Characterization of α-L-Arabinofuranosidase (AbfA) Variant (Q46R; D205E; K285E) Biochemical Properties and in Silico Study on the Effect of Mutation to Its Structure

Ratna Melinda, Purkan, Handoko Darmokoesoemo, and N. N. T. Puspaningsih

***Abstract*—This study aimed to determine the biochemical properties of the AbfA variant (Q46R; D205E; K285E) also the correlation between change in biochemical properties and its tertiary structure modification. The enzyme expressed in *E. coli* BL21 (*DE3*)/ pBM5abf variant was partially purified by heat treatment for 1 hour at 70°C. The partially purified enzyme was characterized for its biochemical properties. Protein tertiary structure model was built by homology modeling method using *Geobacillus stearothermophilus* T-6 α-L-arabinofuranosidase crystal structure (PDB: 1PZ3) as template. The protein structure model then subjected for in silico study. Partial purification showed an increase of purity by 17.60 fold. The partially purified enzyme showed optimum activity towards p-nitrophenyl-α-L-arabinofuranoside (pNPA) at pH 7 and 70°C. AbfA variant (Q46R; D205E; K285E) was stable for 24 hours at pH 6-9 (at 4°C) and lost almost 70% of its activity on 16 hours incubation at 70°C. Compared to its wildtype, AbfA variant (Q46R; D205E; K285E) showed decrease on thermostability. Superimpose of AbfA variant (Q46R; D205E; K285E) to its wildtype showed RMSD 0.05. Tertiary structure assessment showed that mutations caused reduction of 3 Hydrogen bonds and 5 Van der Waals interactions also formation of 1 salt bridge and 1 weak electrostatic interaction. In silico analysis of AbfA variant (Q46R; D205E; K285E) protein model revealed that the decrease on thermostability was related to the reduction of some non covalent interactions especially Hydrogen bonds and Van der Waals interaction, due to modification in AbfA variant (Q46R; D205E; K205E) structure.**

***Index Terms*—Biochemical properties, α-L-arabinofuranosidase (AbfA), in silico, non covalent interaction.**

I. Introduction

Alpha-L-arabinofuranosidase (EC 3.2.1.55) are the enzymes catalyze the hydrolysis of terminal non reducing α-L-1, 2-, α-L-1,3- L-arabinofuranosidic bonds in hemicelluloses such as arabinoxylan, L-arabinan and other L-arabinose-containing polysaccharides. The α-L-arabinofuranosidase acts synergistically with other hemicellulases and pectic enzymes for complete degradation of hemicelluloses and pectin [1]. Its synergistic action with other lignocelluloses degrading enzymes are promising tool in agro-industrial processes including improvement of animal feedstock quality, production of bioethanol from lignocelluloses material, production of important medicinal compounds and pulp treatment [2]. Since pulping and bleaching are both performed at high temperature, the application in paper industry needs a thermophilic hemicelulases preferably those active above pH 7 [3].

A xylanolytic gene cluster from a thermophilic bacterium *Geobacillus thermoleovorans* IT-08 that had been isolated from Gunung Pancar hotspring (Bogor, West Java, Indonesia) was successfully cloned into plasmid pTP510 in *Escherichia coli* DH5α. This gene cluster encodes exo-xylanase (geneBank accession number DQ387047), β-xylosidase (DQ345777) and α-L-arabinofuranosidase (DQ387046) [4]. In order to increase the activity of AbfA on basic condition, *abfa* gene had subjected to direct evolution using PCR-errorprone method, followed by cloning to M5 plasmid (excretory expression system)[5] and transformation to *E. coli* BL21(*DE3*). Screening of the extracellular AbfA, had showed some variants with increasing activity on pH 9, one of which is variant A9 [6]. Nucleotide sequencing and alignment of *abfa* variant A9 to its wildtype (DQ 387046.1) showed three point mutations: A137G, A853G and T615A. In silico translation of the gene showed three amino acids substitution: Gln46Arg; Asp205Glu; Lys285Glu.

Amino acid substitution might affect the fold or function of the protein by disrupting the interacting forces within the protein as well as between protein and its environment. Studies on the effects of mutations have mostly been performed experimentally e.g. biochemical characterization of the mutations, which able to provides some insight into how the mutation affects activity; however, without structural information it is rare that the exact mechanism responsible for a change in activity can be characterized. Due to difficulties in experimental determination of protein structure, a bioinformatics approach is very useful in predicting the protein structure [7]. An understanding of correlation between mutation and protein function and stability is important for designing, engineering or developing a new enzyme [8]. This study aims to determine the biochemical properties of the AbfA variant (Q46R; D205E; K285E) as well as identify the correlation between biochemical property changes and modification on its tertiary structure, using protein tertiary structure model.

II. Materials and Methods

A. Microorganism and Media Culture

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E. coli BL21(*DE3*)/ pBM5abf variant (A137G, A853G and T615A) was grown in sterile Luria-Bertani (LB) medium supplemented with 100 µg/ml Ampicillin, 2.5 mM IPTG and 10 mM Glycine. Sterile LB medium and Glycine were prepared by autoclaving the medium for 15 min at 121^{o}C while the sterile IPTG was prepared by filtering the IPTG solution using 0.22 µm filter.

