

COMPUTATIONAL ANALYSIS ON PROTEIN-LIGAND INTERACTION OF  
XYLITOL-PHOSPHATE DEHYDROGENASE ENZYMES FOR XYLITOL  
PRODUCTION

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PRODUCTION

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“To my wonderful family for their endless support and motivation.  
Ummi and Abah, thank you for your love and patience”

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## ABSTRACT

Xylitol is a high-value low-calorie sweetener used as sugar substitute in food and pharmaceutical industry. Xylitol phosphate dehydrogenase (XPDH) catalyses the conversion of D-xylulose 5-phosphate (XU5P) and D-ribulose 5-phosphate (RU5P) to xylitol and ribitol respectively in the presence of nicotinamide adenine dinucleotide hydride (NADH). Although these enzymes have been shown to produce xylitol, however there is a limited understanding of the mechanism of the catalytic events of these reactions and the detailed mechanism has yet to be elucidated. Understanding of the catalytic activity of these enzymes would provide novel information for protein engineering to improve xylitol production. The main goal of this work is to analyse the conformational changes of XPDH-bound ligands such as  $Zn^{2+}$ , NADH, XU5P, and RU5P to elucidate the key amino acids involved in the substrate binding. *In silico* modelling, comparative molecular dynamic simulations, interaction analysis and conformational study were carried out on three XPDH enzymes of the Medium-chain dehydrogenase (MDR) family; XPDH from *Lactobacillus rhamnosus* (LrXPDH) and *Clostridium difficile* (CdXPDH, Cd1XPDH) in order to elucidate the atomistic details of conformational transition, especially on the open and closed state of XPDH. The critical residues involved in substrate binding and conformational changes were mutated using *in silico* site-directed mutagenesis. The result showed that residues Cys37, His58, Glu59, and Glu142 form an active site pocket within the catalytic domain. In the coenzyme domain, NADH is shown to bind to highly conserved glycine-rich motif; GXGXXG (residues 166-171). The results also revealed that XPDH consists of a dual mechanism that can catalyse hydride transfer to dissimilar substrates (XU5P and RU5P), which His58 and Ser39 would act as the proton donor for reduction of XU5P and RU5P respectively. The structural comparison and MD simulations displayed a significant difference in the conformational dynamics of the catalytic and coenzyme loops between Apo and XPDH-complexes and highlight the contribution of newly found triad residues (W48, I259, and W285). The study also identified the effect of S39A and W285A mutations on substrate binding and conformational changes. The study successfully elucidated the mechanistic aspect of catalysis mechanism and dynamical event of XPDH enzymes at molecular level. The results from this study would assist future mutagenesis study and enzyme modification work to increase the catalysis efficiency of xylitol production in the industry.

