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CLONING AND EXPRESSION OF MINIATURIZED HALOALKANE DEHALOGENASE ENZYME

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PENGESAHAN

Dengan ini adalah disahkan bahawa tesis projek yang bertajuk “Pengklonan dan Expreksi miniatur Haloalkane Dehalogenase Enzim” telah disiapkan serta dikemukakan kepada Jabatan Biokimia oleh Lee Ser Leng (161108) sebagai syarat untuk kursus BCH 4999 (Projek).

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ABSTRACT

Haloalkane dehalogenase (HLD) belongs to a group of hydrolases class of enzymes that catalyses the hydrolysis of haloalkanes. Haloalkane dehalogenases are useful for many bioremediation applications. Mini proteins design of less than 100 amino acids residues are a subject of interest as they confer better stability, has a minimized loss of entropy and the structures are easier to study. In this study, the design of a minimized structure from DhaA haloalkane dehalogenase was done to obtain a functional protein with a smaller structure. A 60 residues amino acids structure was designed from 296 residues through structural modelling with YASARA software. Through homology modelling, docking and molecular dynamics (MD) simulation, the 3D structure of the mini protein was designed, cloned, expressed in pET32b vector, and transformed to *E. coli* transformants. Protein expression was optimized at 20 ℃ and inducer concentration of 0.01 mM. The kinetics of the mini protein were analysed with Isothermal Titration Calorimetry (ITC) method and it was found out that the mini protein had some interactions with various substrates concentration. At a much lower efficient protein as compared to the native structure, it is recommended that further modifications of protein should be done to achieve better functionality.

ABSTRAK

Haloalkane Dehalogenase (HLD) tergolong dalam kumpulan kelas enzim hydrolase yang menjadi pemangkin hidrolisis haloalkane. Haloalkane Dehalogenase berguna untuk aplikasi industri bioremediasi. Reka bentuk protein mini kurang daripada 100 amino asid adalah menarik kerana mereka mempunyai kestabilan yang lebih baik, mempunyai pengurangan rendah entropi dan struktur lebih mudah dikaji. Dalam kajian ini, reka bentuk struktur DhaA haloalkane dehalogenase dipendekkan untuk mendapatkan protein yang berfungsi dengan struktur yang lebih kecil. Satu 60 residu amino asid direka daripada 296 residu melalui pemodelan struktur dengan perisian YASARA. Melalui model homologi, dok dan simulasi dinamik molekul (MD), struktur 3D protein mini direka, diklon, diekspresikan dalam vektor pET32b, dan ditransformasi kepada transformant *E. coli*. Ekspresi protein dioptimumkan pada 20 ℃ dan kepekatan perangsang 0.01 mM. Kinetik protein mini dianalisis dengan kaedah Pentitratan Kalorimetri Sesuhu (ITC) dan didapati bahawa protein mini ini mempunyai interaksi dengan pelbagai kepekatan substrat. Untuk mendapatkan protein yang boleh berfungsi lebih baik berbanding struktur asli, adalah disyorkan bahawa pengubahsuaian selanjutnya dilakukan untuk mencapai fungsi yang lebih baik.

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LIST OF ABBREVIATIONS

%	percentage
3D	three dimensional
µL	microlitre
µJ	micro Joule
^{0}C	degree of Celcius
Å	Angstrom
Asn	Asparagine
Asp	Aspartate
bp	base pair
EC	Enzyme Commission
et al.	and friends
Glu	Glutamate
HCl	hydrogen chloride
His	Histidine
IPTG	Isopropyl β-D-1-thiogalactopyranoside
kb	kilo base
kDa	kilo Dalton
mg	milligram
min	minute
mL	millilitre
mM	millimolar
Ni-Sepharose	Nickel Sepharose
nM	nanomolar
OD	Optical Density
rpm	revolutions per minute
s	second
Trp	Tryptophan
w/v	weight over volume
x g	times gravity
YASARA	Yet Another Scientific Artificial Reality Application

CHAPTER 1

INTRODUCTION

Haloalkane dehalogenases (HLDs; EC3.8.1.5) are enzymes that catalyse hydrolytic dehalogenation in a wide range of halogenated compounds, yielding an alcohol, a proton and a halide. HLDs mainly originated from microbial sources. There are many types of haloalkane dehalogenases such as LinB, DbjA, DhlA, DhaA, DbeA, DmbA, DatA, DmbC, and DrbA haloalkane dehalogenases.

Mini proteins are polypeptides shorter than 100 residues which nevertheless exhibit a well-defined tertiary structure (Wang *et al.*, 2008). Mini proteins represent simple and useful model systems to study the structural determinants of protein folding and stability and to illustrate such principles within biochemistry and molecular biology educational context (Polticelli *et al*., 2001) The study of mini proteins also allows pinpointing of specific interactions that stabilize their three-dimensional organization. The structural analysis of mini proteins can be a valuable tool to understand the physico-chemical determinants of protein stability (Polticelli *et al*., 2001).

However, HLD has many different families and not all of the structures are annotated. To date, X-ray structures of three different bacterial HLDs have been solved, and several (putative) dehalogenases have been cloned or detected by genome sequence analysis (Janssen *et al*., 2004). HLDs have drawn considerable interest because they catalyse a reaction of great environmental significance: the conversion of an alkyl halide functionality to an alcohol group (Janssen *et al.*, 2004). In this study, the minimized structure of haloalkane dehalogenase will enable us to study and tackle problems related to haloalkane pollutions and degradations.

Objectives

- To design a minimized haloalkane dehalogenase structure from *Rhodococcus rhodochrous* (PDB ID: 4E46) by YASARA software.
- To clone and express the designed mini protein gene in *E. coli.*

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