Microbiological Research 194 (2017) 29–37

Contents lists available at ScienceDirect

Microbiological Research

journal homepage: www.elsevier.com/locate/micres

Δ9 desaturase from *Trypanosoma cruzi*: Key enzyme in the parasite metabolism. Cloning and overexpression

Mariela R. Woelke[a], Natalia S. Paulucci[a], Armentano Selva[a], Hermes Garban[b], Mirta García de Lema[a,*]

[a] Departamento de Biología Molecular, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto, CPX5804BYA, Río Cuarto, Córdoba, Argentina
[b] Department of Medicine, Division of Dermatology and Jonsson Comprehensive Cancer Center, David Geffen School of Medicine at the University of California, Los Angeles (UCLA), Los Angeles, CA, United States

ARTICLE INFO

Article history:
Received 14 December 2015
Received in revised form 3 July 2016
Accepted 31 July 2016
Available online 27 October 2016

Keywords:
Trypanosoma cruzi
Δ9 desaturase
Heterologous expression
Desaturase gene
Unsaturated fatty acids
Ketoconazole

ABSTRACT

Desaturases, key enzymes in the metabolism of fatty acids, regulate the physical and biochemical properties of membranes. They adjust the composition of saturated and unsaturated fatty acids in response to changes in the environmental.

We demonstrated the existence of Δ9 desaturase activity in epimastigotes of the *Trypanosoma cruzi* Tulahuen strain. In the present study, showed that this enzyme has an approximate molecular mass of 50 kDa and a pI value of approximately 9. In order to characterize the Δ9 desaturase of *Trypanosoma cruzi,* (TcΔ9DES) we have cloned, sequenced and expressed in *Escherichia coli*. The gene consists of 1300 bp and encodes a peptide of 433 amino acids with a molecular weight of 50 kDa. Analysis of the amino acid sequence revealed three clusters of histidine and two hydrophobic regions, characteristic of membrane-bound desaturases.

Gene expression studies showed that TcΔ9DES was overexpressed as an active protein. Fatty acid analysis showed that the expressed protein was confirmed to be functional with Δ9 desaturase activity. This enzyme changed the fatty acid profile of TcΔ9DES-expressing *E. coli*, decreasing the levels of palmitic (16:0) and stearic (18:0) acids and enhancing palmitoleic (16:1Δ9) and monounsaturated 18 carbons fatty acids. When [1–14C]palmitic or [1–14C]stearic acid was used as substrate, TcΔ9DES-expressing *E. coli* exhibited high desaturase activity associated with increased levels of monounsaturated fatty acids, suggesting that the TcΔ9DES enzyme was actively expressed in *E. coli*.

To check the commitment of TcΔ9DES against sterol biosynthesis inhibitors we tested the activity under ketoconazole effect. Native TcΔ9DES, showed a significant activity inhibition.

Since TcΔ9DES has shown active participation under different environmental factors, among them, ketoconazole, we consider that it plays a critical role in the metabolism of the parasite

1. Introduction

American trypanosomiasis, also known as Chagas disease, is caused by *Trypanosoma cruzi*, a flagellate parasite of the order of the kinetoplastida. The infection is transmitted by the blood-sucking *Hemiptera Reduviidae* insect family, subfamily Triatominae (Miles et al., 2003). Chagas disease is considered endemic to South and Central America, although it has now spread to other continents due to immigration flows. About 6 million to 7 million people are estimated to be infected worldwide, mostly in Latin America, [WHO World Health Organization, 2015. http://www.who.int/entity/mediacentre/factsheets/fs340/en/.]

