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ORIGINAL ARTICLE

Fetal Bovine Serum Concentration Affects Δ^9 Desaturase Activity of *Trypanosoma cruzi*

Ana L. Villasuso · Patricio Romero · Mariela Woelke · Patricia Moyano · Estela Machado · Mirta García de Lema

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Abstract Fetal bovine serum (FBS) is an important factor in the culture of *Trypanosoma cruzi*, since this parasite obtains and metabolizes fatty acids (FAs) from the culture medium, and changes in FBS concentration reduce the degree of unsaturation of FAs in phosphoinositides. When *T. cruzi* epimastigotes were cultured with 5% instead of 10% FBS, and stearic acid was used as the substrate, Δ^9 desaturase activity decreased by 50%. Apparent K_m and V_m values for stearic acid, determined from Lineaweaver–Burk plots, were 2 μM and 219 pmol/min/mg of protein, respectively. In studies of the requirement for reduced pyridine nucleotide, Δ^9 desaturase activity reached a maximum with 8 μM NADH and then remained constant; the apparent K_m and V_m were 4.3 μM and 46.8 pmol/min/mg of protein, respectively. The effect of FBS was observed only for Δ^9 desaturase activity; Δ^{12} desaturase activity was not affected. The results suggest that decreased FBS in culture medium is a signal that modulates Δ^9 desaturase activity in *T. cruzi* epimastigotes.

Keywords *Trypanosoma cruzi* · Δ^9 Desaturase · Δ^{12} Desaturase · Fetal bovine serum

Abbreviations

ATP	Adenosine triphosphate
FBS	Fetal bovine serum
EDTA	Ethylenediaminetetraacetic acid
FAME	Fatty acid methyl esters
FFA	Free fatty acid
FAs	Fatty acids
HPLC	High performance liquid chromatography
HEPES	(4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
NL	Neutral lipid
PL	Phospholipid
PMSF	Phenylmethylsulphonyl fluoride
PUFAs	Polyunsaturated fatty acids
PC	Phosphatidylcholine
SEM	Standard error of the mean
TLC	Thin layer chromatography

A. L. Villasuso · P. Romero · M. Woelke · P. Moyano · E. Machado · M. García de Lema (✉)
Departamento de Biología Molecular, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto, CPX5804BYA Río Cuarto, Córdoba, Argentina
e-mail: mgarcia@exa.unrc.edu.ar

Introduction

Protozoan parasites often encounter unpredictable changes in their environment. Modification of their membrane lipid composition helps maintain the biophysical properties for optimal membrane function, allowing them to cope with environmental changes [1, 2]. *Trypanosoma cruzi*, the etiological agent of Chagas' disease, is an intracellular protozoan that undergoes a complex life cycle between a hematophagous insect vector, *Triatoma infestans*, and a mammalian host. In the intestinal tract of the vector, the replicative non-infectious epimastigotes differentiate to the infectious non-dividing metacyclic forms, a process denominated metacyclogenesis.

It is well known that differentiation of these parasites involves changes in the shape of the cell; consequently the membrane fluidity might be essential for trypanosome

transmission. In *T. cruzi*, it has been demonstrated that the oleic acid present in the intestinal extracts of *T. infestans* induce cell differentiation of *T. cruzi* epimastigotes into the infective metacyclic form [3]. As part of a strategy for surviving in these different environmental conditions, *T. cruzi* adjusts the balance between saturated and unsaturated fatty acids (FAs) in certain membrane lipids [4, 5]. Desaturases are key enzymes in FA metabolism required to regulate physical and biochemical properties of membranes [6]. Desaturases are present in most living cells, and play critical roles in the biosynthesis of polyunsaturated fatty acids (PUFAs). PUFAs serve as precursors of biologically active molecules involved in the activation of a variety of signalling mechanisms that affect cellular functions [7].

The endoplasmic reticulum contains membrane-bound enzymes that remove two hydrogen atoms from the aliphatic chain of a FA to produce a *cis* double bond [8]. Δ^9 Desaturase is of particular interest since FAs that contain a double bond at the central C9–C10 position have a maximal disordering effect on membrane physical properties [9]. Since FAs are the main constituents of membrane glycerolipids, modulation of the number and position of double bonds in acyl chains by individual FA desaturases helps maintain the proper dynamic state of the membrane bilayer during environmental impacts [10]. Temperature changes have been shown to modulate the ratio of saturated to unsaturated FAs in *T. cruzi* [5]. The concentration of fetal bovine serum (FBS) in the culture medium also affects the degree of unsaturation. When the FBS concentration in the culture medium is reduced from 10 to 5%, the proportion of linoleic acid in phosphoinositides decreases [4], suggesting that desaturase activity may be modulated by FBS concentration.

