UNIVERSIDADE DE SÃO PAULO

FACULDADE DE CIÊNCIAS FARMACÊUTICAS DE RIBEIRÃO PRETO

Structural and biochemical characterization of *Schistosoma mansoni* class II fumarate hydratase enzyme

Caracterização estrutural e bioquímica da enzima fumarato hidratase classe II de *Schistosoma mansoni*

IARA AIMÊ CARDOSO

Ribeirão Preto
2019

UNIVERSIDADE DE SÃO PAULO

FACULDADE DE CIÊNCIAS FARMACÊUTICAS DE RIBEIRÃO PRETO

IARA AIMÊ CARDOSO

Structural and biochemical characterization of *Schistosoma mansoni* class II fumarate hydratase enzyme

Caracterização estrutural e bioquímica da enzima fumarato hidratase classe II de *Schistosoma mansoni*

Master dissertation presented to the Graduate Program of School of Pharmaceutical Sciences of Ribeirão Preto/USP for the degree of Master in Sciences.

Concentration Area: Chemistry and Biological Physics

Supervisor: Profa. Dra. Maria Cristina Nonato

Corrected version of master dissertation presented to the Pharmaceutical Sciences Graduate Program, on 08/11/2019. The original version is available at School of Pharmaceutical Sciences of Ribeirão Preto/USP.

Ribeirão Preto
2019

Cardoso, Iara Aimê

Structural and biochemical characterization of *Schistosoma mansoni* class II fumarate hydratase enzyme. Ribeirão Preto, 2019.

64 p.; 30 cm.

Master dissertation presented to the Graduate Program of School of Pharmaceutical Sciences of Ribeirão Preto/USP for the degree of Master in Sciences. Concentration Area: Chemical and Biological Physics

Supervisor: Nonato, Maria Cristina

1. Fumarate hydratase 2. *Schistosoma mansoni* 3. X-ray crystallography 4. Kinetic assay

APPROVAL PAGE

Iara Aimê Cardoso

Structural and biochemical characterization of *Schistosoma mansoni* class II fumarate hydratase enzyme

Master dissertation presented to the Graduate Program of School of Pharmaceutical Sciences of Ribeirão Preto/USP for the degree of Master in Sciences.

Concentration Area: Chemistry and Biological Physics

Supervisor: Prof. Dr. Maria Cristina Nonato

Approved on:

Examiners

Prof. Dr. _____

Institution: _____ Signature:_____

Prof. Dr. _____

Institution: _____ Signature:_____

Prof. Dr. _____

Institution: _____ Signature:_____

Prof. Dr. _____

Institution: _____ Signature:_____

Abstract

Cardoso, I. A. **Structural and biochemical characterization of *Schistosoma mansoni* class II fumarate hydratase enzyme.** 2019. 64f. Dissertation (Master). Faculdade de Ciências Farmacêuticas de Ribeirão Preto – Universidade de São Paulo, Ribeirão Preto, 2019.

Schistomiasis is a neglected tropical disease caused by trematodes worms from the genus *Schistosoma*. Schistosomiasis is the second most devastating parasitic disease after malaria. The disease has a high economic burden and affects mainly poor population without access to proper sanitation. Praziquantel is the only drug approved for the treatment of schistosomiasis and resistance is already reported. Fumarate hydratases or fumarases are enzymes that catalyze the reversible hydration of fumarate to L-malate. This enzyme participates in DNA repair and important metabolic processes such as the urea and the tricarboxylic acid cycles. Fumarases are divided in two classes, and *Schistosoma mansoni* possess both, being class I localized in mitochondria, while class II is cytosolic. The fundamental role of fumarases in the metabolism make them potential target for drug design against schistosomiasis. This work describes, for the first time, the cloning, expression and purification protocol for the class II fumarate hydratase from *Schistosoma mansoni* (*Sm*FH_{II}). In order to estimate the contribution of the reverse reaction, the enzyme was kinetically characterized using both substrates concomitantly. *Sm*FH_{II} was shown to follow a Michaelis-Menten mechanism of catalysis with k_{cat}^{MAL} of 19 $mM^{-1}s^{-1}$ and k_{cat}^{FUM} of 49 $mM^{-1}s^{-1}$, and K_m^{MAL} of 0.56 mM and K_m^{FUM} of 0.15 mM. Differential scanning fluorimetry (DSF) performed under different chemical environments shows that the highest thermal stability is reached at pH 7.5 and at higher ionic strength. The significant thermoshift observed for *Sm*FH_{II} in presence of well known ligands makes DSF the adequate technique for ligand screening. *Sm*FH_{II} structure in complex with L-malate was determined by single crystal X-ray diffraction, at 1.85 Å resolution. A new construct [*Sm*$FHII_{(\Delta 263\text{-}277)}$] lacking the additional portion only found in trematode worms was also evaluated by kinetic and DSF experiments. Although not essential for activity, the results suggest that the removal of this region impacts on protein stability and may has influence on L-malate catalysis. The differences between *Sm*FH_{II} and human fumarase are distributed all over the structure, and could be explored to design new selective inhibitors.

Keywords: Fumarate hydratase, *Schistosoma mansoni*, X-ray crystallography, kinetic characterization.

Resumo

Cardoso, I. A. **Caracterização estrutural e bioquímica da enzima fumarato hidratase classe II de *Schistosoma mansoni*.** 2019. 64f. Dissertação (Mestrado). Faculdade de Ciências Farmacêuticas de Ribeirão Preto – Universidade de São Paulo, Ribeirão Preto, 2019.

A esquistossomose é uma doença tropical negligenciada causada por parasitas trematódeos do gênero *Schistosoma*. A esquistossomose é a segunda doença parasitária mais devastadora do mundo, atrás apenas da malária. A doença tem um alto impacto econômico, afetando principalmente a população pobre sem acesso a saneamento adequado. Praziquantel é o único medicamento aprovado para o tratamento da esquistossomose e já existem relatos de parasitas resistentes a esse fármaco. Fumarato hidratases ou fumarases são enzimas que catalisam a hidratação reversível de fumarato em L-malato. Essa enzima participa do reparo ao dano do DNA e de processos metabólicos importantes, como os ciclos da uréia e do ácido tricarboxílico. As fumarases são divididas em duas classes e o *S. mansoni* possui ambas, sendo a classe I mitocondrial, enquanto a classe II é citosólica. O papel fundamental da fumarase no metabolismo faz dela um alvo potencial para o planejamento de fármacos contra a esquistossomose. Este trabalho descreve, pela primeira vez, o protocolo de clonagem, expressão e purificação da fumarato hidratase classe II de *Schistosoma mansoni* (*Sm*FH_{II}). De forma a estimar a contribuição da reação reversa, a enzima foi caracterizada cineticamente utilizando os dois substratos concomitantemente. A *Sm*FH_{II} demonstrou seguir o mecanismo de catálise de Michaelis-Menten, tendo um k_{cat}^{MAL} de 19 $mM^{-1}s^{-1}$ e k_{cat}^{FUM} de 49 $mM^{-1}s^{-1}$, e K_m^{MAL} de 0,56 mM e K_m^{FUM} de 0,15 mM. Fluorimetria de varredura diferencial (DSF) realizada em diferentes ambientes químicos demonstrou que a maior estabilidade térmica da proteína é alcançada em pH 7,5 e também com o aumento alta força iônica, além de ser uma técnica útil para a triagem de ligantes. A estrutura da *Sm*FH_{II} foi determinada por difração de raios-X de monocristal, com uma resolução de 1,85 Å. Uma nova construção [*Sm*$FH_{II(\Delta 263\text{-}277)}$] sem a porção adicional, encontrada apenas em vermes de trematódeos, também foi avaliada por ensaios cinéticos e de DSF. Embora não seja essencial para a atividade enzimática, os resultados sugerem que a remoção dessa região afeta a estabilidade da proteína e pode ter influência na catálise do L-malato. As diferenças entre *Sm*FH_{II} e fumarase humana estão distribuídas por toda a estrutura e podem ser exploradas para delinear novos inibidores seletivos.