B. Production of AbfA Variant

AbfA variant was produced using the method described on [6] with some modification. The cultures were grown in LB medium containing 100 µg/ml Ampicillin and incubated under shaking condition (150 rpm) at 37 ℃ until the optical density at 600nm (OD_{600}) measured by spectrophotometer (UV-1800, Shimadzu) was 0.5. Once the OD_{600} has reached 0.5, sterile IPTG was added to the culture until final concentration 2.5 mM. The incubation continued under shaking condition (150 rpm) at 30 ℃ up to 24h. At the 4th hour, sterile Glycine was added until final concentration 10 mM. Culture were taken after 24 h incubation and centrifuged (3500rpm) for 10 min. The cell pellet obtained was then resuspended in phosphate buffer (pH 7). After that cell suspension was lysed by ultrasonicator (Soniprep 150 Sanyo) and centrifuged (12000rpm) for 10 min to produce crude enzymes.

C. Partial Purification

The crude enzyme was partially purified by heat treatment at 70^{o}C in an hour. The denatured protein was separated with the undenatured one by centrifugation at 4^{o}C (12000rpm, 15 min). The partially purified enzyme was used for the biochemical properties characterization including determination of optimum pH and temperature as well as pH stability and thermostability.

D. Enzyme Activity Assay

Enzyme activity was measured by incubating 20 µL enzyme with 180 µL pNPA 1mM in phosphate buffer pH 7, at 70^{o}C for 30 minutes. After 30 minutes incubation, 200 µL Na_2CO_3 0.5 M was then added, followed by 600 µL aquadest. The absorbance was measured at 405 nm using spectrophotometer. One unit of enzyme activity was defined as µmol p-nitrophenol liberated by 1 mL of enzyme per minute at incubation condition.

E. Determination of Optimum pH and Temperature

The optimum temperature of enzyme was determined by measuring the enzyme activity towards pNPA diluted in phosphate buffer pH 7 at various temperatures (50-90^{o}C). The optimum pH of enzyme was determined by measuring the enzyme activity at various pH (6-10). Incubation of enzyme and substrate were held at 70^{o}C. The buffers used were: phosphate citrate buffer (pH 6), phosphate buffer (pH 7-8), Glycine-NaOH buffer (pH 9-10).

F. Determination of pH Stability

pH stability was assessed by diluting the enzyme at various pH and same concentration for each pH then stored at 4^{o}C for 24 h. On 0h and 24 h, the enzyme activity was measured at its optimum pH and temperature. Residual activity at 24 h is the comparison between activity at 24 h and activity at 0h.

G. Determination of Temperature Stability

Thermostability was assessed by incubating the enzyme at 70^{o}C up to 24 hours. The activity of enzyme measured at 0, 5th, 9th, 16th and 24th hour using same procedure as described in enzyme activity assay at optimum and pH temperature. Residual activity at x hour is the comparison between activity at x hour and activity at 0h.

H. Protein Tertiary Structure Modeling

Protein tertiary structure was modeled using homology modeling method by Swiss Model [9] using α-L-arabinofuranosidase from *Geobacillus stearothermophilus* T-6 as template [10].

I. Determination of RMSD

The RMSD changes was determined using Super Pose [11]

J. Determination of Non Covalent Interaction and Solvent Accessibility Surface

Solvent Accessibility Surface and non covalent interactions including Hydrogen bonds, Van der Waals Interactions were determined using Chimera 1.6.7. Electrostatic interaction were determined by measuring the distance between charged atom using Chimera 1.6.7 [12]

K. Determination of ΔΔG

The changes in ΔG upon mutation (ΔΔG) were determined using PoP MuSiC-2.0 [13].

III. Results and Discussion

A. Expression and Characterization of AbfA Variant

Expression of intracellular AbfA variant (Q46R; D205E; K285E) was detected by using SDS PAGE. A 61 KDa band was present, corresponded to intracellular AbfA variant (data not shown). The molecular weight of AbfA produced by *G. thermoleovorans* IT-08 is 58 kDa but since *abfa* variant gene was on M5 plasmid system [5], the intracellular protein synthesized would still have the signal peptide, gave an addition to the molecular weight [14], [15]. The band showed at polyacrylamide gel (61KDa) was in agreement with the theoretical molecular weight of protein plus signal peptide estimated by ProtParam [16]. Partial purification by heat treatment at 70^{o}C was based on thermophilic property of AbfA [4]. The heat treatment would denature most mesophilic proteins produced by *E. coli* but not the termophilic AbfA protein, resulted in increase of purity by 17.60 fold (Table I).

AbfA variant displayed an optimum activity at pH 7 (Fig. 1) and 70^{o}C (Fig. 2). AbfA variant showed no differences in optimum temperature with extracellular AbfA synthesized by *E. coli* BL21(*DE3*)/pBM5abf. The optimum pH of AbfA variant was relatively same as the extracellular AbfA synthesized by *E.* coli BL21(*DE3*)/ pBM5abf, which is between the range of neutral to slightly basic (pH 7-8) [6].

Mutation on *abfa* gene did not affect the optimum condition for catalysis since the substituted amino acids were not located at or around the active site [17]-[19], as shown on Fig. 3. Residues involved in catalysis are Glu29, Arg69, Asn74, Asn174, Glu175, His244, Tyr246, Glu294, and

Gln351 [10].

