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Research Article

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Bioinformatics Analysis of Aceto Hydroxyl Acid Synthase as an Alternative Enzyme for Pyruvate Decarboxylase

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ABSTRACT

The aim of this study was to evaluate the potential of using Aceto hydroxyl acid synthase as an alternative of Pyruvate decarboxylase (PDC) for production of L-phenyl acetyl carbinol. After preparing 3D structures of AHAS and PDC and alignment of sequences, the molecular docking of PDC and AHAS were done in order to find the most active protein. All molecules were optimized before docking and the highest Mol Dock score was reported. It was confirmed that AHAS had valuable affinity to pyruvate molecule which could subsequently resulted in high L-PAC production yield. The estimated production yield of L-PAC using AHAS obtained according to previous articles was reasonably higher than PDC. This study revealed the valuable affinity of AHAS to benzaldehyde and presented estimation of its production yield of this enzyme in compare to PDC.

Keywords: Pyruvate decarboxylase; Aceto hydroxy acid synthase; L-phenyl acetyl carbinol

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INTRODUCTION

L-phenyl acetyl carbinol is an important compound used as precursor of pseudoephedrine, L-ephedrine and norephedrine. These drugs have anti asthmatics and decongestants properties [1]. Conventional extraction from Ephedra plants and chemical reactions are the main ways of L-PAC production. But extraction and purification of L-PAC is costly, time consuming and complicated. L-PAC could be produced by yeast cultures through a simple biotransformation reaction [2].

The acetaldehyde resulted from decarboxylation of pyruvic acid, undergoes condensation reaction with benzaldehyde and lead to L-PAC. The biotransformation process is not 100% complete and by products like benzyl alcohol and benzoic acid lead to reduction in yield of process. There are lots of studies like finding new more reproductive enzymes, immobilizing enzymes and cells and optimization of benzaldehyde and acetaldehyde in microbial culture to reduce the byproduct formation and so increase the yield of process [3].

Biotransformation of benzaldehyde to L-PAC using bacteria and yeast cells [4,5], purified enzymes [6,7] and immobilized cells [8] had been investigated in order to reach to valuable L-PAC production yield. Engel et al. reported the potential effect of *E. coli* AHAS for conversion of benzaldehyde to L-PAC. This enzyme produced a little acetaldehyde in compare to pyruvate decarboxylase and so converts highest percent of substrate to L-PAC [9].

We aligned the sequence of AHAS with genome of yeast *saccharomyces cervisiae* and found that ILV-2 is completely identical to *E. coli* AHAS. We produced petit mutants and demonstrate that this mutant is more efficient in bioconversion of benzaldehyde. We believed that the main thing that leads to this observation is that petit mutants have lots of proteins including ILV-2 which cannot transform to mitochondria because of damage in protein transporters of mitochondrial membrane [5]. Finding and comparing transformation activity of AHAS and PDC is

very important for optimizing bioconversion process using point mutation. In this paper we tried to compare sequence, structure and binding activity of these two enzymes using bioinformatics methods.

MATERIALS AND METHODS

Proteins Structure and Ligand Preparation

The crystallographic structure of Pyruvate decarboxylase (PDC) and Acetohydroxy acid synthase (AHAS) obtained from protein data bank (http://www.rcsb.org/) with PDB IDs of: 1QPB and 5INU, respectively. The crystallography of proteins was X-Ray diffraction with resolution of 2.4 and 1.98 A^0 for PDC and AHAS, respectively. Model optimization performed using GaussView software.

The 3D structure of Pyruvate sketched with Chem Bio Draw and geometry optimized with Hyperchem software using semi empirical method of AM1 and PM3.

Alignment of Two Proteins

In order to find how much the two proteins have similarity in their amino acid sequences, protein sequence alignment performed using Expassy webserver (http://web.expasy.org/cgi-bin/sim/sim.pl?prot) with Blosum 62 comparison matrix.

ProtParam Tool

The physiochemical properties of proteins including isoelectric point, aliphatic and instability indexes, and gravity average hydropath city (GRAVY) were calculated using ProtParam server (http://web.expasy.org/protparam).

GOR IV Server

The GOR IV server (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_gor4.html)) was used to predict the percentage of secondary structures at intended proteins.