Palavras-chave: Fumarato hidratase, *Schistosoma mansoni*, cristalografia de raios-x, caracterização cinética.

1. INTRODUCTION

1.1. Neglected tropical diseases

The term neglected tropical diseases (NTDs) emerged in the 20th century to describe a group of infectious diseases that were found endemic in the tropical and subtropical areas of the globe. NTDs specially affect the poorest population with no adequate sanitation and constant contact with infectious vectors[1; 2]. These diseases cause important morbidity and mortality, being a serious public health problem in many countries of Africa, Asia, and Latin America[3]. NTDs also reflect the scarce investments in research and development of new therapies or programs to their control[2].

Currently, the World Health Organization (WHO) recognizes 20 diseases as NTDs, including schistosomiasis, Chagas disease, dengue and chikungunya, leishmaniasis, among others. These diseases affect more than one billion people, which represents one sixth of the world population[1].

Neglected tropical diseases cause huge human suffering and numerous cases of death, remaining a serious impediment to socioeconomic development[1]. The indifference to these diseases only aggravates the scenario of global inequality, which shows the need to searching new drugs that are more effective and accessible to the low-income populations, as well as to encourage prevention and control programs for NTDs.

1.2. Schistosomiais

1.2.1. History

Schistosomiasis (also known as bilharziasis) is a parasitic neglected disease caused by blood flukes (trematode worms) of the genus *Schistosoma*, that can cause acute and chronic disease[4]. The disease is recognized as one of the oldest still existing infections. *Schistosoma haematobium* eggs were found in Egyptian mummies as old as 5,000 years[5], while *Schistosoma mansoni* eggs were found in a latrine dated AD 1450-1550 in France[6]. The parasite was first described by the german parasitologist Theodor Maximilian Bilharz in 1851 during an autopsy performed at Cairo, and it was firstly named *Distomum haematobium*.[7; 8]

In Brazil, the first identification of *S. mansoni* worms was made by the doctor and researcher Manuel Augusto Pirajá da Silva in 1908[9]. The introduction of schistosomiasis in Brazil occurred through the trade of slaves originating from the west coast of Africa, who entered the country mainly through the ports of Recife and Salvador to work in sugarcane crops. The disease initially spread throughout the northeastern of Brazil, forming an extensive transmission area along the states of Rio Grande do Norte and Bahia. In the 18th century, with the decline of sugar production in the Northeast and the beginning of the gold and diamond cycle, an intense migratory flow introduced the disease in Minas Gerais state, and after that to the others states of Southeast region[10].

1.2.2. Epidemiology

Schistosomiasis is second only to malaria as most devastating parasitic neglected disease in the world[3]. The disease has been reported from 78 countries, which affects almost 240 million people worldwide, and more than 700 million people live in endemic areas[11]. The disease is prevalent in tropical and subtropical areas, and especially affects poor communities with no access to adequate sanitation and drinkable water[12]. Furthermore, according to the number of disability-adjusted life years (DALYs), an important measure of overall disease burden, schistosomiasis resulted in losses of 1.4 million years of full health among global population in 2017[13].

In Brazil, schistosomiasis is distributed over 19 states (**Figure 1**) and affects about 1.5 million individuals, with the highest incidence in the Northeast and Southeast regions[14]. Schistosomiasis in Brazil represents a great economic burden, and its major impact is related to productivity loss. It was estimated in Brazil a total cost of US$ 41.7 million in 2015, with 94.6% belonging to indirect costs as leave, disease aid and premature dead[15].

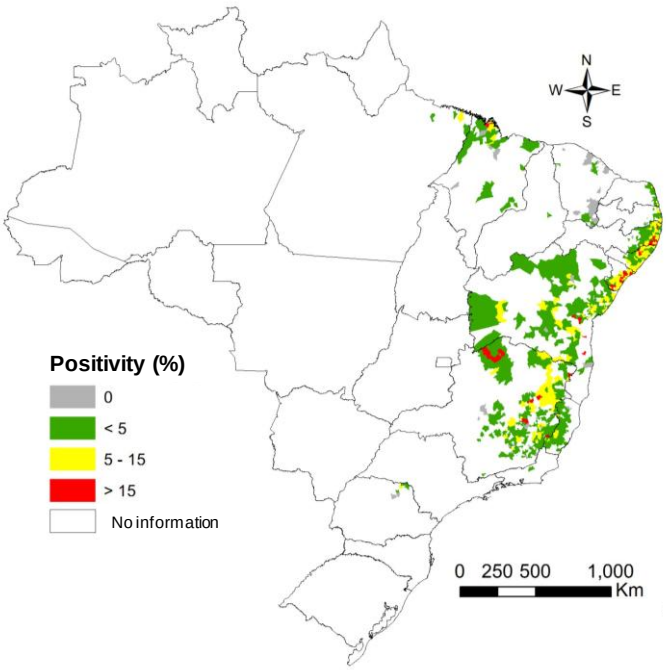

Figure 1. Distribution of schistosomiasis, according to the positivity range - Brazil, 2010 - 2015. (Extracted from Portal da saúde – SUS[16]).

1.2.3. Infection and transmission

Schistosomiasis is caused by the infection of dioecious trematode platelminths, and six species are capable of causing the disease in humans: *S. mekongi*, *S. intercalatum*, *S. guineensis*, *S. mansoni*, *S. haematobium* and *S. japonicum*. The last three species cited are responsible for the largest number of disease cases, and only *S. mansoni* is found in Brazil.

The transmission cycle (**Figure 2**) begins when *Schistosoma* eggs are eliminated with feces or urine, depending on the species. The eggs hatch, releasing the ciliated larval form, called miracidium, which swim and penetrate specific snail intermediate hosts (snail of the genus *Biomphalaria*, in Brazil). The stages in the snail include the generations of sporocysts and the asexual reproduction generating cercariae. The cercariae are released from the snail and penetrate the skin and/or mucous membranes, losing their forked tail and becoming schistosomulae. The schistosomulae migrate through venous circulation to the lungs and the heart till reach the liver and develop into sexed forms. Male and female adult worms exit the liver via the portal vein system when mature, copulate and reside in the mesenteric venules. *S. haematobium* most often inhabits in the vesicular and pelvic venous plexus of the bladder, but can also be found in the rectal venules. Finally, females release the

eggs in the small venules of the portal and perivesical systems. The eggs are moved through the lumen of the intestine (*S. mansoni*, *S. japonicum*, *S. mekongi*, *S. intercalatum/guineensis*) or the bladder and ureters (*S. haematobium*), and are eliminated with feces or urine, restarting the cycle[3].