TABLE I: ABFA VARIANT PURIFICATION TABLE

SAMPLE	[PNP] (μG/ML)	[PROTEIN] (μG/ML)	ACTIVITY (U/ML)	VOLUME (ML)	TOTAL ACTIVITY	SPECIFIC ACTIVITY (U/ML.MG)	YIELD	PURIFICATION FOLD
CRUDE	0.154	32968.42	0.36868	5	1.8434	0.011183	100%	1
PARTIALLY PURIFIED	0.110	1336.67	0.26303	3.5	0.92060	0.1967812	50%	17.60

TABLE II: SUMMARY OF INTERMOLECULAR HYDROGEN BONDS, VAN DER WAALS INTERACTION AND ELECTROSTATIC INTERACTION FORMED BY RESIDUE 46, 205, AND 285

Interaction	Residue number:					
	Gln46	Arg46	Asp205	Glu205	Gln285	Glu285
Hydrogen bond	1	1	5	2	0	0
Van der Waals	24	21	33	32	2	1
Electrostatic	0	0	0	1	0	1 (weak)

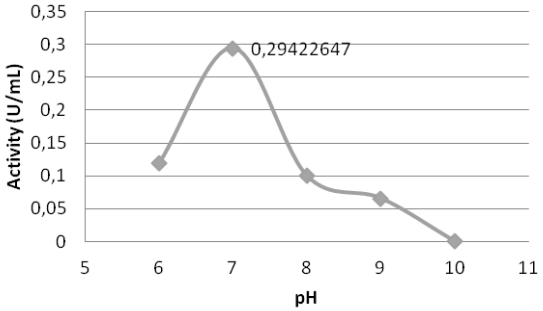

Fig. 1. The activity of AbfA variant on various pH.

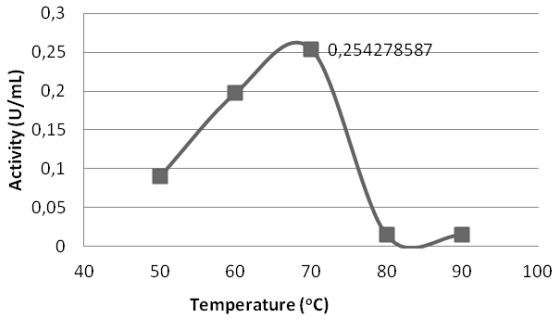

Fig. 2. The activity of AbfA variant on various incubation temperatures.

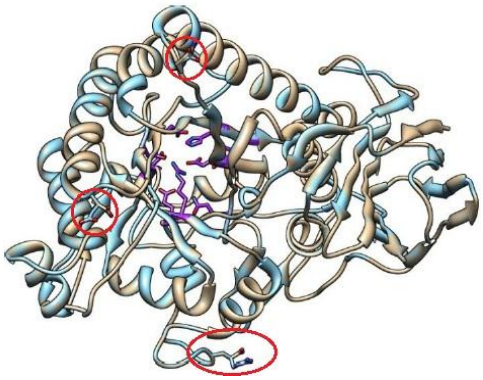

Fig. 3. Superimpose of AbfA wildtype (brown) and variant (blue). Substituted amino acids were showed inside red circle while the amino acids involved in catalysis were purple colored.

AbfA variant lost 25% of its activity at 70°C after 9 h and retained only 34% of its activity over 16 h. Meanwhile, AbfA wildtype still showed 95% activity after 9 h and retained 73% of its activity over 16 h (Fig. 4). The pH stability assessment showed that both AbfA variant and wildtype maintained more than 86% of activity on incubation at pH 6-9 over 24 h thus assumed as stable at pH 6-9 (Fig. 5).

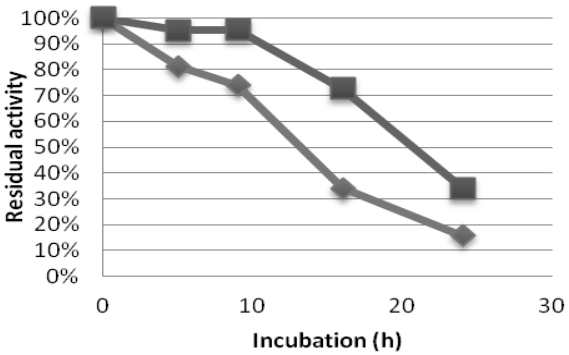

Fig. 4. Effect of temperature at 70°C on the stability of AbfA wildtype (■) and variant (♦).

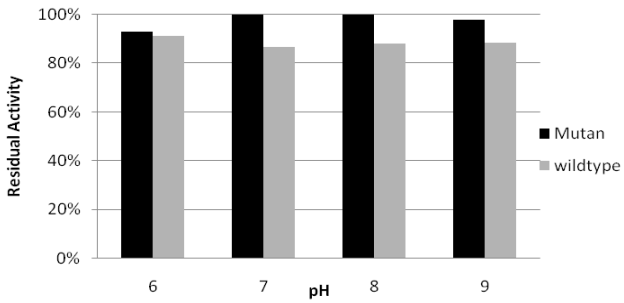

Fig. 5. Effect of pH 6-9 on the stability of AbfA wildtype and variant.

B. *In Silico Study of AbfA Variant*

AbfA from *Geobacillus thermoleovorans* IT-08 was found to be 96% identical to α-L-arabinofuranosidase from *Geobacillus stearothermophilus* T-6 thus it was used as template for construction of homology model of AbfA variant and wildtype. Superimpose of AbfA variant to its wildtype showed total RMSD 0.05 (Fig. 3). The amino acids replacement due to gene mutation resulted in several changes in Hydrogen bonds, Van der Waals interaction and electrostatic interaction, as summarized on Table II. Solvent accessibility area of AbfA variant and wildtype were 19000.7 and 19011.8, respectively. Solvent accessible surface area is one of the parameters characterizing the compactness of protein [20]-[21]. The hypothesis in protein flexibility stated that rigidity is a prequisite for high protein thermostability, supported by some studies conclude that hyperthermophilic

proteins are more rigid enzymes [3]. Increase in solvent accessible surface area of AbfA mutant showed a less compact protein structure, affecting the thermostability of AbfA variant.