Current medical treatment of trypanosomiasis is associated with high toxicity and, in some cases, low efficiency. Consequently, there is an urgent priority to develop new chemotherapy components against this disease. Lipids have been considered important chemotherapeutic targets (Urbina, 2009). Among used

Abbreviations: FA, fatty acids; FID, flame ionization detector; FAME, fatty acid methyl esters; FBS, fetal bovine serum; IPTG, isopropyl-β-D-thiogalactopyranoside; SBI, sterol biosynthesis inhibitors; TLC, thin layer chromatography; PMSF, phenylmethylsulphonyl fluoride; LB, Luria-Bertani; TcΔ9DES, Δ9 desaturase of Trypanosoma cruzi; TcΔ9DES, Δ9 desaturase gene of Trypanosoma cruzi.

* Corresponding author.
E-mail addresses: mgarcia@exa.unrc.edu.ar, mirta_b_garcia@yahoo.com (M.G. de Lema).

http://dx.doi.org/10.1016/j.micres.2016.07.012
0944-5013/

drugs against the protozoan parasite *T cruzi,* sterol biosynthesis inhibitors (SBI) cause antiproliferative effects on the parasite. Contreras et al. (1997) showed that, one of the primary effects of the depletion of endogenous sterols induced by SBI in *T. cruzi* is a modification of the cellular phospholipid composition as a consequence of a reduced activity of PE-PC-*N*-methyl transferase and probably of the acyl Δ9 and Δ6 desaturases.

Delving into the development of future drugs, have been identified a number of genes of the sterol biosynthesis pathway (Cosentino and Agüero, 2014).

Desaturases, which are proteins distributed universally among living organisms, are responsible for the biosynthesis of unsaturated fatty acids and play a central role in the regulation of lipid metabolism. Because fatty acids (FA) are the major constituents of membrane phospholipids, modulation of the number and position of double bonds by desaturases plays an important role in maintaining the dynamic state of membranes (Sajbidor, 1997; Denich et al., 2003). It has been shown that Δ9 desaturase activity is increased at low temperature (McCartney et al., 1996). Additionally, *Tetrahymena thermophila* responds to temperature changes modifying, in part, Δ9 desaturase activity (Nakashima et al., 1996).

Previous studies done by our research group contributed to the knowledge of lipid metabolism in *T. cruzi*. The existence of Δ9 and Δ12 desaturases in epimastigotes of *T. cruzi* was determined in our laboratory (de Lema and Aeberhard, 1986) by adding radioactive FA to the culture medium, which were incorporated and metabolised. In addition, our studies on *T. cruzi* epimastigotes revealed that environmental factors modify the proportion of unsaturated to saturated FA through changes in desaturase enzyme activity (Racagni et al., 1995; Villasuso et al., 2005). Moreover, we have shown that *T. cruzi* is able to desaturate different substrates using Δ9 desaturase by modifying the concentration of fetal bovine serum (FBS) suggesting the importance of this enzyme in the metabolism of the parasite (Villasuso et al., 2010).

Despite Δ9 desaturase of *T. cruzi* (TcΔ9DES) being partially biochemically characterized, the associated gene with this activity has not been identified. While Δ9 desaturase is present in *T. cruzi* and its mammalian host, this enzyme appears to have structural differences, suggesting that this enzyme could be targeted in order to design selective therapies (Alloatti et al., 2009).

Isolation and characterization of this enzyme have imposed a difficult task due to its persistent association with cell membranes and its fragility. Early studies on the purification of this enzyme demonstrated that it was extremely labile, even at high purity and stored at −80 °C. These observations suggested the presence of a microsomal protease that might be responsible for the degradation of the enzyme in addition to the potential loss of desaturase activity by denaturation of the enzyme (Heinemann and Ozols, 2002). One way to overcome this inherent limitation is the overexpression of these proteins in heterologous hosts, such as *E. coli*, with subsequent purification and characterization by conventional methods (Schertler Gebhard, 1992). A large number of membrane desaturases, including Δ9 desaturases, such as those of *Pseudoalteromonas sp.* (Li et al., 2009), rat (Strittmatter et al., 1988), *Cyanobacteria Synechocystis* sp. (Maali et al., 2007) and S-ACP-desaturase of *Arabidopsis thaliana* (Yujin et al., 2010) have been characterized by using heterologous expression systems. Trypanosomatide desaturases have been, also, overexpressed. Thus Petrini et al. (2004), have described the isolation and functional characterization of a *Trypanosoma brucei* oleate desaturase by heterologous expression in *Saccharomyces cerevisiae.* In addition Maldonado et al. (2006), reported the molecular characterization of *T. cruzi* oleate desaturase and they also showed the presence of homologous *T. cruzi* oleate desaturase genes in various pathogenic and nonpathogenic trypanosomatids.