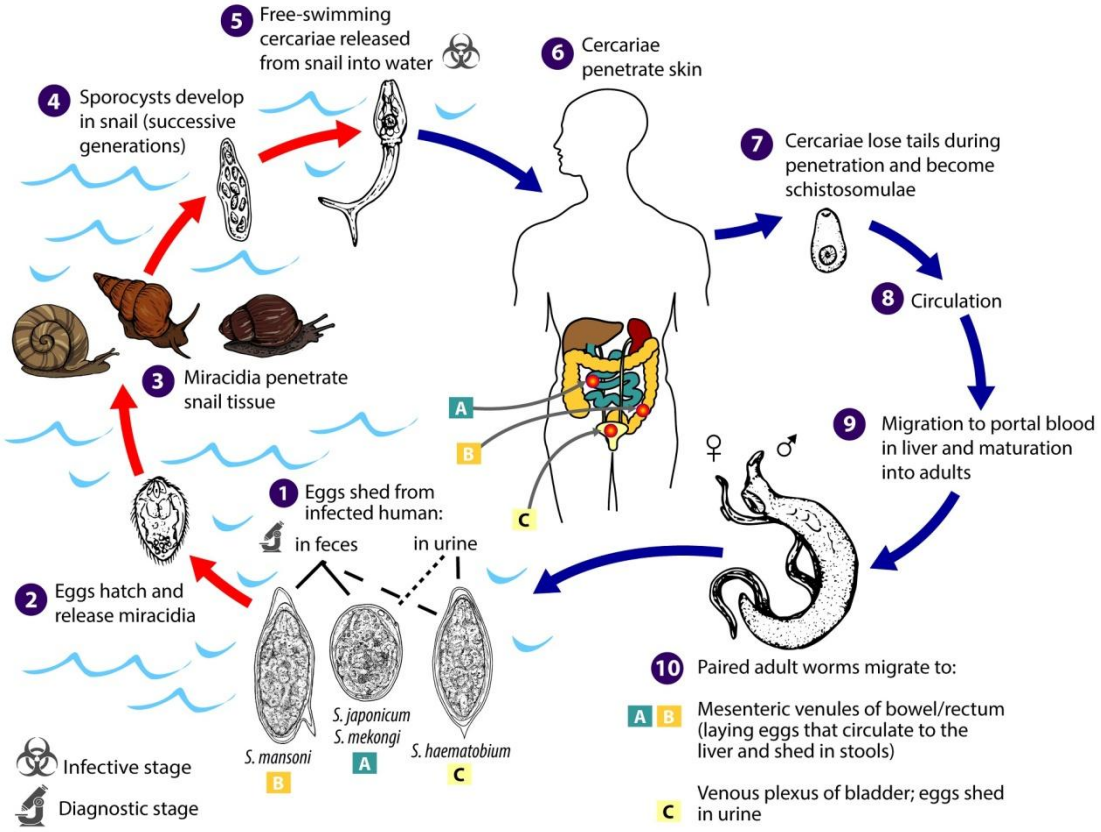

Figure 2. *Schistosoma* spp. life cycle scheme. (Extracted from Centers for Disease Control and Prevention[3]).

Male adult parasites are robust, tuberculate and measure approximately 6-12 mm in length and 10 mm in width (**Figure 3**). The female has a cylindrical body, slender and longer than male (7-17 mm in length)[3] (**Figure 3**).

Figure 3. Schistosome worm pair. (Extracted from the Trustees of the Natural History Museum).

1.2.4. Symptoms, diagnosis, and treatment

Most people do not have any symptoms when they are first infected. Within a few days after infection, individuals may develop a rash or itchy skin, called cercariae dermatitis, characterized by reddish micropapules that resemble insect bites. About one to two months later, nonspecific symptoms appear, such as fever, headache, anorexia, nausea, asthenia, myalgia, cough and diarrhea, characterizing schistosomiasis in the acute form[3; 14].

The symptoms of schistosomiasis are not caused directly by the worms themselves, but due to the reaction of the immune system to the eggs. Eggs that are not eliminated by feces may lodge in the intestine, liver or bladder, causing inflammation and fibrosis[3].

If not treated, schistosomiasis become chronic and can persist for years. In the chronic phase, the disease may have different manifestations, and the liver is the most frequently affected. Signs and symptoms of chronic schistosomiasis include: abdominal pain, enlarged liver, bloody stools, bloody urine, and problems passing urine. In the most severe form of the disease, eggs lodge in the brain or spinal cord and can cause seizures, paralysis, or inflammation of the spinal cord[3; 14].

The most commonly technique used for diagnosing schistosomiasis is the Kato-Katz method, which consists of identifying and counting the parasite's eggs in the feces or urine samples. Such method is a quick and simple way of diagnosis of the disease. Also, a serologic test (antibodies and/or antigens detected in blood samples) can be performed to confirm the infection[3; 12; 14].

Praziquantel is the only treatment for human schistosomiasis recommended by the World Health Organization. However, it fails to prevent immediate reinfection, a common feature of the disease for people who live in tropical areas with poor sanitary conditions.

Moreover, the emergence of drug resistant and praziquantel-insensitive parasites has increased due to its continuous and large-scale use for almost four decades[17; 18; 19]. In Brazil, praziquantel is available as 600 mg tablets and it is administered orally as a single oral dose of 50 mg/kg for adults and 60 mg/kg for children[10].

Although praziquantel have been used as large-scale treatment, also called preventive chemotherapy, its molecular mechanism of action remains unclear. Some studies suggest that schistosome worms calcium ion channels are the target of praziquantel, causing a rapid Ca^{2+} influx and severe spasms and paralysis of the worms' muscles[20; 21; 22]. Other study demonstrated that praziquantel can cause morphological alterations (vacuolation and blebbing) near on the worm surface[20; 23].

Although there are no current commercially available vaccines for schistosomiasis, there are few initiatives for vaccine development in different stages of clinical trials [24; 25; 26; 27]. One example is the Sm14/GLA-SE schistosomiasis vaccine, which has successfully completed phase I and phase IIa clinical trials, with phase IIb/III trials in progress. The vaccine was formulated with recombinant protein Sm14 and with glucopyranosyl lipid A (GLA) adjuvant in an oil-in-water emulsion (SE). Sm14 is a protein member of the fatty acid binding protein (FABP) family that plays an important role in the uptake, transport and compartmentalization of fatty acids from the host into the parasite, since helmints are not capable of synthesizing fatty acids by themselves. Besides being constituents of membranes, lipids also have important roles in the development of different lifecycle stages and the evasion of immune responses by adult worms and larvae. The research and development of Sm14/GLA-SE vaccine have been carried by the coordination of FIOCRUZ, a public institution linked to the Brazilian Ministry of Health[28].

Victims of schsistosomiasis are concentrated in low and middle income country markets, and thus the disease remains away from the spotlights. With scarce investments in research dedicated to schistosomiasis, the world is far to achieve concrete improvements in prevention, treatment and quality of life for this representative fraction of vulnerable people of our society.

