKGV	I'	Yes
Ser230-Thr233	SPLT	VIII	
Asn234-Asp237	NPLD	I	Yes
Asp251-Asn254	DEAN	IV	
Asn254-Leu257	NDGL	IV	
Asp255-Val258	DGLV	IV	
Gly259-Ser262	GRCS	I	
Arg260-Ser263	RCSS	I	
Cys261-His264	CSSH	I	Yes
Leu265-Val268	LGMV	IV	
Asn276-Asp279	NHLD	I	
Asn282-Leu285	NQVL	IV	
Gln283-Gly286	QVLG	IV	
Val284-Leu287	VLGL	I'	Yes
Ser289-Glu292	SLFE	IV	Yes

29 beta turns

Turn	Sequence	Turn type	H-bond
Gly29-Ala32	GYTA	I	
Thr33-Pro36	TKYP	VIII	
Gly42-Gly45	GMLG	II	Yes
Ser48-Gly51	SLLG	IV	
Leu49-Ile52	LLGI	I'	Yes
Asp53-Tyr56	DYWY	VIII	
Trp55-Ile58	WYGI	II	Yes
Ser75-Asn78	SQLN	VIII	
Ser131-Ala134	SVGA	IV	
Pro135-Gly138	PHKG	I	Yes
Pro149-Ser152	PEGS	II	Yes
Asn176-Ala179	NSSA	I	Yes
Pro203-Ile206	PQGI	II	Yes
Pro207-Ala210	PTSA	IV	
Ser209-Gly212	SACG	I	
Ala215-Val218	AYVV	VIII	
Val217-Gly220	VVKG	IV	
Val218-Val221	VKGV	I'	Yes
Ser230-Thr233	SPLT	VIII	
Asn234-Asp237	NPLD	I	Yes
Asp251-Asn254	DEAN	IV	
Asn254-Leu257	NDGL	IV	
Asp255-Val258	DGLV	IV	
Leu265-Val268	LGMV	IV	
Asn276-Asp279	NHLD	I	
Asn282-Leu285	NQVL	IV	
Gln283-Gly286	QVLG	IV	
Val284-Leu287	VLGL	I'	Yes
Ser289-Glu292	SLFE	I	

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IV. CONCLUSION

Prediction of the 3D structure of *Pseudomonas stutzeri* ZS04 lipase was carried out using the Swiss Model web and evaluation results suggest improvements to the 3D structure. This aims to obtain a better impact on the 3D structure of the protein.

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