Ligand Binding Site Detection

The Coach Site server (http://zhanglab.ccmb.med.umich.edu/COACH/) which is a Meta site server for detecting binding sites was used by submitting the PDB files of proteins [10]. Thiamin pyro phosphate (TPP), pyruvic acid and its derivatives, and Flavin adenine dinucleotide (FAD) are the common ligands of PDC and AHAS. Also MVD was used for detecting cavities. The probe size was set at 1.2, and maximum and minimum numbers of ray checks were 16 and 12, respectively. The Grid resolution was set on 0.8.

Molecular Docking

Protein-Ligand docking was done using Molegro Virtual Docker. Pyruvate Decarboxylase and aceto hydroxyl acid synthase were docked separately against pyruvate as ligand setting maximum cavities on 5 and water molecule was included in docking test. The molecular structure of ligand (pyruvate) was checked manually before docking, and corrected if it had failure notice. The cavity volume restricted between 10 to 16 A^0. Geometry optimization performed on pyruvate using MM+ method. Also geometry optimization was done with RMS of 0.01 kcal/mol.

RESULTS AND DISCUSSION

Sequence Analysis

The results showed that these two proteins have 24.8% similarity in 298 residues overlap with score of 145 and reached to 50.0% similarity in 14 residue overlap with score of 34. The two sequences with highest similarity are presented here:

AHAS 548 TELSSAVQAGTPVK
PDC 353 TPANAAVPASTPLK

The physiochemical properties of two proteins are summarized in Table 1. AHAS contain both more acidic and basic residues than PDC, but its instability index is not much higher than PDC which is because of nearly the same values of acidic to basic amino acids ratio. The average hydropathicity index (GRAVY) of AHAS is very lower than PDC average hydropathicity which means that this enzyme is more hydrophilic than PDC. The average percent of different secondary structures of PDC and AHAS were the same.

Table 1: ProtParam and ROC analysis results

Molecule Name	Total number of (Asp + Glu)	Total number of (Arg + Lys)	Instability index	Aliphatic index	GRAVY	Alpha helix	Extended strand	Random coil
PDC	118	99	30.25	93.91	-0.045	0.3339	0.1852	0.4809
AHAS	156	140	34.5	81.26	-0.295	32.79%	20.38%	46.82%

Analyzing proteins based on binding specific substrate comparison (TM site) and sequence profile alignment (S site) showed that there is 4 ligand binding site for PDC with C-scores of 0.27 and 0.21, and only one ligand binding site for AHAS with C-scores of 0.25 (Tables 1 and 2).

C-score is the confidence score of predicted binding site. C-score ranges 0-1, where a higher score indicates a more reliable prediction. These primary results indicate that there is same number of pyruvic acid binding sites for PDC and AHAS (Tables 2 and 3).

Table 2: TM- site results for PDC

Rank	C-score[a]	Cluster size[b]	Rep Templ[c]	Mult templ[d]	Predicted binding site residues
1	0.27	3	2vjyA_BS04_ALK	PYR(2), ALK(1)	9,12,20,22,42,85,28,63,00,000
2	0.22	2	3oe1A_BS02_TDL	TDL(2)	387,388,389,412,413,414,442,443,444,445,448,470,472,473,474,475,476,
3	0.21	2	2q5oA_BS04_PPY	PPY(2)	62 ,223,246,248,249,250,382,404,405,406,407
4	0.2	2	2vjyA_BS05_ALU	ALU(2)	15161764156

Table 3: TM- site results for AHAS

Rank	C-score[a]	Cluster size[b]	Rep Templ[c]	Mult templ[d]	Predicted binding site residues
1	0.36	15	5ahkA_BS02_FAD	FAD(14), FAB(1)	159,220,221,222,226,247,248,249,264,265,267,26,287,288,289,291,293,294,320,321,322, 325,338,33,340,414,415,432,433,434,436
2	0.25	6	1jscA_BS05_TPP	TPP(4), YF4(1), PYD(1)	31,32,33,57,80,83,87,120
3	0.21	3	1t9cB_BS01_1SM	1SM(1), CIE(1), 1TB(1)	34,35,109,110,113,118,119,120,169
4	0.19	3	2ji7A_BS03_ADP	ADP(3)	99,159,160,220,221,222,225,247,287,288,289,293,320,321,338,339,340
5	0.18	2	1jscA_BS02_2HP	2HP(2)	33,34,120

According to Tables 1 and 2, 91 Glu, 220 Ala, 224 Arg, 285 Val, 286 Gly, 309 Phe, 310 His and 31 Met, 32 Asp, 33 Ser, 57 Glu, 80 Val, 83 Leu, 87 Gln, 120 Asp are predicted as ligand binding site of PDC and AHAS, respectively.