The amino acids replacement due to gene mutation resulted in several changes in Hydrogen bonds, Van der Waals interaction and electrostatic interaction, as summarized on Table II. Complete list of the non covalent bonds is provided on supplementary data.

Substitutions of Gln46Arg is not affecting the polarity but the charge [22], gave a chance to the residue to make an electrostatic interaction. However, the amino acid changes did not make any change in electrostatic interaction because the three negatively charged residues around Arg46 (Asp48, Asp55, Glu58) were at 6.5-11Å in distance to Arg46 while the maximum distance for salt bridge formation is 4Å [23], [24]. The only Hydrogen bond formed, was between atoms at protein backbone so it was not affected by the mutation (Fig. 6).

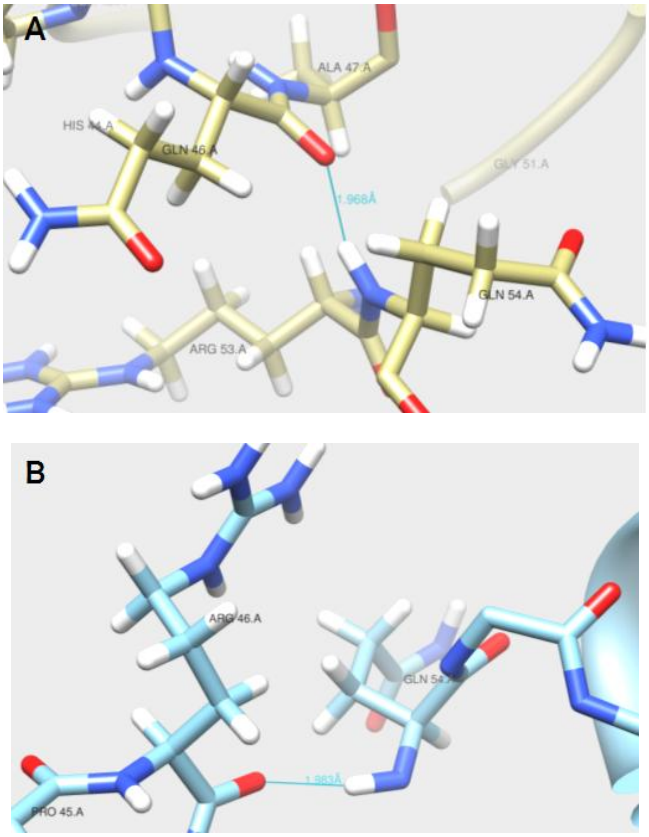

Fig. 6. Hydrogen bond (blue line) formed by residue 46 (A: before mutation; B: after mutation).

The only change at this position was reduction of 3 Van der Waals interactions (see supplementary data). Prediction of mutant stability using ΔΔG showed the ΔΔG was +0.22.

It means that mutation in this position directing to destabilization, most probably caused by reduction of Van der Waals interaction.

Theoretically, the Asp205Glu substitution might not have a huge effect since those two has similar side chain, differ only by a methylene group [22]. However, the in silico study showed reduction of 3 hydrogen bonds (Fig. 7) and 1 Van der Waals interaction (picture not shown), also addition of 1 electrostatic interaction (His146-Glu205) due to the replacement (Fig. 8). A repulsive force arose from free electron pair at His146 N and Glu205 O, compensating the attractive force formed by electrostatic interaction. Replacement at this position showed a destabilization with ΔΔG +2.32. The destabilization was mainly because of the reduction of 3 Hydrogen bonds.

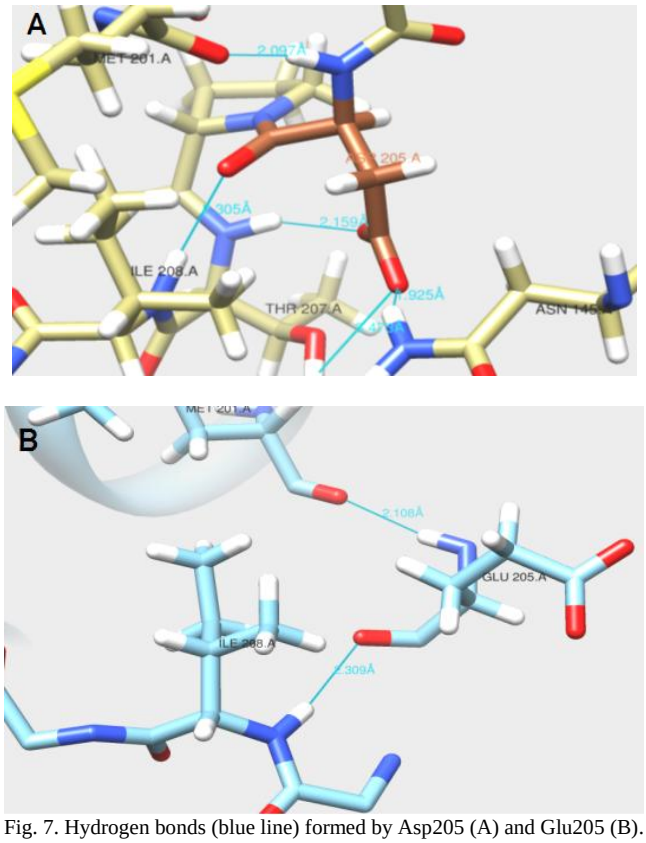

Fig. 7. Hydrogen bonds (blue line) formed by Asp205 (A) and Glu205 (B).

