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Homology Modeling and Blind Docking Approach Studies of Pig Heart Fumarase

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Abstract. The fumarase is an enzyme that could be used as target in drug design to treat infections by *Helicobacter pylori* and *Trypanosoma brucei*. In the first step, homology modeling was employed to build the 3D structure of pig heart fumarase (FUM P). Then, Q-Site Finder program was used to identify the potential binding sites of FUM P and fumarase of *Saccharomyces cerevisiae* (FUM Y). Further, molecular docking of arylderivatives substituted at the aromatic ring with an electron withdrawing or donating groups were evaluated on FUM P and on FUM Y to validate the homology model. The homology model of FUM P showed a structure very similar (70.33 % of identity in sequence) to the crystal structure of FUM Y. Some active sites were identified by Q-Site Finder server on FUM P and on FUM Y which could correspond to sites A and B. The docking results showed that some compounds were bonded at the site A on FUM P and FUM Y being those with electron withdrawing groups with more affinity on FUM P, suggesting that electronic effects are the more important ones during the recognition process by FUM P.
Key words: Homology, docking, arylderivatives, pig heart fumarase.

Resumen. La fumarasa es un enzima que podría ser usada como blanco en el diseño de drogas para el tratamiento de infecciones por *Helicobacter pylori* y *Trypanosoma brucei*. En este trabajo, se creó la estructura en tercera dimensión (3D) de la fumarasa de corazón de cerdo (FUM P) usando modelado por homología tomando como templete a la fumarasa de *Saccharomyces cerevisiae* (FUM Y). Después, se usó el programa Q-Site Finder para identificar los posibles sitios de unión de FUM P y FUM Y. Posteriormente, se efectuaron estudios de acoplamiento molecular de un grupo de derivados aril monosustituidos con grupos electrodonadores y electroatractores, sobre la FUM P y la FUM Y, con el fin de validar el modelo por homología. La estructura en 3D de la FUM P mostró alta semejanza estructural (70.33 %) con la estructura cristalina de la FUM Y. Algunos sitios de unión de FUM P identificados con el programa Q-Site Finder corresponden a los sitios A y B de FUM Y. Los resultados de *docking* mostraron que algunos compuestos interactúan en el sitio A de FUM P FUM Y, siendo los de mayor afinidad aquellos que tienen electroatractores sobre FUM P, lo que sugiere que los efectos electrónicos son los más importantes durante el proceso de reconocimiento para FUM P.
Palabras clave: Homología, acoplamiento, arilderivados, fumarasa de corazón de cerdo.

Introduction

Fumarase (fumarate hydratase, E.C. 4.2.1.2), which functions as a component of the Krebs cycle, catalyses the reversible stereospecific addition of water to fumarate to form L-malate [1]. In prokaryotes, there are two classes of fumarase. Class I is heat-labile, Fe^{+2} dependent, is dimeric with subunits of 60 kDa, and does not have a sequence homology to the eukaryotic fumarase. Class II is heat-stable, Fe^{+2} independent, is tetrameric with subunits of 50 kDa, and has an extensive homology to the eukaryotic fumarase. The three-dimensional structure of several types of class II fumarase has been obtained by crystallographic studies, but two have been widely studied: one from *Escherichia coli* (FUM C) and the other from *Saccharomyces cerevisiae* (FUM Y) [2,3]. In the same studies, it was possible to identify the catalytic site (A) and a possible allosteric site (B) [4]. Site A contains Lys324 and Asn326 amino acid residues, which play an important role in the catalytic activity because they recognize the carboxyl groups of the ligands and stabilize the carbanion species formed in the transition state [2].

Another class II fumarase obtained from pig heart and refereed here as FUM P [5], has a similar primary structure as FUM Y and FUM C. Although FUM P has not yet been crystallized, its tertiary structure was proposed in this work by homology modeling.

To explore the binding site and the interaction of some arylderivatives (stereoisomer aryl-*E*-butenoic acids, structurally related to fumarate) in sites A and B of FUM P and FUM Y, docking simulation studies were done using blind docking procedure in order to explore their recognition by some amino acids involved in the binding site of these enzymes, paying special attention to the electronic effects, which are related to Hammet effects (r), atomic charges and frontier orbitals (HOMO-LUMO). And also, other chemical properties (partition coefficient and steric effects) of the compounds were taken into account

Methods

2.1. Modeling

A three-dimensional structural model for FUM P is currently unavailable. Therefore, a homology model of this enzyme was constructed based on the three-dimensional structure of FUM Y (PDB code: 1yfm).