We showed previously that the ratio of unsaturated to saturated FAs increases with growth in culture, as indicated by an increased percentage of linoleic acid (18:2), and that carbamoylcholine increases [^{14}C] labelling of triacylglycerols and diacylglycerols [11]. These findings indicate that unsaturated FAs are important factors during parasite aging and response to environmental stimuli. Initial studies from our laboratory demonstrated de novo biosynthesis of palmitic acid in *T. cruzi* [12], and we showed later that epimastigotes of *T. cruzi* are able to incorporate and metabolize exogenous FAs; palmitic acid is elongated to stearic acid and then desaturated to oleic acid and linoleic acid. These data support the existence of Δ^9 and Δ^{12} desaturases [12, 13]. Molecular characterization of oleate desaturase was conducted in *T. brucei* by Petrini et al. [14], using heterologous expression, and in *T. cruzi* by Maldonado et al. [15].

Genomic analysis of trypanosomatids revealed the presence of front-end desaturase genes, tentatively designated as $\Delta^8\Delta^5\Delta^6$ desaturases for *Leishmania major* and Δ^6 for *T. brucei and T. cruzi*, on the basis of sequence similarity. The desaturases were later characterized as $\Delta^6\Delta^5\Delta^4$ for *L. major,* while only Δ^4 is present in *Trypanosoma*. Functional predictions are never conclusive for desaturases; i.e., biochemical characterization is essential for correct assignment of enzyme regioselectivities [16]. Study of biochemical properties of a parasite's desaturases helps provide insight into their role in parasite response to environmental conditions. Since *T. cruzi* is able to sense changes in FBS concentration, and consequently modulate the degree of unsaturation of phosphoinositide FAs, we used FBS concentration change as a tool to elucidate Δ^9 and Δ^{12} desaturase activities. Reduction of FBS concentration in the parasite culture medium decreased Δ^9 desaturase activity by 50%. Therefore we suggest that the Δ^9 desaturase activity would play a possible role as a regulator of oleic acid level and could be implied in the regulation of membrane fluidity necessary for parasite transmission. Partial biochemical characterization of *T. cruzi* Δ^9 and Δ^{12} desaturases using subcellular fractions and radioactive FAs is also described.

Experimental Procedure

Materials

Solvents were either analytical or HPLC grade. Lipid standards were from Sigma Chemical Co. (St Louis, MO, USA). Culture media were from Merck (Germany) or Difco (USA). FBS was from Natocor (Argentina).

Parasite Strain and Growth Conditions

The Tulahuen strain of *T. cruzi* was used. Epimastigote forms were grown at 28 °C in modified Warren's medium [17] as described by Racagni et al. [18]. The medium was supplemented with 5 or 10% FBS and 1,000,000 U penicillin per 4×10^7 parasites. Cells in the logarithmic growth phase (5 days old) were harvested by centrifugation at 4,500*g* for 10 min. The weight of harvested cells and the number of mobile cells per mL culture medium was measured.

Enzyme Extraction

Cells were weighed, frozen at −180 °C and thawed three times. Broken cells were homogenized 1:5 (w/v) in 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), pH 7.4 containing 0.25 M sucrose, 5 mM KCl, 1 mM ethylenediaminetetraacetic acid (EDTA), and protease inhibitors (1 μg/mL leupeptin, 1 mM phenylmethylsulphonyl fluoride (PMSF), 1 μg/mL aprotinin). The

homogenate was centrifuged using three sedimentation steps: 1,000*g* for 10 min, 25,000*g* for 12 min, and 105,000*g* for 60 min. Supernatants of 25,000*g*, and 105,000*g*, and the pellet of the 105,000*g* centrifugation, were used for determination of desaturase activity.

Enzymatic Assays

Δ^9 desaturation of [1-^{14}C]palmitic acid or [1-^{14}C]stearic acid was estimated in subcellular fractions of *T. cruzi* by measuring the formation of [1-^{14}C]palmitoleic acid or [1-^{14}C]oleic acid, respectively. The reaction mixture consisted of 41.7 mM potassium phosphate buffer (pH 7.4), 0.25 M sucrose, 0.15 M KCl, 41.7 mM NaF, 5 mM $MgCl_2$, 1.6 mM *N*-acetyl-cysteine, 60 μM CoA (sodium salt), 1.3 mM adenosine triphosphate (ATP), 0.87 mM nicotinamide adenine dinucleotide (NADH), 3.1 μM of [1-^{14}C] palmitic or stearic acid, and 0.2 mg microsomal protein, in a total volume of 750 μL [19]. For determination of Δ^{12} desaturase activity, [1-^{14}C]oleic acid as substrate and 0.8 mg protein were used. After 1 min preincubation at 37 °C, the reaction was initiated by addition of microsomal protein, and mixtures were incubated in open tubes for 15, 5, or 25 min, respectively, for stearic, palmitic, or oleic acid as substrate. The desaturation reaction was stopped by:

(a) 10% KOH in ethanol, followed by saponification at 80 °C for 45 min under N_2 atmosphere. The unsaponified fraction was extracted twice with 2 mL petroleum hydrocarbon (b.p. 30–60 °C), and discarded. After acidification with HCl, FAs were extracted three times with petroleum hydrocarbon, solvent was evaporated under N_2, and FAs were dissolved in 50 μL petroleum hydrocarbon. Finally, FAs were separated on thin layer chromatography (TLC) plates of silica gel G impregnated with 4% $AgNO_3$ (w/v), using toluene as solvent.