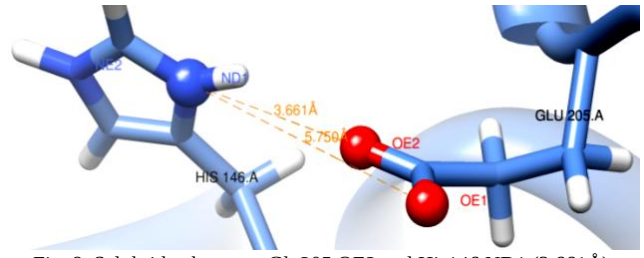

Fig. 8. Salt bridge between Glu205 OE2 and His146 ND1 (3.661Å).

Lys285Glu changes caused a side chain charge inversion, from positively to negatively charged [22]. The replacement at this position actually was more favorable since most charged amino acids around position 285 are positively charged: Lys279, Lys281, Lys282, Arg283 and Lys286. The charge inversion reduced the repulsive force arose from same charges around residue 285. Although amino acid at this position was charged, they were not involved in any salt bridge because of spatial hindrance. The closest negative amino acid to Lys285 was Asp276, whose charged atom was 12.3-13.5Å to Nz Lys285 while the closest positive amino acid to Glu285 was Lys279, whose NZ was 4.6Å to Glu285 OE1. It was assumed that Glu285 OE1 create a weak electrostatic interaction with Lys279 NZ because the charged atom distance was longer than salt bridge's maximum distance. The other change following replacement at this position was reduction of 1 Van der Waals interaction. This

residue (either before or after mutation) did not form any Hydrogen bond. The facing-out side chain packing cause the atoms of residue 285 were at unfavourable distance to form interaction (Fig. 9). Despite Glu285 formed less Van der Waals interaction than Lys285 did, but the weak electrostatic interaction formed and the charge inversion were able to compensate the destabilization caused by reduction of a Van der Waals interactions. The ΔΔG value of amino acid substitution on residue 285 was -0.09.

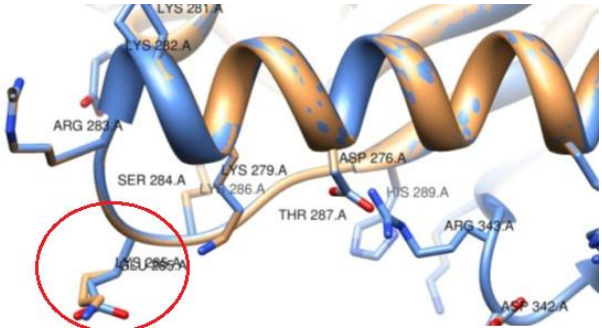

Fig. 9. Side chain packing of residue 285(before mutation: orange; after mutation: blue colored).

Qualitatively, there were reductions in non covalent interaction due to amino acid replacement, causing ΔHfolding of AbfA variant smaller than its wildtype. The maximum approximate bond energy for Hydrogen bond, Van der Waals interaction and electrostatic interaction are 10, 5, and 20 kJ/mol, respectively [22]. Reduction of 3 hydrogen bonds and 5 Van der Waals interaction also formation of 2 electrostatic interactions prior to amino acids substitution would reduce 15 kJ/mol in bond energy. According to the equation $\Delta G = \Delta H - T.\Delta S$ [22], a small ΔH (less heat released) causing a small ΔG value. The $\Delta G_{folding}$ depict amount of energy released to convert unfolding to folding state. Hence, the smaller $\Delta H_{folding}$ value, the smaller $\Delta G_{folding}$. Small value of $\Delta G_{folding}$ means unfolding is easier to occur. The smaller $\Delta H_{folding}$ of AbfA variant cause AbfA variant was more susceptible to unfolding than AbfA wildtype when treated with same heating treatment or in other words, cause AbfA variant has less thermostability than AbfA wildtype.

IV. Conclusion

The partially purified AbfA variant (Q46R, D205E, K285E) displayed optimum activity at pH 7 and 70°C, was stable for 24 hours at pH 6-9 (at 4°C) and lost almost 70% of its activity on 16 hours incubation at 70°C. The decrease in thermostability was prior to reduction in non covalent interaction driven by changes in structure.

Appendix

A. Supplementary 1. Hydrogen Bonds on AbfA

1) On residue 46

Donor (D)	Acceptor (A)	Hydrogen	Distance D..A	Distance D-H..A	notes
Gln 54 N	Gln 46 N	Gln 54 H	2.934	1.968	Before mutation
Gln 54 N	Arg 46 O	Gln 54 H	2.946	1.983	After mutation

2) On residue 205

Donor (D)	Acceptor (A)	Hydrogen	Distance D..A	Distance D-H..A	notes
Asn 145 ND2	Asp 205 OD2	Asn 145 HD2	2.835	1.925	Before mutation
Asp 205 N	Met 201 O	Asp 205 H	3.049	2.097	
Thr 207 N	Asp 205 OD1	Thr 207 H	3.111	2.159	
Ile 208 N	Asp 205 O	ILE 208 H	3.298	2.305	
Thr 207 OG	Asp 205 N	Thr 207 HG	3.440	2.473	
Glu 205 N	Met 201 O	Glu 205 H	3.062	2.108	After mutation
Ile 208 N	Glu 205 O	Ile 208 H	3.303	2.309	

3) On residue 285

No Hydrogen bond found.

B. Supplementary 2. Van der Waals Interaction on AbfA

VDW overlap >= -0,4 angstroms.