In the recent decades, academic research has been playing an important role in the identification, characterization and validation of new therapeutic targets against schistosomiasis[29]. Different potential targets from *Schistosoma spp* have been described and have been widely studied: dihydroorotate dehydrogenase[30; 31], dihydrofolate reductase[32],

histone deacetylases[33], cathepsin B1[34], glutathione S-transferase[35], thioredoxin glutathione reductase[36], among others.

In this work, we want to bring up to discussion the relevance of exploiting the fumarases as potential drug targets against schistosomiasis.

1.3. Fumarate Hydratase

Fumarate hydratases (EC 4.2.1.2), or fumarases, are enzymes that catalyze the estereospecific and reversible hydratation of fumarate to L-malate (**Figure 4**).

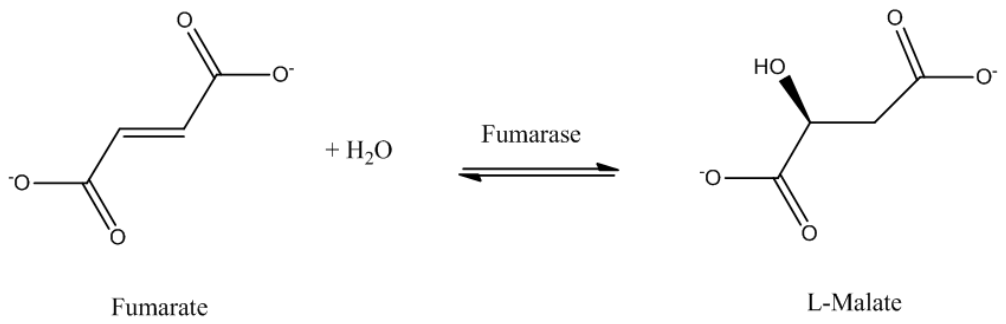

Figure 4. Reversible reaction catalyzed by fumarase enzyme.

The fumarases are classified in two distinct classes: class I fumarases (FH_Is) are homodimeric, contain an iron-sulfur cluster, and have a molecular weight around 120kDa[37; 38; 39], while class II fumarases (FH_{II}s) are homotetrameric, iron independent, have a molecular weight around 200kDa, and are characterized by a conserved amino acid signature (GSSxxPxKxNPxxxE) that contain the catalytic SS-loop sequence (**Figure 5**), common to all aspartase/fumarase superfamily members[37; 38; 40; 41].

Eukaryotic cells express two isoforms of fumarase[42; 43]: the canonical role of fumarase is taken by the mitochondrial echoform that participates in the tricarboxylic acid (TCA) cycle and can also take part in the succinic fermentation pathways by providing fumarate for the enzyme fumarate reductase[44; 45]; the cytosolic echoform has been described as having an important role in the maintenance of genome integrity. By migrating from the cytosol to the nucleus, the cytosolic FHs play a key role in DNA damage response (DDR) to DNA double strand breaks (DSBs)[46]. Moreover, cytosolic fumarase was suggested to participate as a scavenger of fumarate from the urea cycle and catabolism of amino acids[47].

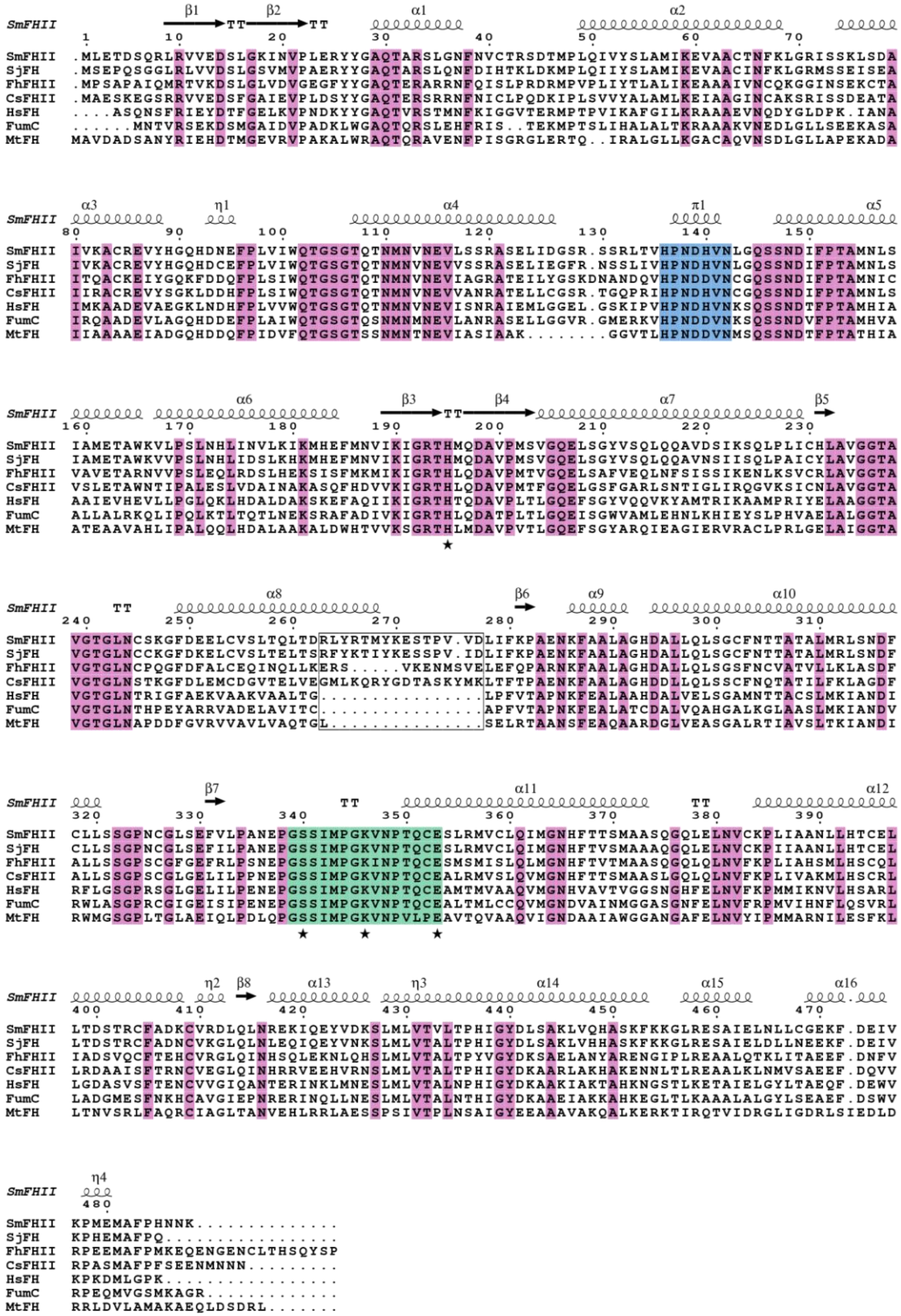

Figure 5. Sequence alignment of class II fumarases. SmFHII (*S. mansoni*), SjFH (*S. japonicum*), FhFHII (*Fasciola hepatica*), CsFHII (*Clonorchis sinensis*), HsFH (*Homo sapiens*), FumC (*Escherichia coli*), MtFH (*Mycobacterium tuberculosis*). The conserved residues are indicated in pink boxes. The conserved B site is indicated in blue boxes. The amino acid signature (GSSxxPxKxNPxxxE) is indicated in green box. The additional fragment is indicated by a dark line box. The catalytic residues are indicated by star symbols. The alignment was performed using MULTALIN and graphically displayed using ESPript[48].