BLAST and Protein Data Bank (PDB) were used to search the sequence of FUM P, which has high sequence similarity with FUM Y. The homology model of FUM P was generated using the SWISS MODEL server [6-8]. Then, hydrogens at ~pH7.4 of FUM Y and FUM P were added and then minimized in 500 steps by using the steepest descendent protocol employing GROMOS96 43B1 parameters that are implemented in the Swiss-PDBViewer version 3.7. Finally FUM Y and FUM P were evaluated from their Ramachandran diagrams using Swiss-PDBViewer program [6].

The three-dimensional structure of the ligands in their minimum-energy conformation was obtained by means of the Gaussian 98 software by using HF/6-31G* level [9].

To identify the ligand recognition binding sites, docking simulations were done based on the structure of both enzymes tested. First, all the possible rotable bonds, the torsional degrees and the atomic partial charges (Gasteiger) of the ligands were assigned by using AutoDock tool. The Kollman charges for all atoms of the enzyme were assigned by using the AutoDock tool, a program included in AutoDock 3.0.5. Then, the ligands were docked under the blind docking (a rectangular grid box was constructed over all protein ($126 \times 126 \times 126$ Å^3) with the grid points separated by 0.375A) procedure on both enzymes by using the AutoDock software under the hybrid Lamarckian Genetic Algorithm, with an initial population of 100 randomly placed individuals and a maximum number of energy evaluations of 1.0×10^7. Resulting docked orientations within a root-mean square deviation of 0.5 Å were clustered together. The lowest energy cluster returned by AutoDock for each compound was used for further analysis. All other parameters were maintained at their default settings [10].

Finally, the electronic effects of the ligands were taking up from the literature [11], whereas their partition coefficient and steric effects were calculated. The first was calculated online by using the molinspiration site (Molinspiration, Bratislava, Slovak Republic) [12]. Then, the steric effect was calculated by using the electrostatic potential as was reported by Suresh [13].

Additionally, the Q-Site Finder server was used to identify the possible active sites; this program is available at online [14]. All protein visualizations were achieved by using Visual Molecular Dynamics (VMD) program [15].

Results and discussion

The homology model of FUM P has high similarity with several crystal structures reported previously for other fumarases (Fig. 1). It shares a 70 % similarity with FUM Y and therefore both enzymes were evaluated by Ramachandran maps that showed very similar regions (data not shown). Figure 2 shows that FUM P (B) has the same shape as FUM Y (A) when they were aligned (C). As can be seen in the same figure, each monomer of fumarase has three domains (D): D1 at the bottom, D2 in the core of the tetrameric enzyme forming a unique five-helix bundle, and D3 at the top of the enzyme [3]. The results from Q-site Finder server showed that FUM P has several sites very similar to FUM Y (see Fig. 3). It is well known that among domains there are sites where the ligands can be fitted. Hence, we decided to use the Q-site Finder program in order to explore the sites A and B for FUM P and FUM Y [4]. However, to confirm this hypothesis one needs more theoretical and biological experiments are desirable. The docking studies have been one of the most used tools to identify active sites on proteins.