## ABSTRAK

Xylitol adalah pemanis rendah kalori yang bernilai tinggi dan digunakan sebagai pengganti gula dalam industri makanan dan industri farmaseutikal. Xylitol fosfat dehidrogenase (XPDH) menjadi pemangkin penukaran xilulosa 5-fosfat (XU5P) kepada xylitol dan D-ribulosa 5-fosfat (RU5P) kepada ribitol dengan menggunakan nikotinamida adenina dinukleotida hidrida (NADH). Walaupun enzim ini telah terbukti menghasilkan xylitol, tetapi pemahaman terhadap mekanisme tindak balas ini adalah terhad dan belum dijelaskan secara terperinci. Pemahaman terhadap pemangkinan ini akan memberikan maklumat baru dalam kejuruteraan protein untuk meningkatkan pengeluaran xylitol. Matlamat utama kajian ini adalah untuk menganalisis perubahan bentuk enzim XPDH dan ligan seperti  $Zn^{2+}$ , NADH, XU5P, dan RU5P serta menjelaskan jujuk asid amino yang terlibat dalam pengikat substrat. Pemodelan dalam siliko, perbandingan simulasi dinamik molekul, analisis interaksi dan kajian sama bentuk telah dijalankan pada tiga enzim XPDH daripada keluarga dehidrogenase Medium (MDR); iaitu XPDH dari *Lactobacillus rhamnosus* (LrXPDH) dan *Clostridium difficile* (CdXPDH, Cd1XPDH) untuk menjelaskan peralihan bentuk secara butiran atom, terutamanya dalam keadaan terbuka dan tertutup XPDH. Asid amino yang terlibat dalam pengikat substrat dan perubahan bentuk telah dimutasi menggunakan tapak siliko mutagenesis berarah. Hasil kajian menunjukkan bahawa jujuk asid amino Cys37, His58, Glu59, Glu142 membentuk poket tapak aktif dalam domain pemangkin. Dalam domain koenzim, NADH terikat dengan motif terabadi, GXGXXG (jujuk amino 166-171) yang kaya dengan glisina. Kajian ini juga mendedahkan XPDH mempunyai dwi mekanisme yang boleh memangkinkan pemindahan hidrida ke substrat yang berbeza (XU5P dan RU5P), iaitu His58 dan Ser39 akan bertindak sebagai penderma proton untuk pengurangan XU5P dan RU5P. Perbandingan struktur dan simulasi MD mendedahkan perbezaan yang signifikan dalam bentuk dinamik dari gelung mangkinan dan koenzim antara apo dan kompleks XPDH serta menonjolkan sumbangan jujuk amino triad yang baru dijumpai (W48, I259, dan W285). Kajian ini juga mengenal pasti kesan mutasi S39A dan W285A pada perubahan pengikat substrat dan analisis perubahan bentuk. Kajian ini berjaya menjelaskan aspek mekanisma pemangkinan mekanistik dan peristiwa dinamik enzim XPDH di peringkat molekul. Hasil dari kajian ini akan membantu kajian mutagenesis di masa depan dan kerja pengubahsuaian enzim untuk meningkatkan kecekapan pemangkinan pengeluaran xylitol dalam industri.

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**LIST OF ABBREVIATIONS**

<i>LrXPDH</i>	-	<i>Lactobacillus rhamnosus</i> xylitol phosphate dehydrogenase
<i>CdXPDH</i>	-	<i>Clostridium difficile</i> xylitol phosphate dehydrogenase
<i>XU5P</i>	-	D-xylulose 5-phosphate
<i>RU5P</i>	-	D-ribulose 5- phosphate
<i>WT</i>	-	Wild-type
<i>MDR</i>	-	Medium-chain dehydrogenase
<i>NAD</i>	-	Nicotinamide adenine dinucleotide
<i>XDH</i>	-	Xylitol dehydrogenase
<i>ArDH</i>	-	Arabitol dehydrogenase
<i>GPDH</i>	-	Galactitol-1-phosphate 5-dehydrogenase
<i>PDH</i>	-	Polyol dehydrogenase
<i>EC</i>	-	Enzyme Commission
<i>CDD</i>	-	Conserved domain database
<i>GOR</i>	-	Garnier-Osguthorpe-Robson
<i>BLAST</i>	-	Basic Local Alignment Search Tool
<i>GO</i>	-	Gene Ontology
<i>RMSD</i>	-	Root mean square deviations
<i>SPC</i>	-	Simple point charge
<i>PME</i>	-	Particle Mesh Ewald
<i>LINC</i>	-	LINear Constraint Solver
<i>GRAVY</i>	-	Grand average of hydrophaticity
<i>NPS</i>	-	Network Protein Sequence
<i>MSA</i>	-	Multiple sequence alignment

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## CHAPTER 1

### INTRODUCTION

#### 1.1 Overview

Today, an increasing number of researchers are focusing on xylitol production as an alternative sugar for healthy eating. Because of their unique properties, they have potential and desirable for food industry such as sugar-free chewing gum, cookies, desserts and soft drink (Mussatto, 2012). Xylitol can also improve the storage properties, taste, and colour of food product (Ur-Rehman *et al.*, 2015). For the pharmaceutical industry, xylitol is the suitable low-calorie sweetener that is recommended for the diabetic patient as it can be metabolized in the absence of insulin (Storey *et al.*, 2007). The global market for xylitol is currently estimated to be over US\$750 million per year and priced at US\$ 6-7 per kg (Global Market Insights, 2016). Xylitol has 12% share of total polyol market, which is the second largest after sorbitol (Albuquerque *et al.*, 2014).