Despite the functional relevance of fumarases, its reaction mechanism is not fully understood yet. The fumarate to L-malate conversion involves the hydration of fumarate by trans-1,4-addition of a hydroxyl group and a proton across the carbon-carbon double bond of fumarate resulting in the formation of L-malate. The reverse reaction proceeds with the elimination of a molecule of water from L-malate, generating fumarate[49].

The first class I fumarase three-dimensional structure was just described in 2016 for cytosolic *Leishmania major* fumarase (LmFH-2)[39]. The LmFH-2 structure is distinct from class II fumarases, revealing a dimeric architecture that resembles a heart, with each lobe containing two domains that are arranged around the active site. Recently, the structure of mitochondrial *Leishmania major* fumarase (LmFH-1) was also solved, showing high structural similarity with LmFH-2[50].

Class II fumarases are known to share high structural similarity, having a tertiary and quaternary fold common to all aspartase/fumarase superfamily members. Each tetramer subunit has three domains: an N-terminal domain, a large central domain composed of 5 α-helices, and a C-terminal domain. The association of the four central domains in the tetramer forms a paired 20 α-helices core that is structurally rigid (**Figure 6**).

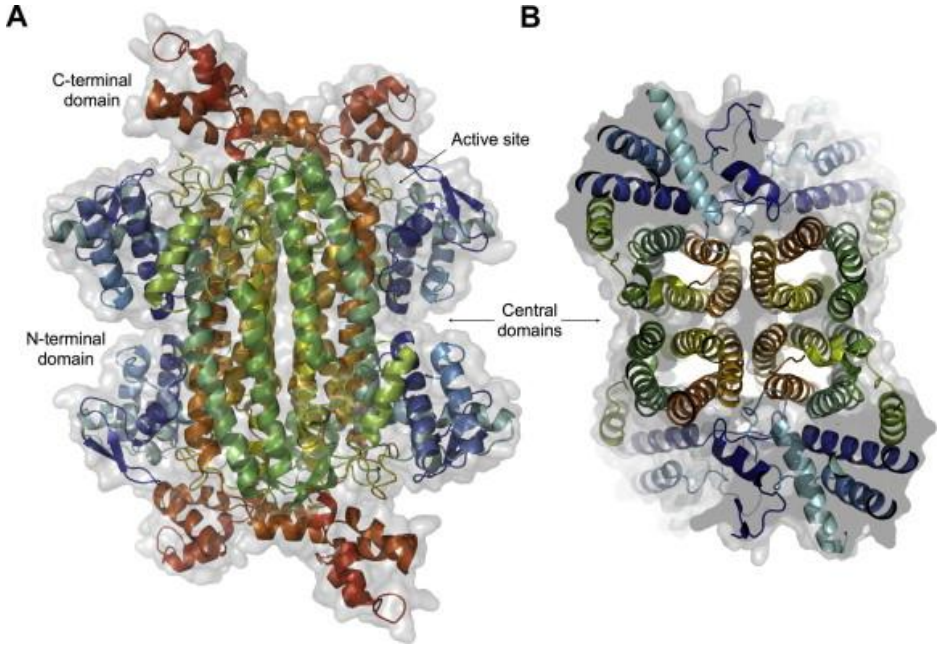

Figure 6. Fumarase structure encoded by Rv1098c *Mycobacterium tuberculosis* gene. The figure shows the central, N-terminal, and C-terminal domains in two different views. (Extracted from Mechaly, et al. 2012[51]).

The class II fumarases active site is composed by residues from three different chain regions, highly conserved among this class, and each region belongs to a distinct tetramer subunit. Thus, there are 4 active sites in the functional tetrameric enzyme.

1.3.1. Fumarase as a target to schistosomiasis

Fumarases are considered potential drug/therapeutic targets, since they are involved in important biological pathways. Recently, studies have been reported promising selective inhibitors to both classes: class II fumarases from *Mycobacterium tuberculosis*[52], class I fumarases from *Leishmania major*[50], *Trypanosoma cruzi*[53] and *Plasmodium falciparum*[54].

Schistosoma mansoni, as well as other trematode worms, possesses the two classes of fumarases. Class I fumarase from *S. mansoni* (*Sm*FH_I) is the mitochondrial enzyme and as such is predicted to contain an oxygen sensitive [4Fe-4S] cluster as a cofactor and to be involved in energy metabolism in *schistosoma spp*. Class II fumarase from *S. mansoni* (*Sm*FH_{II}) is predicted to be the cytosolic enzyme responsible for metabolizing fumarate in the cytosol and when migrating to the nucleus may play an important role in maintaining genomic stablity in the parasite.

Giving those distinct and relevant metabolic pathways in which $SmFH_I$ and $SmFH_{II}$ participates, we are interested in evaluating the inhibition of fumarase activity as a strategy to treat schistosomiasis.

Important to emphasize that since eukaryotes have the ability to shuttle malate and fumarate between mitochondria and cytosol, it is expected that the inhibition of only one isoform ($SmFH_I$ or $SmFH_{II}$) would be inefficient to generate a complete deleterious effect on fumarase activity to the parasite. In fact, this hypothesis has been corroborated by different studies. For instance, fumarase knock out studies performed in *Trypanosoma cruzi* demonstrated that, although the cytosolic or mitochondrial fumarase activities are individually dispensable, their combined activity is essential for parasite viability[53]. Moreover, studies using class II fumarase of *Bacillus subtilis* showed that when this single bacterial enzyme is expressed in a mutant yeast strain it can complement both TCA cycle and DDR eukaryotic functions.[55]

Deeper comprehension of the relevance of fumarase activity in *Schistosoma* spp. will help us to fully understand the function of fumarases for the parasite and validate the

fumarases as drug targets to treat schistosomiasis. This work focused on the structural and kinetic characterization of *Sm*FH_{II} and represents the very first step towards this goal.

2. CONCLUSION

This work presents the first step towards structural and kinetic characterization recombinant cytosolic class II fumarase from *Schistosoma mansoni* (*Sm*FH_{II}).

We successfully established a protocol for *Sm*FH_{II} expression in *E. coli* BL21(DE3), and we obtained soluble protein with a good yield. The gel filtration chromatography showed that the enzyme is tetrameric, as expected for class II fumarases.

Differential Scanning Fluorometry (DSF) was performed to quantify the change in thermal denaturation temperature under varying pH and salt concentrations, and the results reveal that pH 7.5 and high salt concentration increases the thermal stability of *Sm*FH_{II}. Also the DSF has proved to be a useful technique to find new ligands.

We also determined the optimal pH for *Sm*FH_{II} activity, and the results indicates that the maximum activity is reached in the range (7 and 7.5) using fumarate as substrate, and pH 8 using L-malate. Enzyme kinetics using both substrates (L-malate and fumarate) were performed, and the results reveal K_m of 0.56 ± 0.08 mM and 0.15 ± 0.02 mM for L-malate and fumarate, respectively. Comparing with the human fumarase, the K_m values were found to be similar, although the k_{cat} values were much higher for *Hs*FH than *Sm*FH_{II}, probably due the differences between the residues around the catalytic pocket.