(b) Alternatively, the enzyme reaction was stopped by the addition of an appropriate volume of chloroform/methanol (2:1, by vol) [20] if phospholipids (PL) were to be isolated. The lower phase was dried down under stream of nitrogen and PL were isolated from this total lipid extract by TLC.

Lipid Extraction

Total lipids were extracted from washed parasites by the acidified extraction procedure of Bligh and Dyer [20], and 0.1 M KCl in 50% methanol was added to obtain a lower chloroform phase and an upper phase. The lower phase, containing lipids, was washed once with KCl solution, dried under N_2, and dissolved in an appropriate volume of chloroform/methanol (2:1, by vol).

Processing of Radioactive Samples

Separation and Analysis of PL and Neutral Lipids

Aliquots of the total lipid extracts were subjected to TLC to separate the total PL fraction from the neutral lipid (NL), using hexane/ethyl ether/acetic acid (80:20:1, by vol) as solvent. Following TLC, lipids were located by exposing the plates either to iodine vapour (for radioactivity analysis), or to UV light after spraying with 2,7′dichlorofluorescein in methanol (for further analysis of FAs).

Separation and Analysis of Labelled Fatty Acids from Lipids

Fatty acid methyl esters (FAME) were prepared from the lipid fractions separated by TLC with 10% BF_3 in methanol [21], or from FAs saponified with 10% KOH in ethanol. Labelled FAME were resolved according to their degree of unsaturation on TLC plates of silica gel G impregnated with 4% $AgNO_3$ (w/v), using toluene as solvent. FAME bands were located under UV light after spraying the plates with dichlorofluorescein, eluted [22], and evaporated to dryness at 35 °C, in counting vials. Then, 3 mL of Optiphase Hisafe 2 (PerkinElmer, USA), liquid scintillant was added to each vial, and the radioactivity was measured using a liquid scintillation counter (Beckman LS 60001 C, USA) [23].

Analysis of FBS Fatty Acids

Aliquots of FBS total lipids were subjected to methanolysis as described for labelled samples, in order to prepare FAME from FAs. BF_3 in methanol was added to lipids previously evaporated to dryness in screw-cap tubes. Tubes were added with N_2, sealed, and kept overnight at 45 °C. The resulting FAME were purified by TLC using hexane/diethyl ether (95:5, by vol), on plates of silica gel G pre-washed with methanol/diethyl ether (75:25, by vol). FAME were recovered from the silica support by agitation with water/methanol/hexane (1:1:1, by vol), followed by centrifugation, hexane extraction was repeated three times. FA analysis was performed using a Varian 3700 gas chromatograph equipped with two glass columns (2 m × 2 mm) packed with 15% SP 2330 on Chromosorb WAW 100/120 (Supelco Inc., Bellefonte, PA) and two flame ionization detectors. The column oven temperature was programmed from 155 to 230 °C at a rate of 5 °C/min. Injector and detector temperatures were 220

and 230 °C, respectively; the carrier gas was N_2 with a flow rate 30 mL/min.

Statistical Analysis

Results are shown as the means ± SEM for at least three independent experiments. Statistical analysis of data was carried out using Origin Pro 8 Copyright © 1991–2008.

Results

Effect of FBS on Δ^9 and Δ^{12} Desaturase Activities

FBS is essential for culturing *T. cruzi* epimastigote forms. The parasite obtains and metabolizes FAs from the culture medium, and a decrease in FBS concentration alters the degree of FA unsaturation of the signalling lipid [4]. In order to test the hypothesis that FBS concentration modulates desaturase activities, we first determined the FA composition of FBS. The major FAs were palmitic acid (16:0; 23.56%), stearic acid (18:0; 12.33%), and oleic acid (18:1n-9; 23.92%). Arachidonic acid (20:4n-6) and other long-chain FAs were detected in small amounts. To assess the effect of FBS concentration on desaturase activities, parasites were cultured with 5 or 10% FBS (control). 5% FBS decreased Δ^9 desaturase activity by 50% when stearic acid was used as the substrate. The FBS concentration had no effect on activities of Δ^9 with palmitic acid or Δ^{12} with oleic acid as substrates (Fig. 1). Specific activity of Δ^9 desaturase with 5% FBS and stearic acid as substrate was lower than that with 10% FBS, and was higher in the 105,000*g* pellet than in the supernatant (Table 1). The