This sugar is found naturally in fruits and vegetables as well as in yeast, seaweed, and mushrooms. It can be extracted by solid-liquid extraction, but it becomes a major economic problem due to its small proportion of the raw materials (Winkelhausen and Kuzmanova, 1998). Industrially, xylitol produced by catalytic reduction of pure D-xylose, however the chemical method of xylitol manufacturing is laborious and expensive (Rafiqul and Sakinah, 2013a; X.-H. Qi *et al.*, 2016).

Alternatively, this problem could be solved by using D-glucose as the low-cost raw material (Cheng *et al.*, 2014a). D-glucose can be converted into xylitol by using xylitol-phosphate dehydrogenase from *Lactobacillus rhamnosus* and *Clostridium difficile* with the highest yield 22-23% (Povelainen and Miasnikov, 2007a).

The study of XPDH classification is needed in order to know the remarkable mechanism and metabolic pathway to produce xylitol. Oxidoreductases are divided into three classes which are short-chain dehydrogenase (SDR), medium chain dehydrogenase/reductase (MDR) and long-chain dehydrogenase. These enzymes are specifically acting on the CHOH group of a donor molecule with  $\text{NAD}^+$  or  $\text{NADP}^+$  as the acceptor (Auld and Bergman, 2008). Xylitol-phosphate dehydrogenase from *Lactobacillus rhamnosus* ATCC 15820 (LrXPDH), XPDH from *Clostridium difficile* CD630 (CdXPDH) and XPDH from *Clostridium difficile* CD196 (Cd1XPDH) belong to the MDR family. All these three proteins consist of two domains; a catalytic domain and a nicotinamide cofactor (NADH) binding domain. The 3D structure and the active site of XPDH enzymes remained to be identified and the interaction of substrate binding has not been studied in detail at the atomic level. The present research is the first study of the sequences and structural characterization, protein-ligand interaction and protein engineering of XPDH enzymes that can produce xylitol from D-glucose.

Combination of comparative modelling, molecular docking, and molecular dynamics simulation can help to understand the action mode of substrates and the catalytic mechanism of XPDH enzymes. The computational study has been powerful tools for researchers to predict protein structure and ligand-protein interaction. *In silico*, site-directed mutagenesis will establish novel strategies to increase efficiency of XPDH enzymes activity and improve xylitol production.

## 1.2 Problem Statement

Xylitol-phosphate dehydrogenase from *Lactobacillus rhamnosus* ATCC 15820 (LrXPDH), XPDH from *Clostridium difficile* CD630 (CdXPDH) and XPDH from *Clostridium difficile* CD196 (Cd1XPDH), three enzymes from Medium-chain dehydrogenase family that are capable to catalyze the reduction of both D-xylulose 5-phosphate and D-ribulose 5-phosphate to xylitol (Povelainen and Miasnikov, 2007a; Abdullah, 2018). However, the three dimensional (3D) structures of all XPDH enzymes are relatively unknown and the interaction of substrate binding has not been studied in detail at the atomic level. Hence, the comparative modelling and molecular docking studies may reveal the structural active site and interaction of XPDH enzymes with their ligands.

Due to the substrate specificity of XPDH, the xylitol production was accompanied by co-production of ribitol. *In silico* site-directed mutagenesis is required for the understanding rationale of the conversion. Furthermore, the effect of the mutation on the stability of XPDH enzymes has remained unexplored. Molecular dynamic simulations are powerful tools to study the stability of the mutants. It is important to highlight that there is no computational approach for XPDH enzyme to date. *In silico* study of XPDH may provide biotechnologically interesting potential as well as improve the production of xylitol.