Therefore, to identify sites A and B [2,4] in these enzymes, docking studies were done using several arylderivatives as ligands, which have structural relations with fumarate. Such arylderivatives have electron withdrawing or electron donating substituents at the aromatic ring (see Table 1).

```
Extracting template sequences

Running pair-wise alignments with target sequence
Sequence identity of templates with target:

1yfm_.pdb: 70.33 % identity
1fuqA.pdb: 55.43 % identity
1fupA.pdb: 55.43 % identity
1fuoA.pdb: 55.43 % identity
1kq7A.pdb: 55.33 % identity
1furA.pdb: 55.33 % identity
2fusA.pdb: 55.33 % identity
1fupB.pdb: 56.57 % identity
1fuoB.pdb: 56.57 % identity
1fuqB.pdb: 56.57 % identity
1kq7B.pdb: 56.47 % identity
2fusB.pdb: 56.47 % identity
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Fig. 1. Alignment of pig heart fumarase (FUM P) with *Sacharomyces cerevisiae* fumarase (FUM Y) used for building the homology model.

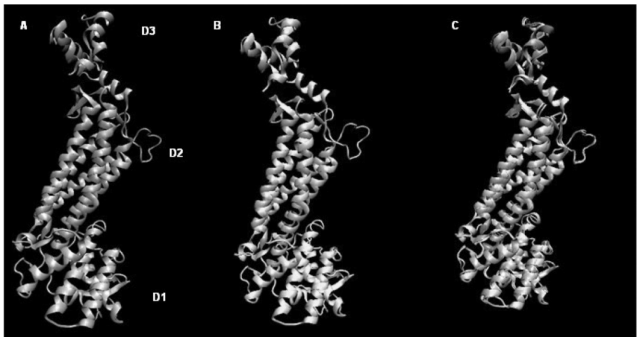


Fig. 2. Stereo view of FUM Y (A), homology model of pig heart fumarase (B) and both aligned (C).

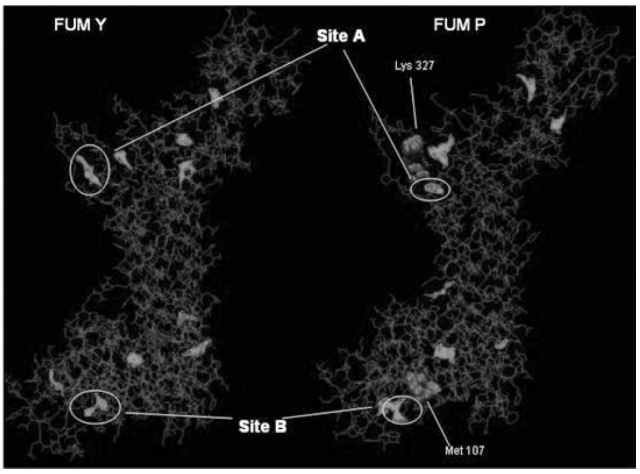


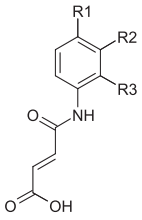
Fig. 3. Binding sites identified by Q-site Finder program of FUM Y and FUM P.

Depending on the recognition site, these molecules could function as fumarase inhibitors in microbial diseases caused by *H. pylori* [16,17]. A classic example is *H. pylori* treatment, where several combinations of several drugs (furazolidone, amoxicillin, bismuth subcitrate, omeprazole) have been employed, but with poor results [18]. This could be because *H. pylori* has developed drug resistance [19], due to these drug-targets show polymorphism [20]. Whereas that fumarase is an enzyme very important that scarcely can modified due to its importance at tricarboxylic acid cycle of *H. pylori* [21]. Furthermore, it is important to identify the treatment to eradicate the *H. pylori* given that it is associated with several gastric diseases, such as cancer, gastritis, and peptic ulcer among others [22]. In addition, it is well-know that this enzyme is essential to *Trypanosoma brucei* (a parasitic protist responsible for sleeping sickness in humans) to convert most of the consumed glucose into excreted succinate, by succinic fermentation [23].

The docking studies showed that the ligands tested bind at active site A of FUM P which is located in the near the second domain, the same region as for FUM Y and FUM C (Fig. 4). This can be explained by the fact that fumarase enzymes have three regions with increased identity that mark the location of the active site. These regions are integrated by 129-146, 181-200, and 312-331 residues characterized by having certain chemical properties to fit the ligands [2,4].

The crystallographic studies show that FUM C has residues from Asn326, Lys324, and Asp331 in site A, whereas FUM Y has Asn351, Lys349, and Glu356 where several ligand were fitted with high affinity for the later enzyme (Table 1). Docking studies with arylderivatives on FUM P show that these compounds were recognized in site A (Lys327, Glu334, Glu318, Glu316, Asn317) as can be seen in Fig. 4 and 5. This site has the same residues as that of FUM Y and FUM C.

Table 1. K_d and ΔG values from the Arylderivatives docked on FUM P and FUM Y.