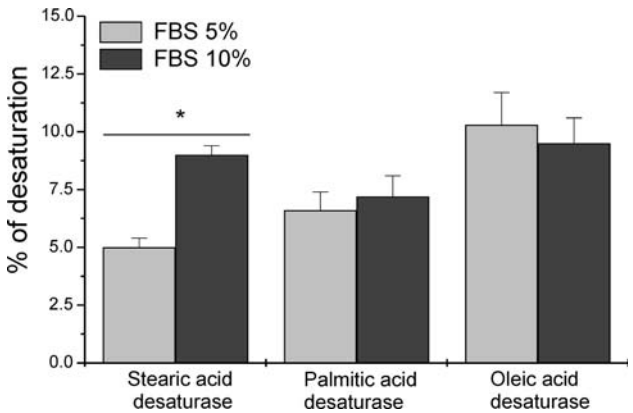

Fig. 1 Effect of fetal bovine serum (FBS) concentration on *T. cruzi* desaturase activities. Parasites were grown with 5 or 10% FBS and harvested at the logarithmic phase of growth (5 days). Δ^9 Desaturase was determined using [1-^{14}C]palmitic acid or [1-^{14}C]stearic as substrate. Δ^{12} Desaturase was assayed using [1-^{14}C]oleic acid as substrate. Results are expressed as percent of desaturation ± SEM, $n = 4$, * $P < 0.05$, *t* test

number of parasites decreased by 30% when they were cultured with 5% instead of 10% FBS (data not shown).

Kinetic Parameters of Desaturases

Δ^9 Desaturase

In view of our previous finding that palmitic acid is elongated to stearic acid and then desaturated to oleic acid and linoleic acid when these substrates are added to culture medium [13], we tested the possibility that both palmitic and stearic acids are substrates of Δ^9 desaturase. Δ^9 Desaturase activity increased up to 140 pmol/min/mg of protein within a stearic acid concentration range of 2.5–3.0 μM (Fig. 2). Apparent K_m and V_m values, determined from a Lineaweaver–Burk plot, corresponded to 2 μM and 219 pmol/min/mg of protein, respectively (Fig. 2, inset). In a study of requirement for reduced pyridine nucleotide, Δ^9 desaturase activity reached a maximum value with 8 μM NADH, and then remained constant (Fig. 3). Apparent K_m and V_m were 4.3 μM and 46.8 pmol/min/mg of protein (Fig. 3, inset). Reduced coenzyme nicotinamide adenine dinucleotide phosphate (NADPH) had the same effect as NADH; both formed 38% of oleic acid. Δ^9 Desaturase required a shorter incubation time when the substrate was palmitic acid; desaturase activity increased until 5 min, and decreased at longer times (data not shown). For palmitic acid, apparent K_m and V_m were 1.33 μM and 58.8 pmol/min/mg of protein (data not shown).

Δ^{12} Desaturase

In view of our finding that the level of diunsaturated FAs was altered by 5% FBS in phosphoinositides, we examined the possibility that Δ^{12} desaturase could be responsible for these changes and be affected by FBS. Δ^{12} Desaturase activity as a function of substrate concentration is shown in Fig. 4. Enzyme activity deviated from Michaelis–Menten kinetics at oleic acid concentrations above 10 μM; however, such an effect is unlikely to occur in vivo since the endogenous substrate concentration is much lower. Apparent K_m and V_m values determined from a Lineweaver–Burk plot were 1.03 μM and 2.8 pmol/min/mg of protein (Fig. 4, inset). There was no difference between NADPH and NADH requirements (data not shown).

Effects of Protein Concentration and Preincubation Time on Desaturase Specific Activities

The effects of the variation in microsomal protein concentration on enzyme activity were tested at 3.02 μM for stearic acid and at 2.6 μM for oleic acid. When the

Table 1 Effect of fetal bovine serum concentration on *T. cruzi* desaturase activities in different fractions

Fatty acids transformation	Specific activity (pmol/min/mg of protein)					
Cell fractions	Stearic acid		Palmitic acid		Oleic acid	
	5% FBS	10%	5% FBS	10%	5% FBS	10%
Supernatant 25,000*g*	65 ± 11	222 ± 37	ND	ND	ND	ND
Supernatant 105,000*g*	34.8 ± 2.2	123.0 ± 7.6	ND	ND	ND	ND
Pellet 105,000*g*	206 ± 10	538 ± 27	141 ± 15	155.9 ± 4.5	7.84 ± 0.94	8.82 ± 0.88

Cell fractions were obtained by differential centrifugation from *T. cruzi* epimastigotes grown with either 5 or 10% of FBS. Values are means ± SEM of three separate experiments