The additional portion present in the *Sm*FH_{II} structure, and also other trematode worms, is a prolongation of an α-helix and part of a loop that seems not to be essential to enzyme activity, but its absence compromises the protein stability.

SmFH_{II} crystals were obtained using PEG as precipitant agent, and the structure was solved for the first time, by molecular replacement at 1.85 Å, complexed with its substrate L-malate. The final structure was deposited in PDB (accession code 6U4O).

Although class II fumarases share a high level of structural similarity, the main differences between *Sm*FH_{II} and *Hs*FH structure could be explored to map new allosteric sites and design selective inhibitors.

The results obtained are promising and consisted in the development of a new pipeline to evaluate the potential of *Sm*FH_{II} as a drug target against schistosomiasis.

3. REFERENCES

1 ORGANIZATION, W. H. Neglected tropical diseases. Disponível em: < https://www.who.int/neglected_diseases/en/ >. Acesso em: Aug 17, 2019.

2 BODIMEADE, C.; MARKS, M.; MABEY, D. Neglected tropical diseases: elimination and eradication. **Clinical Medicine,** v. 19, n. 2, p. 157-160, 2019. ISSN 1470-2118.

3 Centers for Disease Control and Prevention. Disponível em: < https://www.cdc.gov/ >. Acesso em: Jun 27, 2019.

4 SOUZA, F. et al. Esquistossomose mansônica: aspectos gerais, imunologia, patogênese e história natural. **Rev Bras Clin Med,** v. 9, n. 4, p. 300-7, 2011.

5 DEELDER, A. et al. Detection of schistosome antigen in mummies. **Lancet (British edition),** v. 335, n. 8691, p. 724-725, 1990. ISSN 0140-6736.

6 BOUCHET, F. et al. First recovery of Schistosoma mansoni eggs from a latrine in Europe (15–16th centuries). **Journal of Parasitology,** v. 88, n. 2, p. 404-406, 2002. ISSN 0022-3395.

7 DI BELLA, S. et al. History of schistosomiasis (bilharziasis) in humans: from Egyptian medical papyri to molecular biology on mummies. **Pathogens and global health,** v. 112, n. 5, p. 268-273, 2018. ISSN 2047-7724.

8 TAN, S.; AHANA, A. Theodor Bilharz (1825-1862): discoverer of schistosomiasis. **Singapore medical journal,** v. 48, n. 3, p. 184, 2007. ISSN 0037-5675.

9 PIRAJÁ DA SILVA, M. Contribuição para o estudo da schistosomiase na Bahia. **Brasil Med,** v. 22, p. 281-283, 1908.

10 SAÚDE, M. D. Vigilância da esquistossomose mansoni: diretrizes técnicas. **Biblioteca Virtual em Saúde do Ministério da Saúde**, 2014.

11 ORGANIZATION, W. H. Schistosomiasis.

12 _____. Schistosomiasis - Fact sheet, 2019.

13 HAY, S. I. et al. Global, regional, and national disability-adjusted life-years (DALYs) for 333 diseases and injuries and healthy life expectancy (HALE) for 195 countries

and territories, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. **The Lancet,** v. 390, n. 10100, p. 1260-1344, 2017. ISSN 0140-6736.

14 SAÚDE, M. D. Esquistossomose: causas, sintomas, tratamento, diagnóstico e prevenção. Disponível em: < http://www.saude.gov.br/saude-de-a-z/esquistossomose >. Acesso em: Aug 17, 2019.

15 NASCIMENTO, G. L. et al. The cost of a disease targeted for elimination in Brazil: the case of schistosomiasis mansoni. **Memórias do Instituto Oswaldo Cruz,** v. 114, 2019. ISSN 0074-0276.

16 Esquistossomose. Situação Epidemiológica - Dados. Portal da Saúde 2014.

17 WANG, W.; WANG, L.; LIANG, Y.-S. Susceptibility or resistance of praziquantel in human schistosomiasis: a review. **Parasitology research,** v. 111, n. 5, p. 1871-1877, 2012. ISSN 0932-0113.

18 WANG, T. et al. Proteomic analysis of the Schistosoma mansoni miracidium. **PloS one,** v. 11, n. 1, p. e0147247, 2016. ISSN 1932-6203.

19 KING, C. H. Toward the elimination of schistosomiasis. **New England Journal of Medicine,** v. 360, n. 2, p. 106-109, 2009. ISSN 0028-4793.

20 DOENHOFF, M. J.; CIOLI, D.; UTZINGER, J. Praziquantel: mechanisms of action, resistance and new derivatives for schistosomiasis. **Current opinion in infectious diseases,** v. 21, n. 6, p. 659-667, 2008. ISSN 0951-7375.

21 PAX, R.; BENNETT, J.; FETTERER, R. A benzodiazepine derivative and praziquantel: effects on musculature of Schistosoma mansoni and Schistosoma japonicum. **Naunyn-Schmiedeberg's archives of pharmacology,** v. 304, n. 3, p. 309-315, 1978. ISSN 0028-1298.

22 KOHN, A. et al. Specific sites in the beta interaction domain of a schistosome Ca 2+ channel β subunit are key to its role in sensitivity to the anti-schistosomal drug praziquantel. **Parasitology,** v. 127, n. 4, p. 349-356, 2003. ISSN 1469-8161.

23 BECKER, B. et al. Light and electron microscopic studies on the effect of praziquantel onSchistosoma mansoni, Dicrocoelium dendriticum, andFasciola hepatica (Trematoda) in vitro. **Zeitschrift für Parasitenkunde,** v. 63, n. 2, p. 113-128, 1980. ISSN 0044-3255.

24 BENIGUEL, L. et al. Differential production in vitro of antigen specific IgG1, IgG3 and IgA: a study in Schistosoma haematobium infected individuals. **Parasite Immunol,** v. 25, n. 1, p. 39-44, Jan 2003. ISSN 0141-9838 (Print) 0141-9838.

[25] RIVEAU, G. et al. Safety and immunogenicity of rSh28GST antigen in humans: phase 1 randomized clinical study of a vaccine candidate against urinary schistosomiasis. **PLoS Negl Trop Dis,** v. 6, n. 7, p. e1704, 2012. ISSN 1935-2727.

[26] TRAN, M. H. et al. Tetraspanins on the surface of Schistosoma mansoni are protective antigens against schistosomiasis. **Nat Med,** v. 12, n. 7, p. 835-40, Jul 2006. ISSN 1078-8956 (Print) 1078-8956.

[27] HOTEZ, P. J. et al. Developing vaccines to combat hookworm infection and intestinal schistosomiasis. **Nat Rev Microbiol,** v. 8, n. 11, p. 814-26, Nov 2010. ISSN 1740-1526.

[28] TENDLER, M. et al. Current status of the Sm14/GLA-SE Schistosomiasis vaccine: overcoming barriers and paradigms towards the first anti-parasitic Human (itarian) vaccine. **Tropical medicine and infectious disease,** v. 3, n. 4, p. 121, 2018.