Compound	**R1**	**R2**	**R3**	b1	b2	K_d (μM) on FUM P	ΔG (kcal/mol) on FUM P	*Kd* (μM) on FUM Y	ΔG (kcal/mol) on FUM Y
1a	H	H	H	1.78	3.47	10.00	-2.72	1.29	-8.03
1b	Cl	H	H	2.64	3.53	1.77	-3.75	0.58	-8.50
1c	H	Cl	H	—-	—-	5.79	-3.05	0.83	-8.29
1d	H	H	Cl	2.64	3.53	5.06	-3.13	0.80	-8.31
1e	F	H	H	2.64	3.53	2.49	-3.55	1.10	-8.12
1f	H	F	H	—-	—-	10.40	-2.70	>	—-
1g	H	H	F	2.64	3.04	4.49	-3.20	0.66	-8.42
1h	OH	H	H	2.25	3.07	3.37	-3.37	0.98	-8.19
1i	H	OH	H	—-	—-	7.85	-2.87	1.45	-7.96
1j	H	H	OH	2.25	3.17	2.89	-3.46	0.46	-8.64
1k	OCH_3	H	H	1.78	3.47	2.57	-3.53	1.35	-8.00
1l	H	OCH_3	H	—-	—-	5.99	-3.03	14.92	-6.58
1m **1m**	H	H	OCH_3	—-	—-	4.13	-3.25	1.10	-8.12

[b1]Bond length 1 in Å, [b2]Bond length 2 in Å.

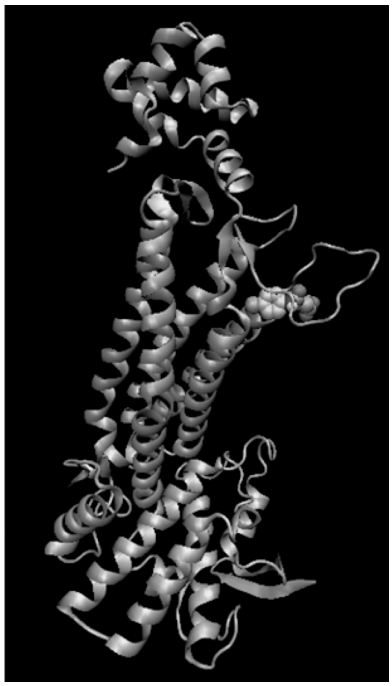


Fig. 4. Compound **1b** binding in the site A near to the second domain of FUM P.

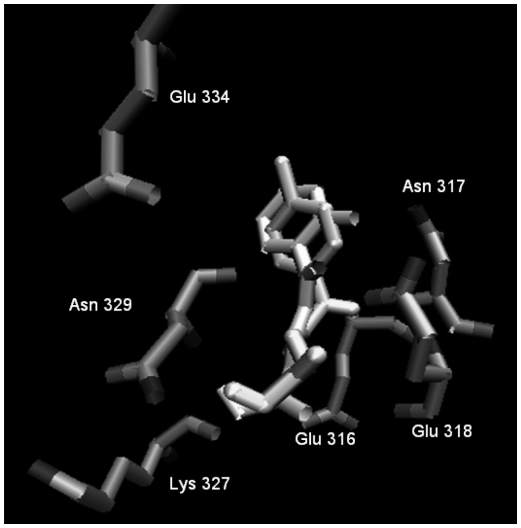


Fig. 5. Interactions of the compound **1b** (yellow) and **1g** (white) with catalytic residues in the site A of FUM P. The compound interacts principally with Asn329 and Glu318.

In addition, the docking results prove that the arylderivatives with substituents at *ortho* or *para* positions bind at site A of FUM P making several interactions with Ans329 and Glu318 (Fig. 6). These results demonstrated that the interaction of the arylderivatives at site A is affected by the kind of substituent located in the ring, whether it be electron withdrawing (**1b, 1d, 1e, 1g)** or electron donating (**1h, 1j, 1k, 1m**). For instance, the compounds with Cl or OCH_3 groups located in *ortho* (**1d, 1m**) or *para* (**1b, 1k**) position generate a negative or positive charge, respectively, to the *ipso* carbon bonded to the amide (Table 2). Such charges can be stabilized by the nitrogen atom of the amide group and through an extension of the conjugation by the π system of the arylderivatives. The hydrogen atom of the amide group of the arylderivative can form a hydrogen bond (b1) (see Fig. 6) with the oxygen atom (acting as an acceptor) of the amide carbonyl group of the backbond of the Glu318 residue. When the charge is stabilized by the amide nitrogen atom, the resonance effects of this bond could help to form a second hydrogen bond (b2) (see Fig. 6) between the oxygen atom of the carbonyl group of arylderivatives and the proton of the backbone amide of Asn329. Also, the formation of a third hydrogen bond (b3) (see Fig. 6) was observed between the oxygen atom of the carbonyl group of the carboxylic acid and the amino group of Lys327 or with Asp329 in some cases.

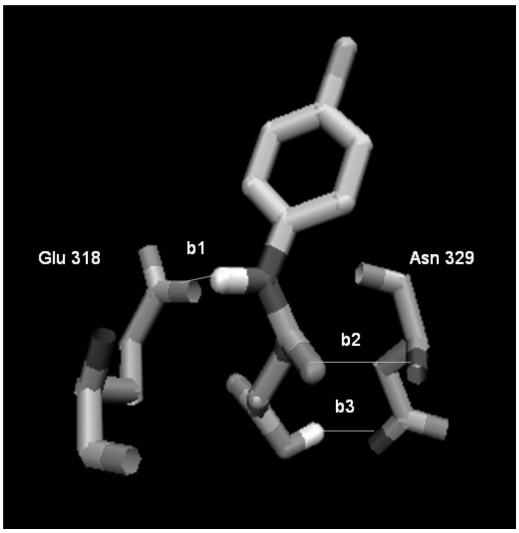


Fig. 6. Interactions and distances between the compound **1b** and the Asn329 and Glu318.

Even though the compounds with electron withdrawing (**1b, 1d, 1e, 1g)** and electron donating (**1h, 1j, 1k, 1m**) groups in *ortho* or *para* positions in the aromatic ring were recognized at site A, the hydrogen bond length between the oxygen atom of the amide group of the arylderivatives and the hydrogen of the backbone of Asn329 (see Fig. 6) was shorter with electron donating groups than with electron withdrawing groups (data not shown).

The K_d and ΔG values obtained by docking studies show that the arylderivatives have more affinity for FUM P (in site A) when they have an electron withdrawing rather an electron donating substituent (Table 1). Furthermore, it is important to recognize that the electronic effects could be significant in binding the ligands by fumarase as is mentioned here. The compounds with substitution in *para* and o*rtho* position were