### 1.3 Research Objectives

The main goal of this research is to analyse the protein-ligand interaction of xylitol-phosphate dehydrogenase enzymes for xylitol production. There are several objectives need to be achieved in this research project:

1. To investigate the primary sequence characteristics and the three-dimensional structures of wild-type and mutant xylitol phosphate dehydrogenase (XPDH) enzymes.
2. To identify the key binding residues and analyse the interaction of the substrates with XPDH-complex at the catalytic and coenzyme domain.
3. To elucidate the details mechanism of xylitol phosphate dehydrogenase (XPDH) enzymes.
4. To study the effect of the mutation on the stability of XPDH enzymes based on amino acid substitution and comparative molecular dynamic simulation.
5. To elucidate the atomistic details of conformational changes on the open and closed state of XPDH enzymes

### 1.4 Scope of Study

This study is exclusively bioinformatics and computational analysis which include model development, protein interaction, protein engineering, protein stability and dynamics. All the data were derived from the primary database and analyzed using high performance computing facilities in FBME. In this works, three Xylitol-phosphate dehydrogenase (XPDH) enzymes that can produce xylitol were selected; including XPDH from *Lactobacillus rhamnosus* and *Clostridium difficile*. The primary sequence and structural analysis of XPDH enzymes were done to investigate their functional characteristics and elucidate the potential protein engineering for xylitol production. The interaction of substrate binding protein will be studied using molecular docking. The simulations were performed using open source GROMACS

(GROningen Machine for Chemical Simulation) version 5.1.4 software (Abraham *et al.*, 2015) in order to investigate the dynamic signature and conformational behaviour of the protein-ligand complex.

## 1.5 Significance of Study

In this research, the sequence and structural analysis of XPDH enzymes provide the valuable structural information of molecular architecture of XPDH which offer novel details in PDH family and may be relevant to wider MDR superfamily. This analysis also help the fundamental biology on sequence-structure-function relationship of protein families. The study of protein-ligand interaction of XPDH provides an insight into the possible catalytic event, improve specificity of the substrate and provide information for the protein engineering to increase the xylitol production.

This study also successfully elucidate the mechanistic aspect of catalysis mechanism and dynamic event of XPDH enzymes at the molecular level, especially on the open and closed state of XPDH which has been impossible to determine by experimental technique. *In silico* site directed mutagenesis in this study will provide the fundamental information contribution of key residues in XPDH catalysis and molecular dynamic.

Overall, this thesis makes a significant contribution to the field of knowledge by offering information on structural, dynamic and computational study in order to design rational strategies to increase the efficiency of XPDH enzymes activity and improve xylitol production.

## 1.6 Thesis Organization

This thesis is comprised of six chapters. Chapter 1 describes the outline of the research which includes the background of this study and the problem statement. This chapter also emphasized the objectives, the scopes and the significance of this research.

Chapter 2 include the literature review that related to the study. This chapter is focusing on reviewing other related proteins in the same family, the production and the application of the related sugar and the basic concept of this research area.

Chapter 3 present the research methodology which includes the operational frameworks in order to achieve the research goals. All the methods and materials used in this study are described in detail.

Chapter 4 shows the structure and function prediction of Xylitol phosphate dehydrogenase (XPDH). The interaction of protein-ligand binding and molecular dynamic simulation are also discussed in detail. The significant results from this chapter were used to identify the potential protein engineering (Chapter 5) for xylitol production.

Chapter 5 highlights the information of *in silico* site mutagenesis of CdXPDH –complex proteins. The result of the conducted experiments and discussion related to the objectives are included in this chapter.

Chapter 6 gives a conclusion of the thesis by a general discussion of the result obtained. In addition, this chapter discusses the directions for future work in order to improve the production of xylitol.

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