ND not determined

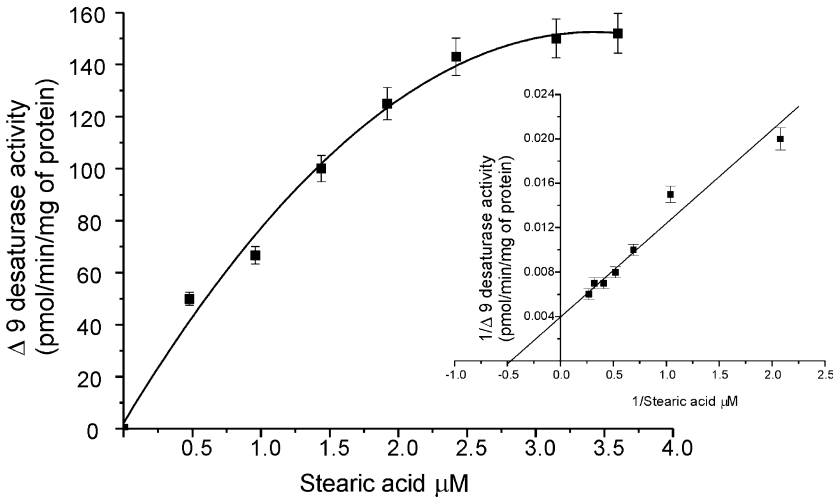

Fig. 2 Effect of concentration of substrate [1-^{14}C]stearic acid on *T. cruzi* Δ^9 desaturase activity. Incubation was performed at 37 °C for 15 min. The assay mixture is described in "Experimental Procedure". A double reciprocal plot was constructed to obtain apparent K_m and V_m (*inset*). Values are means ± SEM from three separate experiments

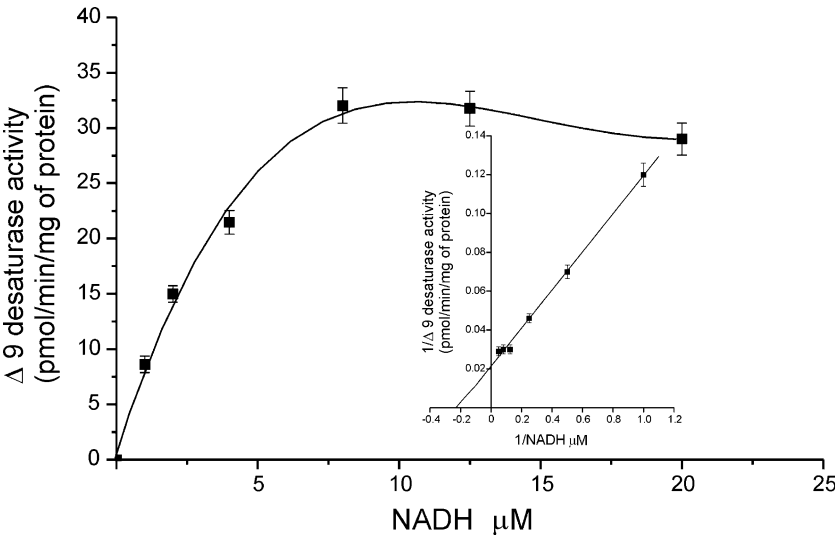

Fig. 3 Δ^9 Desaturase activity as a function of NADH concentration. Incubation was performed at 37 °C for 15 min. The assay mixture is described in "Experimental Procedure". A double reciprocal plot was constructed to obtain apparent K_m and V_m (*inset*). Values are means ± SEM from three separate experiments

microsomal protein concentration increased, Δ^9 desaturase activity increased linearly up to 0.2 mg, and Δ^{12} desaturase activity increased up to 0.8 mg, beyond these protein concentrations, a linear relationship was no longer observed (Fig. 5a, b).

Activity of Δ^9 desaturase required a shorter preincubation time than that of Δ^{12} desaturase. Δ^9 desaturase activity increased up to 15 min, and decreased thereafter (Fig. 6). For Δ^{12} desaturase, maximum activity was observed at 35 min (data not shown). However, it is possible that

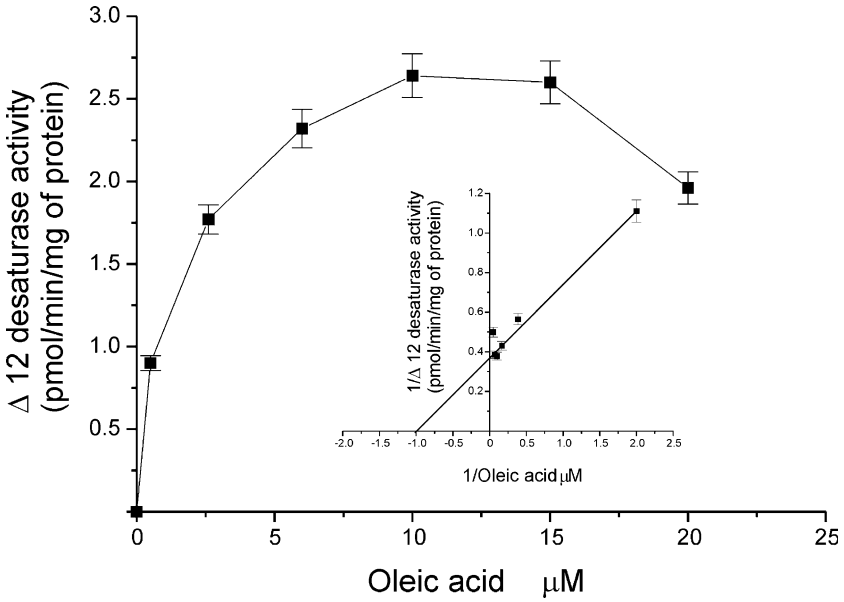

Fig. 4 Effect of [1-^{14}C]oleic acid concentration on *T. cruzi* Δ^{12} desaturase activity. Incubation was performed at 37 °C for 25 min, under the conditions described in "Experimental Procedure". A double reciprocal plot was constructed to obtain the apparent K_m and V_m (*inset*). Values are mean ± SEM obtained from three separate experiments

