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THE SEARCH FOR ENZYMES WITH POTENTIAL IN CLINICAL PRACTICE AND THEIR CORRELATION WITH PATHOGENIC MICROORGANISMS: AN INTEGRATIVE REVIEW

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Abstract: In 1957, the important questions of how some bacteria grow using acetate as a sole carbon source, and how germinating seedlings convert fat to carbohydrate, were answered by the seminal studies. These studies from of Kornberg, Krebs and Beevers identified two new enzymes, isocitrate lyase and malate synthase, which, in conjunction with reactions of the citric acid cycle, allowed for the net synthesis of anapleurotic succinate from two molecules of acetyl-CoA via a pathway named the glyoxylate cycle. The discovery in 1976 by Lazarow and De Duve of the peroxisomal β-oxidation of fatty acids in rats substantiates the possibility of acetyl-CoA formation in these organelles. The glyoxylate cycle was originally described in bacterial cells grown in the medium with C_2-compounds as the only source of carbon. The induction of the glyoxylate cycle in prokaryotes, plants, and fungi occurs in response to the arising carbohydrate requirement in various physiological and stress situations.
Keywords: Glyoxylate cycle, Pathogenc, Isocitrate lyase and Malate synthase.

INTRODUCTION

The glyoxylate cycle is a special variant of the tricarboxylic cycle (TCA) that allows utilization of two carbons compounds in the absence of glucose. The glyoxylate cycle is generally not present in human and animal tissue, and can only be found in plants, bacteria, fungi and protists (Chew *et al*., 2019c). As a shunt in the TCA cycle, the glyoxylate cycle shares three out of five metabolic enzymes with the cycle: malate dehydrogenase (EC 1.1.1.37), citrate synthase (EC 2.3.3.1) and aconitase (EC 4.2.1.3), by-passing the two decarboxylation steps catalyzed by isocitrate dehydrogenase (EC 1.1.1.41) and α-ketoglutarate dehydrogenase complex (EC 1.2.4.2, EC 2.3.1.61, EC 1.8.1.4).

The glyoxylate cycle is a metabolic pathway well characterized in plants, fungi and several microorganisms, that able these organisms to use fats for the synthesis of carbohydrates via the acetate generated during fatty acid β-oxidation (Nakazawa *et al*., 2005). The first enzyme of glyoxylate shunt pathway is isocitrate lyase (EC 4.1.3.1) that transforms isocitrate into glyoxylate. The second enzyme, malate synthase (EC 2.3.3.9), transfers the acetyl group from acetyl-CoA to glyoxylate to further generate malate and CoA (Quartararo *et al.,* 2011; Kumar *et al.*, 2011) (Figure 01).

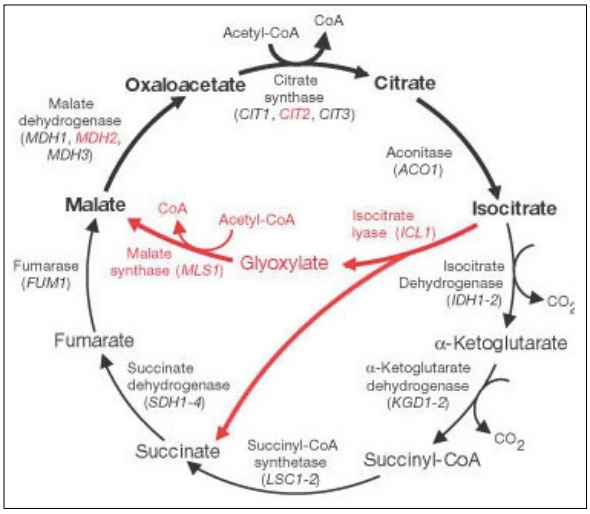

Figure 01 - Schematic drawing of the glyoxylate and tricarboxylic acid cycles (Lorenz and Fink 2002).

Isocitrate lyase is induced during intracellular growth of several pathogens and it has been assumed that isocitrate lyase and the entire glyoxylate cycle are involved in virulence and pathogenicity of human pathogenic bacteria like *Mycobacterium tuberculosis* and fungi such as *Candida albicans* (Lorenz & Fink, 2001; McKinney *et al.,* 2000; Muñoz-Elías & McKinney, 2005).