[29] FERREIRA, L. G.; OLIVA, G.; ANDRICOPULO, A. D. Target-based molecular modeling strategies for schistosomiasis drug discovery. **Future Med Chem,** v. 7, n. 6, p. 753-64, 2015. ISSN 1756-8919.

[30] NONATO, M. C. et al. Structural basis for the design of selective inhibitors for Schistosoma mansoni dihydroorotate dehydrogenase. **Biochimie,** v. 158, p. 180-190, 2019. ISSN 0300-9084.

[31] CALIL, F. A. et al. Ligand-based design, synthesis and biochemical evaluation of potent and selective inhibitors of Schistosoma mansoni dihydroorotate dehydrogenase. **Eur J Med Chem,** v. 167, p. 357-366, Apr 1 2019. ISSN 0223-5234.

[32] SERRÃO, V. H. B. et al. Structure and kinetics assays of recombinant Schistosoma mansoni dihydrofolate reductase. **Acta tropica,** v. 170, p. 190-196, 2017. ISSN 0001-706X.

[33] MAREK, M. et al. Structural basis for the inhibition of histone deacetylase 8 (HDAC8), a key epigenetic player in the blood fluke Schistosoma mansoni. **PLoS pathogens,** v. 9, n. 9, p. e1003645, 2013. ISSN 1553-7374.

[34] ABDULLA, M.-H. et al. Schistosomiasis mansoni: novel chemotherapy using a cysteine protease inhibitor. **PLoS medicine,** v. 4, n. 1, p. e14, 2007. ISSN 1549-1676.

[35] MO, A. X. et al. Schistosomiasis elimination strategies and potential role of a vaccine in achieving global health goals. **The American journal of tropical medicine and hygiene,** v. 90, n. 1, p. 54-60, 2014. ISSN 0002-9637.

36 LI, T. et al. High-throughput screening against thioredoxin glutathione reductase identifies novel inhibitors with potential therapeutic value for schistosomiasis. **Infectious diseases of poverty,** v. 4, n. 1, p. 40, 2015. ISSN 2049-9957.

37 WOODS, S. A.; SCHWARTZBACH, S. D.; GUEST, J. R. Two biochemically distinct classes of fumarase in Escherichia coli. **Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology,** v. 954, p. 14-26, 1988. ISSN 0167-4838.

38 GUEST, J. R. et al. The fumarase genes of Escherichia coli: location of the fumB gene and discovery of a new gene (fumC). **Microbiology,** v. 131, n. 11, p. 2971-2984, 1985. ISSN 1465-2080.

39 FELICIANO, P. R.; DRENNAN, C. L.; NONATO, M. C. Crystal structure of an Fe-S cluster-containing fumarate hydratase enzyme from Leishmania major reveals a unique protein fold. **Proceedings of the National Academy of Sciences,** v. 113, n. 35, p. 9804-9809, 2016. ISSN 0027-8424.

40 SUZUKI, T. et al. Rat liver mitochondrial and cytosolic fumarases with identical amino acid sequences are encoded from a single gene. **Journal of Biological Chemistry,** v. 264, n. 5, p. 2581-2586, 1989. ISSN 0021-9258.

41 NAST, G.; MULLER-ROBER, B. Molecular characterization of potato fumarate hydratase and functional expression in Escherichia coli. **Plant physiology,** v. 112, n. 3, p. 1219-1227, 1996. ISSN 1532-2548.

42 YOGEV, O.; NAAMATI, A.; PINES, O. Fumarase: a paradigm of dual targeting and dual localized functions. **Febs Journal,** v. 278, n. 22, p. 4230-4242, 2011. ISSN 1742-4658.

43 DIK, E. et al. Human Fumarate Hydratase Is Dual Localized by an Alternative Transcription Initiation Mechanism. **Traffic,** v. 17, n. 7, p. 720-732, 2016. ISSN 1600-0854.

44 FELICIANO, P. R. et al. Fumarate hydratase isoforms of Leishmania major: subcellular localization, structural and kinetic properties. **International journal of biological macromolecules,** v. 51, n. 1, p. 25-31, 2012. ISSN 0141-8130.

45 BESTEIRO, S. et al. Succinate secreted by Trypanosoma brucei is produced by a novel and unique glycosomal enzyme, NADH-dependent fumarate reductase. **Journal of Biological Chemistry,** v. 277, n. 41, p. 38001-38012, 2002. ISSN 0021-9258.

46 YOGEV, O. et al. Fumarase: a mitochondrial metabolic enzyme and a cytosolic/nuclear component of the DNA damage response. **PLoS Biol,** v. 8, n. 3, p. e1000328, 2010. ISSN 1545-7885.

47 RATNER, S. Biosynthesis of urea. VI. Enzymatic cleavage of argininosuccinic acid to arginine and fumaric acid. PARKER ANSLOW, W. 204**:** 115-125 p. 1953.

48 GOUET, P. et al. ESPript: analysis of multiple sequence alignments in PostScript. **Bioinformatics (Oxford, England),** v. 15, n. 4, p. 305-308, 1999. ISSN 1460-2059.

49 WEAVER, T.; BANASZAK, L. Crystallographic studies of the catalytic and a second site in fumarase C from Escherichia coli. **Biochemistry,** v. 35, n. 44, p. 13955-13965, 1996. ISSN 0006-2960.

50 FELICIANO, P. R.; DRENNAN, C. L.; NONATO, M. C. Crystal structures of fumarate hydratases from Leishmania major in a complex with inhibitor 2-thiomalate. **ACS chemical biology,** v. 14, n. 2, p. 266-275, 2019. ISSN 1554-8929.

51 MECHALY, A. E. et al. Conformational changes upon ligand binding in the essential class II fumarase Rv1098c from Mycobacterium tuberculosis. **FEBS letters,** v. 586, n. 11, p. 1606-1611, 2012. ISSN 1873-3468.

52 KASBEKAR, M. et al. Selective small molecule inhibitor of the Mycobacterium tuberculosis fumarate hydratase reveals an allosteric regulatory site. **Proceedings of the National Academy of Sciences,** v. 113, n. 27, p. 7503-7508, 2016. ISSN 0027-8424.

53 DE PÁDUA, R. A. et al. Characterisation of the fumarate hydratase repertoire in Trypanosoma cruzi. **International journal of biological macromolecules,** v. 102, p. 42-51, 2017. ISSN 0141-8130.

54 JAYARAMAN, V. et al. Biochemical characterization and essentiality of Plasmodium fumarate hydratase. **Journal of Biological Chemistry,** v. 293, n. 16, p. 5878-5894, 2018. ISSN 0021-9258.

55 YOGEV, O. et al. Fumarase: A Mitochondrial Metabolic Enzyme and a Cytosolic/Nuclear Component of the DNA Damage Response. **Plos Biology,** v. 8, n. 3, Mar 2010. ISSN 1544-9173. Disponível em: < <Go to ISI>://WOS:000278125400008 >.

56 NIESEN, F. H.; BERGLUND, H.; VEDADI, M. The use of differential scanning fluorimetry to detect ligand interactions that promote protein stability. **Nature protocols,** v. 2, n. 9, p. 2212, 2007. ISSN 1750-2799.