1) Gln46

No.	atom1		atom2		overlap	distance
1	GLN 46	H	HIS 44	HD2	0.818	1.182
2	GLN 46	H	HIS 44	CD2	0.614	2.086
3	GLN 46	N	HIS 44	HD2	0.571	2.054
4	GLN 46	O	GLN 54	H	0.512	1.968
5	GLN 46	N	HIS 44	CD2	0.272	3.053
6	GLN 46	O	GLN 54	N	0.171	2.934
7	GLN 46	OE1	ASP 55	HB3	0.142	2.338
8	GLN 46	CA	HIS 44	HD2	0.027	2.673
9	GLN 46	O	GLN 54	CB	-0.010	3.190
10	GLN 46	CB	HIS 44	HD2	-0.024	2.724
11	GLN 46	HB3	HIS 44	CD2	-0.024	2.724
12	GLN 46	H	HIS 44	C	-0.027	2.727
13	GLN 46	N	HIS 44	O	-0.047	3.152
14	GLN 46	HB3	HIS 44	HD2	-0.092	2.092
15	GLN 46	CB	HIS 44	CD2	-0.146	3.546
16	GLN 46	O	GLN 54	HB3	-0.196	2.676
17	GLN 46	OE1	ASP 55	CB	-0.208	3.388
18	GLN 46	H	HIS 44	CG	-0.299	2.999
19	GLN 46	NE2	HIS 44	HE2	-0.301	2.926
20	GLN 46	C	GLN 54	H	-0.302	3.002
21	GLN 46	CA	HIS 44	CD2	-0.328	3.728

22	GLN 46	HB3	HIS 44	NE2	-0.368	2.993
23	GLN 46	H	HIS 44	NE2	-0.381	3.006
24	GLN 46	H	PRO 45	CD	-0.396	3.096

2) Arg46

No.	atom1		atom2		overlap	distance
1	ARG 46	H	HIS 44	HD2	0.823	1.177
2	ARG 46	H	HIS 44	CD2	0.620	2.080
3	ARG 46	N	HIS 44	HD2	0.581	2.044
4	ARG 46	O	GLN 54	H	0.497	1.983
5	ARG 46	N	HIS 44	CD2	0.280	3.045
6	ARG 46	O	GLN 54	N	0.159	2.946
7	ARG 46	HB2	HIS 44	CD2	0.085	2.615
8	ARG 46	CA	HIS 44	HD2	0.048	2.652
9	ARG 46	CB	HIS 44	HD2	0.021	2.679
10	ARG 46	HB2	HIS 44	HD2	0.002	1.998
11	ARG 46	O	GLN 54	CB	-0.009	3.189
12	ARG 46	H	HIS 44	C	-0.036	2.736
13	ARG 46	N	HIS 44	O	-0.060	3.165
14	ARG 46	CB	HIS 44	CD2	-0.090	3.490
15	ARG 46	O	GLN 54	HB2	-0.191	2.671
16	ARG 46	HB2	HIS 44	NE2	-0.262	2.887
17	ARG 46	H	HIS 44	CG	-0.299	2.999
18	ARG 46	C	GLN 54	H	-0.302	3.002
19	ARG 46	CA	HIS 44	CD2	-0.306	3.706
20	ARG 46	H	HIS 44	NE2	-0.370	2.995
21	ARG 46	H	PRO 45	CD	-0.388	3.088

3) Lys 285

No.	atom 1		atom 2		overlap	distance
1	LYS 285	C	LYS 279	HE3	-0.362	3.062
2	LYS 285	H	LYS 286	H	-0.383	2.383

4) Glu 285

No.	atom 1		atom 2		overlap	distance
1	GLU 285	C	LYS 279	HE 3	-0.387	3.087

5) Asp205

No.	atom1		atom2		overlap	distance
1	ASP 205	OD2	ASN 145	HD21	0.555	1.925
2	ASP 205	H	MET 201	O	0.383	2.097
3	ASP 205	OD1	THR 207	OG1	0.327	2.653
4	ASP 205	OD1	THR 207	H	0.321	2.159
5	ASP 205	O	ILE 208	HG12	0.308	2.172
6	ASP 205	OD2	ASN 145	ND2	0.270	2.835
7	ASP 205	C	THR 207	H	0.190	2.510
8	ASP 205	O	ILE 208	H	0.175	2.305
9	ASP 205	HA	PRO 206	HD2	0.096	1.904
10	ASP 205	C	LYS 202	O	0.070	3.110
11	ASP 205	N	MET 201	O	0.056	3.049
12	ASP 205	O	THR 207	N	0.020	3.085
13	ASP 205	O	ILE 208	CG1	0.002	3.178
14	ASP 205	OD1	THR 207	N	-0.006	3.111
15	ASP 205	CG	THR 207	OG1	-0.011	3.211
16	ASP 205	O	ILE 208	HG22	-0.099	2.579
17	ASP 205	CG	ILE 208	HG22	-0.141	2.841
18	ASP 205	OD1	PRO 206	N	-0.187	3.292
19	ASP 205	O	ILE 208	N	-0.193	3.298
20	ASP 205	OD2	ASN 145	HB3	-0.195	2.675
21	ASP 205	O	ILE 208	CG2	-0.234	3.414
22	ASP 205	CG	ASN 145	HD21	-0.238	2.938
23	ASP 205	OD2	THR 207	OG1	-0.253	3.233
24	ASP 205	OD1	THR 207	CB	-0.256	3.436
25	ASP 205	N	LYS 202	O	-0.262	3.367
26	ASP 205	O	THR 207	H	-0.263	2.743
27	ASP 205	O	LYS 202	HA	-0.271	2.751
28	ASP 205	OD2	ASN 145	CB	-0.293	3.473
29	ASP 205	H	LYS 202	C	-0.319	3.019
30	ASP 205	OD1	THR 207	CG2	-0.372	3.552
31	ASP 205	CG	ILE 208	CG2	-0.378	3.778
32	ASP 205	OD2	ILE 208	CG2	-0.383	3.563
33	ASP 205	OD2	ILE 208	HG22	-0.396	2.876