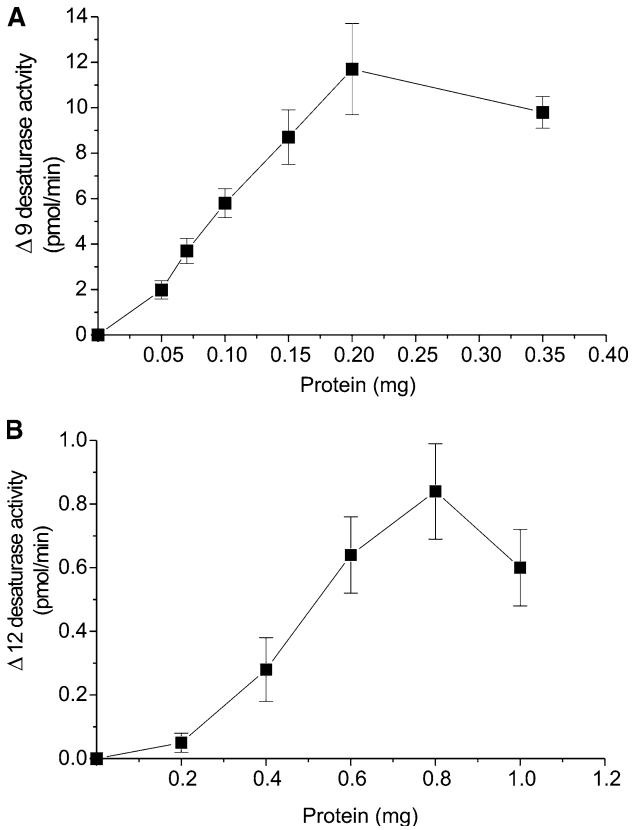

Fig. 5 Effects of 105,000*g* pellet protein concentration on *T. cruzi* Δ^9 (**a**) and Δ^{12} (**b**) desaturase activities. Protein concentration varied from 0.05 to 0.4 mg for Δ^9 desaturase activity, and from 0.2 to 1 mg for Δ^{12} desaturase activity. Incubation was performed at 37 °C for 15 min and 25 min, for Δ^9 and Δ^{12} desaturase, respectively. Reaction conditions are described in "Experimental Procedure". Values are means ± SEM of three separate experiments

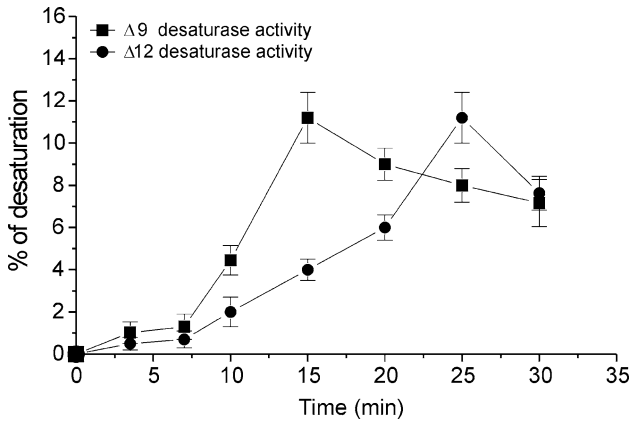

Fig. 6 Effect of incubation time on desaturase activities, determined as described in "Experimental Procedure". 18:0 and 18:1n-9 were used as substrates to test Δ^9 and Δ^{12} desaturase activity, respectively. Values are means ± SEM of three separate experiments

products of non-specific elongation of oleate, linoleate, or FA with the double bond in different positions were formed at this time, giving rise to other desaturase activities. For this reason, Δ^{12} desaturase activity was assayed at 25 min in subsequent experiments.

Other Biochemical Properties of Δ^9 Desaturase

In view of our previous finding that palmitic acid added to the culture medium is metabolized and incorporated into complex lipids by *T. cruzi* epimastigotes [13], we tested the behavior of palmitic acid with the microsomal fraction enzyme source.