57 PEREIRA DE PADUA, R. A.; NONATO, M. C. Cloning, expression, purification, crystallization and preliminary X-ray diffraction analysis of recombinant human fumarase. **Acta Crystallographica Section F: Structural Biology Communications,** v. 70, n. 1, p. 120-122, 2014. ISSN 2053-230X.

58 MCPHERSON, A. **Preparation and analysis of protein crystals**. John Wiley & Sons, 1982. ISBN 0471085243.

59 LUFT, J. R.; NEWMAN, J.; SNELL, E. H. Crystallization screening: the influence of history on current practice. **Acta Crystallographica Section F: Structural Biology Communications,** v. 70, n. 7, p. 835-853, 2014. ISSN 2053-230X.

60 LUFT, J. R.; DETITTA, G. T. A method to produce microseed stock for use in the crystallization of biological macromolecules. **Acta Crystallographica Section D: Biological Crystallography,** v. 55, n. 5, p. 988-993, 1999. ISSN 0907-4449.

61 KABSCH, W. Xds. **Acta Crystallographica Section D: Biological Crystallography,** v. 66, n. 2, p. 125-132, 2010. ISSN 0907-4449.

62 VAGIN, A.; TEPLYAKOV, A. MOLREP: an automated program for molecular replacement. **Journal of applied crystallography,** v. 30, n. 6, p. 1022-1025, 1997. ISSN 0021-8898.

63 COWTAN, K. The" Buccaneer" protein model building software. **CCP4 Newsletter,** v. 44, 2006.

64 MURSHUDOV, G. N.; VAGIN, A. A.; DODSON, E. J. Refinement of macromolecular structures by the maximum-likelihood method. **Acta Crystallographica Section D: Biological Crystallography,** v. 53, n. 3, p. 240-255, 1997. ISSN 0907-4449.

65 EMSLEY, P. et al. Features and development of Coot. **Acta Crystallographica Section D: Biological Crystallography,** v. 66, n. 4, p. 486-501, 2010. ISSN 0907-4449.

66 GENDA, T.; WATABE, S.; OZAKI, H. Purification and characterization of fumarase from Corynebacterium glutamicum. **Bioscience, biotechnology, and biochemistry,** v. 70, n. 5, p. 1102-1109, 2006. ISSN 1347-6947.

67 AJALLA ALEIXO, M. A. et al. Structural, biochemical and biophysical characterization of recombinant human fumarate hydratase. **The FEBS journal,** v. 286, n. 10, p. 1925-1940, 2019. ISSN 1742-464X.

68 KNIEWALD, J.; MILDNER, P. Thermochemistry of fumarase-inhibitor binding. **FEBS letters,** v. 53, n. 2, p. 225-228, 1975. ISSN 1873-3468.

69 MASSEY, V. Studies on fumarase. 4. The effects of inhibitors on fumarase activity. **Biochemical Journal,** v. 55, n. 1, p. 172, 1953.

[70] WEAVER, T. Structure of free fumarase C from Escherichia coli. **Acta Crystallographica Section D: Biological Crystallography,** v. 61, n. 10, p. 1395-1401, 2005. ISSN 0907-4449.

[71] PEREIRA, A. S. et al. Inhibition of histone methyltransferase EZH2 in Schistosoma mansoni in vitro by GSK343 reduces egg laying and decreases the expression of genes implicated in DNA replication and noncoding RNA metabolism. **PLoS neglected tropical diseases,** v. 12, n. 10, p. e0006873, 2018. ISSN 1935-2735.

[72] TURGEON, M.-O.; PERRY, N. J.; POULOGIANNIS, G. DNA damage, repair, and cancer metabolism. **Frontiers in oncology,** v. 8, p. 15, 2018. ISSN 2234-943X.

[73] LESHETS, M. et al. Fumarase: From the TCA cycle to DNA damage response and tumor suppression. **Frontiers in molecular biosciences,** v. 5, p. 68, 2018. ISSN 2296-889X.

[74] JIANG, Y. et al. Local generation of fumarate promotes DNA repair through inhibition of histone H3 demethylation. **Nature cell biology,** v. 17, n. 9, p. 1158, 2015. ISSN 1476-4679.

[75] LESHETS, M. et al. Fumarase is involved in DNA double-strand break resection through a functional interaction with Sae2. **Current genetics,** v. 64, n. 3, p. 697-712, 2018. ISSN 0172-8083.

[76] EOH, H.; RHEE, K. Y. Multifunctional essentiality of succinate metabolism in adaptation to hypoxia in Mycobacterium tuberculosis. **Proceedings of the National Academy of Sciences,** v. 110, n. 16, p. 6554-6559, 2013. ISSN 0027-8424.

[77] RUECKER, N. et al. Fumarase deficiency causes protein and metabolite succination and intoxicates Mycobacterium tuberculosis. **Cell chemical biology,** v. 24, n. 3, p. 306-315, 2017. ISSN 2451-9456.

[78] SULLIVAN, L. B. et al. The proto-oncometabolite fumarate binds glutathione to amplify ROS-dependent signaling. **Molecular cell,** v. 51, n. 2, p. 236-248, 2013. ISSN 1097-2765.

[79] ZHENG, L. et al. Fumarate induces redox-dependent senescence by modifying glutathione metabolism. **Nature communications,** v. 6, p. 6001, 2015. ISSN 2041-1723.

[80] KUNTZ, A. N. et al. Thioredoxin glutathione reductase from Schistosoma mansoni: an essential parasite enzyme and a key drug target. **PLoS Medicine,** v. 4, n. 6, p. e206, 2007. ISSN 1549-1676.

[81] LU, Z. et al. Schistosome sex matters: a deep view into gonad-specific and pairing-dependent transcriptomes reveals a complex gender interplay. **Scientific reports,** v. 6, p. 31150, 2016. ISSN 2045-2322.

[82] ESTÉVEZ, M. et al. X-ray crystallographic and kinetic correlation of a clinically observed human fumarase mutation. **Protein science,** v. 11, n. 6, p. 1552-1557, 2002. ISSN 0961-8368.

[83] KATAYAMA, N.; TAKEYA, M.; OSANAI, T. Biochemical characterisation of fumarase C from a unicellular cyanobacterium demonstrating its substrate affinity, altered by an amino acid substitution. **Scientific reports,** v. 9, n. 1, p. 10629, 2019. ISSN 2045-2322.

[84] WEAVER, T. et al. Alterations within fumarase C reveal newly observed SS Loop conformations. **The FASEB Journal,** v. 33, n. 1_supplement, p. 468.7-468.7, 2019. ISSN 0892-6638.

[85] BAUGH, L. et al. Increasing the structural coverage of tuberculosis drug targets. **Tuberculosis,** v. 95, n. 2, p. 142-148, 2015. ISSN 1472-9792.

[86] PAWSON, T.; NASH, P. Assembly of cell regulatory systems through protein interaction domains. **Science,** v. 300, n. 5618, p. 445-452, 2003. ISSN 0036-8075.

[87] BLOM, N.; GAMMELTOFT, S.; BRUNAK, S. Sequence and structure-based prediction of eukaryotic protein phosphorylation sites. **Journal of molecular biology,** v. 294, n. 5, p. 1351-1362, 1999. ISSN 0022-2836.