recognized in the site A; however, it is worth noting that the affinity was greater when the ring had a substituent in *para* position, as can be seen for the compounds **1b**, **1e**, **1h,** and **1k**. Also it is important to mention that the substitution of the ring improves the affinity between the enzyme and the compound because the compound without such a substituent has less affinity (10 mM) than the compound with Cl (1.7 mM). As one can be seen in Table 2, despite of the is well-known that the other parameters (π and hindrance effects) play a very interesting role in ligand recognition [24], in this case, apparently the arylderivatives could not affect the affinity for both proteins. These mean that these kind of enzymes recognize the ligand independently of hindrance effects due to their site is close to surface contrary to other enzymes such as chloloroperoxidase [25]. In addition, the lipophylic effects aparently do not interfere in the ligand recognition on fumarase which could be due to, these site are no hydrophobic as occur with other enzymes such as Glutathione S-Tranferase family [26].

The arylderivatives with a substituent at *meta* position (**1i, 1l, 1f**) were bonded at a different place (data not shown) in comparison with the arylderivatives that have a substituent at *ortho* or *para* positions of the aromatic ring, possibly because the former arylderivatives did not originate a charge on the *ipso* carbon atom bonded to the amide group. Therefore, it can be said that the resonance effects are more important than the inductive effects, because the OCH_3 in *meta* position did not bind at the same site.

According to this study, the electronic effects could play a very important role in the drug recognition process. In this sense, some quantum-chemical descriptors such as HOMO and LUMO energies could be used to explore the electronic properties more carefully and also it yields data more safe as have been explored for other systems [27,28]. The HOMO and LUMO energies were evaluated for all arylderivatives (see Table 2). As can be seen in this Table, the ligands have high HOMO energies which confirm that the high electron density increase them be recognized at site A. This means that the site A could be electronically poor. On the other hand, the other principal molecular physicochemical parameters involved in ligand recognition such as the partition coefficient and the steric effects could not participate for this enzyme, according these results. Thus, suggesting that the atomic charges regulate the ligand recognition. So, we study the atomic charges of some atoms conserved for all compounds, identifying that the nitrogen and *ipso* carbon atoms play a very important role in the ligand recognition of FUM P, we have get it from optimization geometry results identifying that their charge is maintained negatively and it increases with electro donating group (**1k**) which was reflected in the K_d values.