Table 2 Effect of time on incorporation of [1-^{14}C] palmitic acid in lipids

Time (min)	Incorporation of radioactive substrate (%)		
	2	5	10
Lipid fractions			
FFA	63.0 ± 1.5	55.6 ± 1.1	45.5 ± 1.7
NL	21.5 ± 1.9	24.8 ± 1.5	24.6 ± 0.1
PL	15.5 ± 1.1	19.5 ± 0.4	29.8 ± 1.8

Incorporation of [1-^{14}C] palmitic acid in neutral lipids (NL) and phospholipids (PL) was followed for 2, 5, and 10 min. Lipids were extracted as described by Bligh and Dyer [20], and different fractions were obtained by TLC. Values represent means ± SEM of three independent experiments

Incorporation of [1-C^{14}]palmitic acid in lipids was dependent on incubation time. We observed different effects on the radioactivity detected in free fatty acid (FFA), NL and PL fractions, especially at 2 vs. 10 min incubation time (Table 2). The greatest radioactivity increase in PL (from 15.5 ± 1.1 to 29.8 ± 1.8%, $n = 3$, $P < 0.05$) and the greatest decrease in FFA (from 63.0 ± 1.5 to 45.5 ± 1.7%, $n = 3$, $P < 0.05$) were found at 10 min. In the presence of reduced coenzymes, a 26% increase in radioactivity incorporation in PL was observed at 10 min (data not shown). FFA showed the highest radioactivity at all times tested. Since [1-^{14}C]palmitic acid was incorporated in PL, the function of phosphatidylcholine (PC) as substrate of *T. cruzi* desaturases was confirmed using 1,2 di [1-^{14}C] palmitoylphosphatidylcholine.

Since it is possible that 1,2 di [1-^{14}C] palmitoylphosphatidylcholine is hydrolyzed before serving as a substrate of desaturases, levels of radioactivity on complex lipids and FFA were tested at various incubation times. Over 93% (93.6 ± 2.2, $n = 12$) of total radioactivity was recovered in PL at 30 min, indicating that hydrolysis did not occur. After testing desaturase activity using 1,2 di [1-^{14}C] palmitoylphosphatidylcholine as substrate, FAs were saponified, FAME were obtained, and analyzed by $AgNO_3$ TLC. When the reaction time was 5 min, 17% of the radioactivity was observed in monounsaturated FAs, and the amount of label increased by 29.1% at 10 min. As a consequence, radioactivity in saturated FAs decreased from 64 to 51% between 5 and 10 min. About 19% of radioactivity was found in the diunsaturated FA fraction, suggesting that 1,2 di [1-^{14}C] palmitoylphosphatidylcholine may also be a desaturase substrate in *T. cruzi*.

Discussion

Our previous biochemical studies showed that the FA composition of *T. cruzi* phosphoinositides is significantly altered by decreased FBS concentrations in culture medium [4], and that the FBS concentration is a key factor for growth of *T. cruzi* epimastigote forms in culture. In the present study, we examined the effect of FBS concentration on Δ^9 and Δ^{12} desaturase activities in *T. cruzi* epimastigotes, in relation to our previous findings. When stearic acid was the substrate and the FBS concentration was lowered from 10 to 5%, epimastigotes were able to modify Δ^9 desaturase; consequently, oleic acid levels were also lower than at 10% FBS. Thus, a smaller amount of substrate was available for Δ^{12} desaturase. This could explain the decreased level of linoleic acid in phosphoinositides we had observed previously [4]. Modulation of desaturase activity occurs only in the presence of FAs that serve as substrates of the enzyme. In *T. cruzi*, a decrease in FBS leads to a reduction in Δ^9 desaturase activity, since a decrease in the stearic acid level from FBS occurred when the concentration was 5%. In contrast, when palmitic acid was the substrate, FBS was not able to modify Δ^9 desaturase activity. Since the palmitic acid level is twice that of stearic acid in FBS, its variation in the culture medium may affect Δ^9 desaturase activity for palmitic acid to a lesser extent. The low susceptibility of Δ^{12} desaturase to FBS concentration suggests a significant role of this enzyme in the synthesis of linoleic acid. Availability of oleic acid may be responsible for the decrease in diunsaturated FAs when parasites are cultured with 5% FBS [4].

Furthermore, since the decrease in FBS concentration also implicates a decrease in the oleic acid level, we alternatively considered that the oleic acid level produce by Δ^9 desaturase activity could also be a signal for triggering the differentiation in the parasite [3].

Modification in the degree of unsaturation of FAs in *T. cruzi* was also observed by Florin-Christensen et al. [5] when epimastigotes were transferred from 28 to 37 °C, reflecting a response to environmental conditions. In the present study, desaturase activity was detected in all fractions studied, indicating a complex distribution pattern as shown for *Tetrahymena pyriformis* [24]. In *T. cruzi* epimastigotes, Δ^9 desaturase showed highest specific activity in the 105,000*g* pellet, similarly observed in mammals and yeast [25–27]. K_m values obtained for *T. cruzi* desaturases show that the same amounts of palmitic, stearic, and oleic acids are required for both desaturases to reach 50% of the maximum velocity. However, once K_m is reached, desaturation of stearic acid by Δ^9 desaturase occurs 3.7 times faster than for palmitic and 78 times faster than for oleic acid by Δ^{12} desaturase. Thus, Δ^9 desaturase is capable of using palmitic acid CoA and stearic acid CoA in spite of the differences in the V_m value. This is consistent with results in mice, where four Δ^9 desaturase isoforms capable of using palmitic acid and stearic acid CoA were reported [28]. Palmitic acid desaturation increased up till 5 min,

whereas enzyme activity increased until 15 min when stearic acid was the substrate. These findings suggest that desaturases use palmitic acid first, the product of de novo synthesis in *T. cruzi,* and then stearic acid. These time courses are consistent with a normal precursor–product metabolic relationship, implying sequential synthesis.