The role of the methylcitrate cycle is the apparently simple α-oxidation of propionate to pyruvate. It resembles the part of the glyoxylate cycle in which acetate is oxidized to glyoxylate at the corresponding carbon. The

methylcitrate cycle was initially postulated by studies with mutant strains of *Candida lipolytica*, in which accumulation of either methylcitrate or 2-methylisocitrate was observed during growth on odd-chain fatty acids, which are degraded via propionyl-CoA (Tabuchi *et al*., 1974).

Malate synthase is an important component of the phosphoenolpyruvate-glyoxylate cycle in *Escherichia coli*, which functions under conditions of glucose hunger (Fischer & Sauer, 2003). In addition, it serves an anaplerotic role in glycolate oxidation (Kornberg & Sadler, 1960; Ornston & Ornston, 1969), persistence of *Mycobacterium tuberculosis* is facilitated by the glyoxylate bypass (McKinney *et al*., 2000), and this metabolic pathway is required for high virulence of *Candida albicans* (Lorenz & Fink, 2001).

In this review, we intend to address the state of the art of relevant publications and studies on the glyoxalate cycle and its implications for understanding the virulence mechanism of action of pathogenic microorganisms, as well as the focus on cycle enzymes as potential targets for effective drugs.

MATERIALS AND METHODS

This study is an integrative review. This method aims to synthesize and incorporate the search for the best and most recent evidence and results obtained in research on a topic or issue, in a systematic, orderly and comprehensive manner.

The following databases were accessed to locate the materials: Google Scholar, Scielo, PubMed, NCBI (National Center for Biotechnology Information), Wiley Online Library.

The selection criteria, as well as the keywords for the search, were based on the context focused on the Glyoxalate Cycle and diseases caused by pathogenic microorganisms. After a selective and objective reading, the articles and books were selected, observing the year of publication, place of publication, results and conclusions.

RESULTS AND DISCUSSION

Taking into account the three domains of living organisms (Bacteria, Eukarya and Archaea), the key enzymes of this cycle have been detected in Bacteria and fungi, plants, some invertebrates, and even in some vertebrates (Woese *et al*., 1990; Davis *et al*., 1990). Operation of the glyoxylate cycle in peroxisomes would result in efficient succinate production which in turn can fuel gluconeogenesis in the rat liver (Lazarow *et al*., 1976).

The glyoxylate pathway is important in fungal and bacterial pathogenesis (Lorenz & Fink, 2002; Vereecke *et al*, 2002). The transcriptional profile of phagocytosed populations of human pathogenic fungi *C. albicans* demonstrated that all the steps of the glyoxylate cycle are induced (Lorenz *et al*, 2004).

Isocitrate lyase enzyme activity is determined using Dixon and Kornberg's method (Kornberg & Krebs, 1957). The basic concept of this method is to spectrophotometrically measure the formation of glyoxylate-phenylhydrazone at 324 nm in the presence of phenylhydrazine and isocitrate. Malate synthase enzyme activity is determined as described by Dixon and Kornberg (1959) and modified by Polakis and Bartley (1965). The method is based on the consumption of acetyl-CoA at 232 nm. Those method are efficient, however are time-consuming and expensive.

Northern blot and differential display experiments with *C. albicans* in the presence of macrophages revealed that both ICL and MLS are induced (Lorenz & Fink, 2001; Prigneau *et al*., 2003). In addition, both

enzymes are induced in *C. albicans* exposed to human neutrophils (Fradin *et al*., 2005). High enzymic activities of ICL and MLS were detected in *C. albicans* strains isolated from diabetic patients suffering from vulvovaginal candidiasis (Lattif, *et al*., 2006). Furthermore, evaluation of ICL mutants in a mouse model demonstrated that activity of this enzyme is essential for fungal virulence (Lorenz & Fink, 2001).