6) Glu205

No.	atom1		atom2		overlap	distance
1	GLU 205	HG3	VAL 204	HG11	0.404	1.596
2	GLU 205	H	MET 201	O	0.372	2.108
3	GLU 205	HG2	ASN 145	CB	0.316	2.384
4	GLU 205	O	ILE 208	HG13	0.303	2.177
5	GLU 205	C	THR 207	H	0.216	2.484
6	GLU 205	CD	VAL 204	O	0.175	3.005
7	GLU 205	O	ILE 208	H	0.171	2.309
8	GLU 205	OE2	VAL 204	O	0.143	2.817
9	GLU 205	HG3	VAL 204	C	0.127	2.573
10	GLU 205	HG2	ASN 145	HB2	0.113	1.887
11	GLU 205	HA	PRO 206	HD3	0.074	1.926
12	GLU 205	CG	VAL 204	O	0.070	3.110
13	GLU 205	C	LYS 202	O	0.043	3.137
14	GLU 205	N	MET 201	O	0.043	3.062
15	GLU 205	HG3	VAL 204	CG1	0.030	2.670
16	GLU 205	CG	VAL 204	HG11	0.025	2.675
17	GLU 205	O	THR 207	N	0.019	3.086
18	GLU 205	O	ILE 208	CG1	0.000	3.180
19	GLU 205	HG2	ASN 145	HB3	-0.054	2.054
20	GLU 205	CG	ASN 145	CB	-0.059	3.459
21	GLU 205	HG3	VAL 204	O	-0.092	2.572
22	GLU 205	O	ILE 208	HG22	-0.114	2.594
23	GLU 205	CG	ASN 145	HB2	-0.176	2.876

24	GLU 205	O	ILE 208	N	-0.198	3.303
25	GLU 205	O	ILE 208	CG2	-0.247	3.427
26	GLU 205	CD	VAL 204	C	-0.253	3.653
27	GLU 205	O	LYS 202	HA	-0.257	2.737
28	GLU 205	N	LYS 202	O	-0.266	3.371
29	GLU 205	O	THR 207	H	-0.268	2.748
30	GLU 205	H	LYS 202	C	-0.327	3.027
31	GLU 205	CG	VAL 204	CG1	-0.353	3.753
32	GLU 205	CG	ASN 145	HB3	-0.353	3.053

C. Supplementary 3: Distances between Charged Atoms on Residue 46, 205 Dan 285

Electrostatic interaction on both AbfA wildtype and variant were determined by measured the distances between charged atoms located around charged mutated residue.

1) AbfA wildtype

No.	Atom 1			Atom 2			Distance (Å)
1	Asp	205	OD1	Lys	166	NZ	7.418
2	Asp	205	OD2	Lys	166	NZ	6.664
3	Asp	205	OD1	His	146	ND1	8.015
4	Asp	205	OD2	His	165	ND1	11.480
5	Asp	205	OD1	His	165	ND1	13.640
6	Asp	205	OD2	Lys	168	NZ	7.995
7	Asp	205	OD1	Lys	168	NZ	7.649
8	Lys	285	NZ	Asp	276	OD2	13.550
9	Lys	285	NZ	Asp	276	OD1	12.328

2) AbfA variant (Gln46Arg; Asp205Glu; Lys285Glu)

No.	Atom 1			Atom 2			Distance (Å)
1	Glu	285	OE2	Arg	283	NH1	11.898
2	Glu	285	OE2	Arg	283	NH2	12.989
3	Glu	285	OE2	Lys	279	NZ	6.505
5	Glu	285	OE1	His	289	NE2	12.188
6	Glu	285	OE1	Lys	279	NZ	4.796
8	Glu	285	OE2	Lys	282	NZ	15.598
9	Glu	285	OE2	Lys	286	NZ	12.907
10	Arg	46	NH2	Asp	55	OD2	6.568
11	Arg	46	NH2	Asp	55	OD2	5.980
12	Arg	46	NH1	Asp	55	OD1	6.525
13	Arg	46	NH1	Asp	55	OD1	6.602
14	Arg	46	NH2	Glu	58	OE1	7.955
15	Arg	46	NH2	Glu	58	OE2	7.051
17	Glu	205	OE1	Lys	202	NZ	12.890
18	Glu	205	OE1	Lys	168	NZ	9.775
19	Glu	205	OE1	Lys	166	NZ	6.078
21	Glu	205	OE1	His	146	NE2	7.744
22	Glu	205	OE2	His	146	NE2	5.756
23	Glu	205	OE1	His	146	ND1	5.750
24	Glu	205	OE2	His	146	ND1	3.661

REFERENCES

[1] R. De Vries, H. Kester, C. Poulsen, J. Benen, and J. Visser, "Synergy between accessory enzymes from Aspergillus in the degradation of plant cell wall polysaccharides," *J. Carbohydr. Res.*, vol. 327, pp. 401-410, 2000.

[2] M. Numan and N. Bhosle, "α-L-arabinofuranosidase: the potential applications in biotechnology," *J. Ind Microbial Biotechnol*, vol. 33, pp. 253, 2006.