In addition to acyl-CoA desaturases as found in animals and fungi, FAs in plants and cyanobacteria are desaturated by acyl-lipid desaturases [29].

The phylogenetic relationships among Δ^4/Δ^5 desaturases in lower eukaryotes and Δ^6 desaturase from cyanobacteria were analyzed by Tripodi et al. [16]. Our results suggest that *T. cruzi* can desaturate FAs esterified to a phosphoglycerolipid, similarly to *Synechococcus*. In this algal genus, the acyl-lipid Δ^9 desaturase also uses palmitic acid esterified to phosphoglycerides [30]. In contrast, in *Synechocystis*, acyl-lipid Δ^9 desaturases are specific to stearic acid esterified at the C-1 position of a glycerolipid [29].

Both mono and diunsaturated FAs were produced when 1,2 di [1-^{14}C] palmitoylphosphatidylcholine was used as substrate. Desaturation by *T. cruzi* is comparable to the oleic acid desaturation systems in yeast [31] and in *Tetrahymena pyriformis* [32], in that one substrate may be a phospholipid. The *Acanthamoeba castellanii* desaturase, on the other hand, uses only FAs linked to phospholipids [33]. FAD2 microsomal Δ^{12} desaturases in higher plants use FAs esterified to a phosphoglycerolipid backbone, although the FAD2 enzyme does not display a preference for either *sn*-1 or *sn*-2 position [25]. Biochemical characterization of Δ^{12} desaturases from insects indicates that they use acyl-CoA substrates [34]. In *T. cruzi*, desaturation of stearic acid increased up to 15 min and formation of linoleic acid from oleic acid increased up to 35 min, at a much slower rate, suggesting that the activities of the two enzymes are coupled. Gabrielides et al. [35] obtained similar results for *Neurospora.*

T. cruzi desaturases appear to be related to other microsomal Δ^9 and Δ^{12} desaturases, since they show substrate requirements similar to those of microsomal desaturases in higher plants and fungi [36, 37]. Regarding the influence of microsomal protein concentration, the activities of both enzymes showed the same behavior: a breakdown in the linearity in the same concentration range. Since either stearic acid or oleic acid was rapidly incorporated into phospholipids, less substrate may be available for Δ^9 or Δ^{12} desaturation. This could explain the observation that an increase in microsomal protein above 0.2 mg for Δ^9 desaturase, or 0.8 mg for Δ^{12} desaturase, produces a decrease in conversion of stearic acid to oleic acid, or oleic acid to linoleic acid. It is possible that incorporation of the FA substrate in microsomal lipids competes with the desaturation reaction, as suggested by Irazú et al. [38]. Reduced coenzymes NADH and NADPH showed similar effects on desaturation, indicating that they both yield electrons in similar amounts for the enzymatic system. Similar requirements have been reported by Fukushima et al. [39] for *T. pyriformis* MT-1, and by Shipiro and Prescott [24] for *T. pyriformis* W. In *T. pyriformis* W, NADH is three times more efficient than NADPH as an electron donor for this reaction.

We observed that the decreased percentage of radioactivity in free palmitic acid was due to its incorporation into complex lipids, which depended on incubation time (Table 2). These results are consistent with those for *T. cruzi* by Florin-Christensen et al. [5]. These authors explained the process as part of a mechanism for adaptation to different temperatures, and proposed a role of triacylglycerides and sterol esters for storage of FAs in membrane lipids. In our study, incorporation of radioactivity from [1-^{14}C]palmitic acid into PL was at a maximum at 10 min, and was enhanced in the presence of reduced coenzymes. This increase could have resulted from an increase in (1) desaturase activities that form unsaturated FAs efficiently; those would then be incorporated into PL by specific acyl transferases; (2) activity of elongases, which also require an electron donor, and whose products (FAs with a chain length greater than that of palmitic acid) would be incorporated by specific acyl transferases; (3) acyl transferase activities resulting from an increased concentration of FA substrates formed during NADH-dependent reactions. In view of previous and present findings, possibility (1) seems most likely.

In summary, the present results demonstrate the participation of *T. cruzi* Δ^9 desaturase in responses to environmental changes, which enhance our understanding of lipid metabolism in this parasite. Since 18:1n-9 induces cell differentiation to infective forms in *T. cruzi* [3], Δ^9 desaturase can be a potential target to attack parasite transmission and consequently, the determination of Δ^9 desaturase activity provides information that may also lead to improved design of chemotherapy drugs against Chagas' disease.

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