The site B has been found in FUM C and FUM Y and it has been postulated as an allosteric site [29]. In FUM C, the allosteric site is integrated by Asn131, Glu132, Arg126, Hys129, and Ser137 residues that are located in the same subunit near the first domain. Even though this site has been identified in FUM C and FUM Y [2,4], its role during catalysis is unclear [2]. The results obtained using Q-site Finder server showed several actives sites for FUM Y, (Fig. 3). Some of they have been identified as two important active sites, A and B. However, by using the docking procedure, only one compound was fit in the site B on this enzyme. It could be due to the compounds have a structure very different to L-malate which is the ligand recognized at site B [5]. Furthermore, to identify and study this site could be tested some compounds with these chemical properties. Although FUM P has been widely studied by means of kinetic methods with several ligands [5], its site B has not been identified clearly. The docking results show that any arylderivatives were bonded near the first domain where it could be possible that FUM P has a site B. In this way, other studies could be achieved using L-malate derivatives in order to explore the site B. Thus is due to that by using the Q-site Finder server, there is a site which could

Table 2. Atomic charge values obtained by Mulliken population analysis, HOMO and LUMO energies and ρ, π and steric effects.

Compounds	HOMO (eV)	LUMO (eV)	Nitrogen (Mulliken charges)	ρ	π	$MESP_{steric}$ (kcal/mol)
1a	-0.3105	0.0636	-0.939	0	0	0
1b	-0.3154	0.0574	-0.941	0.23	0.678	-0.005
1c	-0.3224	0.0574	-0.942	0.37	0.654	-0.014
1d	-0.3216	0.0596	-0.967	—-	0.630	0.050
1e	-0.3135	0.0608	-0.939	0.06	0.164	-0.042
1f	-0.3206	0.0584	-0.943	0.34	0.139	-0.018
1g	-0.3190	0.0617	-0.943	—-	0.116	-0.017
1h	-0.2943	0.0656	-0.938	-0.37	-0.479	-0.033
1i	-0.3054	0.0612	-0.945	0.12	-0.511	-0.035
1j	-0.3068	0.0591	-0.964	—-	-0.267	0.012
1k	-0.2910	0.0665	-0.938	-0.27	0.057	-0.073
1l	-0.3010	0.0629	-0.944	0.12	0.033	-0.036
1m	-0.3016	0.0632	-0.975	—-	0.009	-0.066

be site B (Fig 3) due to its closenees to Met 107 where this site is located [5]. And also, this program shows that the site A radius of FUM Y is greater than of FUM P. This could explain the highest affinity of the compounds by FUM Y. This data suggest, that despite of their high similarity, could be possible design drugs with more selectivity.

In conclusion, the homology model showed that FUM P has a high structural similarity with FUM Y, as expected from its sequence identity. Docking studies demonstrated that FUM P has a site A in the same place as FUM C and FUM Y, close to the second domain. The arylderivatives with a substituent in *ortho* or *para* positions were fitted to this site on FUM P judging by docking studies, forming hydrogen bonds principally with the Asn329 and Glu318 residues. Therefore, these compounds could act as competitive inhibitors. On the other hand, any arylderivative were bonded close to the first domain where the site B has been identified in FUM C and FUM Y. The results show that it is possible to obtain good results by docking studies with monomers of the tetrameric enzyme. However, more studies could be done in relation to the binding of the same compounds on the tetrameric enzyme. Finally, it is important mention that a site in FUM P was identified as equivalent to site B reported in other fumarases.

Acknowledgements

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