The results of searching cavities by Molegro Virtual Docker showed that both enzymes have 5 cavities but the cavities of AHAS were clearly larger than PDC and also their positions were near each other (Figure 1). This finding was in coordination with TM and S site prediction results which predicted the 2 and 3 templates for clusters of PDC and 6 templates for cluster of AHAS.

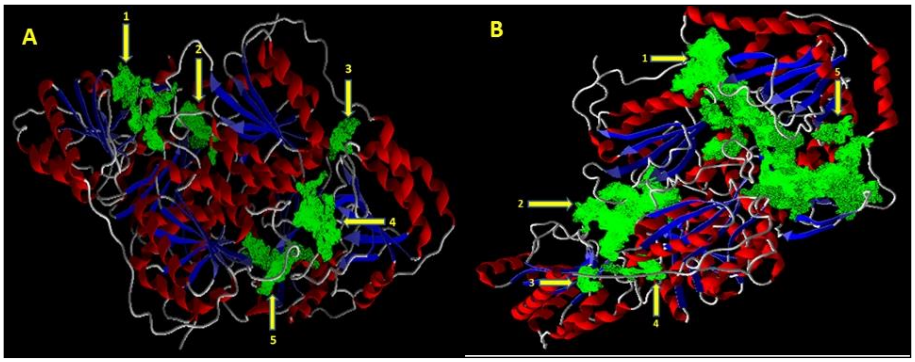

Figure 1: Detected cavities (shown with yellow arrows) of PDC (A) and AHAS (B) using Molegro Virtual Docker

In silico studies performed on sequences and 3D structures of PDC and AHAS in order to compare them and measure their activity. Alignment studies of these two protein sequences showed 24.8% similarity. Docking performed based on ligand binding sites predicted by Coach Site server.
The results interestingly showed close affinity of AHAS (-48.9182 kcal/mol) and PDC (-50.3954 kcal/mol) to pyruvate molecule. The affinity of two enzymes to product (L-PAC) and two by product (benzyl alcohol and benzoic acid) were determined using previous determined cavities. The results showed that AHAS by product production is higher (about 1.5 times) than AHAS while also its L-PAC production is (about 1.1 times) more than PDC (Table 4).

Table 4: Binding affinity of benzaldehyde to cavities of PDC and AHAS

Protein	Ligand	MolDock Score (kcal/mol)	HBond (kcal/mol)
PDC	Benzaldehyde (Substrate)	-50.3954	-3.461
AHAS	Benzaldehyde (Substrate)	-48.9182	-4.12458
PDC	Benzyl alcohol (By product)	-68.7523	-2.5101
AHAS	Benzyl alcohol (By product)	-101.368	-4.8735
PDC	Benzoic acid (By product)	-41.4454	-0.251
AHAS	Benzoic acid (By product)	-64.5849	-0.08581
PDC	L-PAC (Product)	-54.7766	-0.02192
AHAS	L-PAC (Product)	-60.5306	-5.31081

DISCUSSION

Various studies performed on yeast PDC enzyme in order to find its active sites, recognition and catalytic sites and amino acids which are important for its stability [11]. Searching for species with more active PDC, trying to performing point mutations on PDC sequence and looking for new enzymes has been done for enhancing bioconversion process. With respect to the studies performed by Park et al. which we used as reference for comparing production rate and yield, in the case of 99% benzaldehyde conversion, about 52% of conversion is related to benzyl alcohol and only about 17% of bioconversion resulted L-PAC [3]. The production yield of L-PAC and benzyl alcohol using AHAS calculated based on the production yields calculated by Park et al. and relative affinities calculated by molecular docking studies (Table 5).

Table 5: Estimated production yield of AHAS in compare to PDC

Enzyme name	L-PAC yield (%)	Benzyl alcohol yield (%)	Benzaldehyde conversion (%)
PDC	17	52	99
AHAS	18.78	76.6	99

CONCLUSION

Although benzyl alcohol production of AHAS is 1.4 times more than PDC but as the L-PAC production yield increased from 17 to 18.78%, it conclude that overall byproduct production by AHAS decrease to 88.22%.

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