[3] C. Vieille and G. Zeikus, "Hyperthermophillic Enzymes: Sources, Uses and Molecular Mechanisms for Thermostability," *Microbiology and Molecular Biology Reviews*, vol. 33, pp. 8, 2001.

[4] N. N. T. Puspaningsih, "Pencirian Enzim Xilanolitik dan Kloning Gen Penyandi Xilosidase dari *Bacillus thermoleovorans* IT-08," Ph.D Thesis, Sekolah Pascasarjana, Institut Pertanian Bogor, Bogor, Indonesia, 2004.

[5] M. Jonet, N. Mahadi, A. Murad, A. Rabu, F. D. Abu Bakar, K. O. Low, R. Illias, "Optimization of a Heterologous Signal Peptide by Site-Directed Mutagenesis for Improved Secretion of Recombinant Proteins in *Escherichia coli*," *J Mol Microbiol Biotechnol*, vol. 22, pp. 48-58, 2012.

[6] L. N. Yamani, "Konstruksi Sekresi Ekstraseluler dan Peningkatan pH Optimum α-L-arabinofuranosidase dari *Geobacillus thermoleovorans* IT-08," Master Thesis, Departement of Chemistry, Faculty of Science and Technology, Universitas Airlangga, Surabaya, Indonesia, 2011.

[7] M. Berrondo, "Predicting The Structure and Function of Protein Mutants," Ph.D Thesis, John Hopkins University, Departement of Chemical and Biomolecular Engineering, United States of America, 2010.

[8] N. Tokuriki, F. Stricher, J. Schymkowitz, L. Serrano, and D. Tawfik, "The Stability Effects of Protein Mutations Appear to Be Universally Distributed," *J Mol Biol*, pp. 1318, 2007.

[9] T. Schwede, J. Kopp, N. Guex, and M. Peitsch, "Swiss Model: An automated protein homology modeling server," *Nucleic acid research*, vol. 31 no. 13 pp. 3381-3381, 2003.

[10] K. Hovel, D. Shallom, K. Niefind, V. Belakhov, G. Shoham, T. Baasov, Y. Shoham, and D. Schomburg, "Crystal Structure and Snapshots Along The Reaction Pathway of A Family 51 α-L-arabinofuranosidase," *EMBO J.*, vol. 22, pp. 4922-4932, 2003.

[11] R. Maiti, H. Gary, G. V. Domselaar, H. Zhang, and D. Wishart, "SuperPose: a simple server for sophisticated structural superposition," *Nucleic Acids Res.*, pp. 32, July 2004.

[12] E. Pettersen, T. Goddard, C. Huang, G. Couch, D. Greenblatt, E. Meng, and T. Ferrin, "UCSF Chimera--a visualization system for exploratory research and analysis," *J Comput Chem.*, vol. 25, no. 13, pp. 1605-12, 2004.

[13] Y. Dehouck, A. Grosfils, B. Folch, D.Gillis, Ph. Bogaerts, and M. Rooman, "Prediction of protein stability changes upon mutations using statistical potentials and neural networks: PoPMuSiC 2.0," *Bioinformatics*, vol. 25, pp. 2537-2543, 2009.

[14] C. Bailey, L. Gerber, A. Howard, and S. Udenfriend, "Processing at the carboxyl terminus of nascent placental alkaline phospatase in a cell free system: Evidence for specific cleavage of a signal peptide," in *Proc. Natl. Acad. Sci.*, USA, vol. 86, pp. 24, 1989.

[15] B. Westley and R. Weber, "Divergence of the two albumins of *X.laevis*: Evidence for the glycosylation of the major 74K albumin," *Differentiation*, vol. 22, no. 3, pp. 227-30, 1982.

[16] E. Gasteiger, C. Hoogland, A. Gattiker, S. Duvaud, M. R. Wilkins, R. D. Appel, and A. Bairoch, "Protein Identification and Analysis Tools on the ExPASy Server," in *The Proteomics Protocols Handbook*, J. M. Walker, Ed., Humana Press, 2005, pp. 571-607.

[17] Y. Lu, K. Zen, S. Muthukrishnan, and K. Kramer, "Site-directed mutagenesis and functional analysis of active site acidic amino acid residues D142, D144 and E146 in *Manduca sexta* (tobacco hornworm) chitinase," *Insect Biochemistry and Molecular Biology*, vol. 32, pp. 1369-1382, 2002.

[18] J. Nielsen, T. Borchert, and G. Vriend, "The determinants of α-amylase pH-activity profile," *Protein Engineering*, vol. 14 no. 7 pp. 505, 2001.

[19] B. Tynan-Connolly and J. Nielsen, "Redesigning protein pKa values," *Protein Sci.*, 2007, vol. 16, no. 2, pp. 239.

[20] N. Bogatyreva and D. Ivankov, "The Relationship between the Solvent-Accessible Surface Area of a Protein and the Number of Native Contacts in Its Structure," *Molecular Biology*, vol. 42, no. 6, pp. 932, 2008.
[21] A. Lesk and C. Chotia, "Solvent Accessibility, Protein Surfaces and Protein Folding," *Biophysical society*, pp. 35, 1979.
[22] D. Whitford, *Proteins Structure and Functions*, West Sussex, England: John Wiley and Sons, Ltd., 2005, pp. 25, 28, 53-58.
[23] S. Kumar and R. Nussinov, "Close Range Electrostatic Interactions in Proteins," *Chem Bio. Chem.*, vol. 3, no. 7, pp. 605, 2002.
[24] A. A. Karshikoff and I. Jelesarov, "Salt bridges and conformational flexibility: Effect on protein stability," *Biotechnol and biotechnol*, pp. 606, 2008.

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