Therefore, in this study we describe some biochemical features of the native TcΔ9DES enzyme from epimastigotes of *T. cruzi*, Tulahuen strain, the behavior in the presence of a SBI, ketoconazole and its further isolation and functional characterization by heterologous expression in *E. coli.*

2. Materials and methods

2.1. Microorganisms, plasmids and growth conditions

The microorganisms, plasmids and oligonucleotide primers used in this study are listed in Table 1 (Taliaffero and Pizzi, 1955). *T. cruzi* epimastigote forms were grown at 28 °C in modified Warren's medium (Warren, 1960) as previously described (Racagni et al., 1992). The medium was supplemented with 10% FBS and 33000 UI penicillin per 4×10^7 parasites. Cells in the logarithmic growth phase (5 days old) were harvested by centrifugation at 4500 × *g* for 10 min. The weight of the harvested cells and the number of mobile cells per ml culture medium was measured.

The *E. coli* XL 10 GOLD strain was used in all routine DNA manipulations and cloning procedures. *E. coli* BL21 codon Plus was used as a host for the pET28a (+) plasmid containing the putative *TcΔ9DES*. Recombinant *E. coli* strains were grown at 37 °C in Luria-Bertani (LB) broth containing the required antibiotics.

2.2. Partial characterization of the native Δ9 desaturase of T. cruzi

2.2.1. Isolation and partial purification

To determine the characteristics of TcΔ9DES, epimastigotes of *T. cruzi* were subjected to differential centrifugation using a previously described method (Villasuso et al., 2010). Thus, different subcellular fractions were collected, and the 105,000 × *g* pellet was used as a protein source in further analysis.

2.2.2. Electrophoresis

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE; 10% (w/v)) was carried out (Laemmli, 1970) using a Mini-protean IV Bio-Rad system and Kaleidoscope 195–6.4 D (Bio-Rad, Hercules, CA, USA) as a molecular mass standard. The bands were stained with Coomasie Brilliant Blue R-250.

2.2.3. Isoelectric focusing

Isoelectric focusing (IEF) was carried out as previously described (Agostini et al., 2000) and resolved on polyacrylamide gels over a pH range 3–10 using a Bio-Rad Mini IEF System. Samples were previously desalted with Sephadex G-25. The gels were calibrated using the Bio-Rad pI Calibration Kit (4.45–9.60) (Bio-Rad, Hercules, CA, USA). To detect the protein markers for pI, the gel was immersed in a solution containing 0.04% (w/v) Coomasie Brilliant Blue R-250 and 0.05% (w/v) Crocein Scarlet for 10 min, and the protein bands were stained with silver nitrate.

2.2.4. Immunoblotting

The presence of native TcΔ9DES was determined by Western blotting (Toubin et al., 1979); the samples were subjected to SDS-PAGE and IEF electrophoresis, and the bands were electrotransferred to a Hybond ECL nitrocellulose membrane (Amersham Biosciences, Piscataway, NJ, USA) using a Mini Trans-Blot cell (Bio-Rad, Hercules, CA, USA) at a constant voltage of 100 V for 1 h at 4 °C. Blotting was performed using a primary antibody against rat Δ9 desaturase, which was kindly provided by Dr. Omar Rimoldi, INIBIOLP (Instituto de Investigaciones Bioquimicas de La Plata-Argentina). Detection was realised using the amplified alkaline phosphatase Immun-Blot kit (BioRad, Hercules, CA, USA) by SDS