Alber and coworkers focused on *Rhodobacter sphaeroides*, a nutritionally versatile purple non-sulphur photosynthetic bacterium capable of growth with acetate as the sole carbon source, yet known to lack isocitrate lyase. Using a combination of genetics, physiology and biochemistry, the authors reveal a new pathway for acetate assimilation (Alber et al., 2006).

Only isocitrate lyase activity has been clearly detected in some genera of halophilic Archaea. That activity was induced when acetate was the main carbon source. There is an earlier work, in which isocitrate lyase and malate synthase activities were detected in the archaeon *Halobacterium salinarum* (Oren *et al*., 1995; Aitken *et al*., 1969). Acetate could have a role in the nutrition of natural communities of halophilic Archaea, as it is produced from glycerol in hypersaline lakes by some species of halophiles. *Haloferax volcanii* is a halophilic archaeon able to grow in minimal medium with acetate as the sole carbon source. Isocitrate lyase activity was detected in this organism when it was grown on a medium with acetate as the main carbon source (Kauri *et al*., 1990).

In *Escherichia coli* and *Aspergillus nidulans*, propionate is oxidized to pyruvate via the methylcitrate cycle. The last step of this cycle, the cleavage of 2-methylisocitrate to succinate and pyruvate is catalysed by 2-methylisocitrate lyase. The accumulation of either methylcitrate or 2-methylisocitrate was observed during growth on odd-chain fatty acids, which are degraded via propionyl-CoA. Further studies demonstrated the existence of methylcitrate synthase, methylcitrate dehydratase and 2-methylisocitrate lyase (Tabuchi et al., 1995).

The filamentous hemiascomycete *Ashbya gossypii* is a natural overproducer of vitamin B_2 (riboflavin). The ability of plants and microorganisms to grow on fatty acids as carbon source is based on the function of the glyoxylate cycle. Since the precursors of riboflavin, GTP and ribulose 5-phosphate, originate from carbohydrate metabolism, the glyoxylate cycle in concert with gluconeogenesis plays an essential role with respect to growth and riboflavin synthesis of the fungus. Isocitrate lyase, the key enzyme of this metabolic pathway, catalyzes the cleavage of isocitrate to glyoxylate and succinate diverting isocitrate through a carbon-conserving pathway (Wickerham et al., 1946; Schmidt et al., 1996).

Paracoccidioides spp is a thermally dimorphic fungus that causes Paracoccidioidomycosis (PCM), a human systemic granulomatous mycosis (Rippon, 1980; Lutz, 1908). PCM is endemic at Latin America, and at Brazil, it is responsible for 51.2% of the death caused by systemic mycosis (Prado *et al.,* 2009). In yeast cells of the human pathogenic fungus *Paracoccidioides* spp, isocitrate lyase transcript and protein (*Pb*ICL) are highly abundant (Felipe *et al*., 2005; Cruz *et al*., 2011), and up-regulation occurs during transition from mycelium to yeast (Bastos *et al*., 2007; Rezende *et al*., 2011), as well as, during the infection process (Costa *et al*., 2007) and internalization by macrophages (Derengowski *et al*., 2008). In addition, the inactivation of *Pb*ICL by phosphorylation is reversible, denoting a new strategy for the rapid adaptation to changing environmental conditions (Cruz

et al., 2011). From *Paracoccidioides sp.*, RT-PCR analysis showed that transcript levels of the ICL and MLS genes in this fungus increased following phagocytosis by murine macrophages (Derengowski *et al*., 2008).

Malate synthase of *Paracoccidioides* spp (*Pb*MLS) participates in the glyoxylate pathway and in the allantoin degradation pathway of the purine metabolism, which allows the fungus to use nitrogen compounds (Zambuzzi-Carvalho *et al*., 2009). *Pb*MLS is localized in peroxisomes, on the cell surface, and is secreted. In addition, *Pb*MLS plays a role as adhesin, with capacity to mediate adhesion and internalization of the fungus to host cells (Neto *et al*., 2009). *Pb*MLS interacts with proteins from different functional categories, suggesting their multiple roles and locations (Oliveira *et al*., 2013).

Because of the importance of isocitrate lyase and malate synthase, both enzymes are a subject of the potential drug investigation. Some isocitrate lyase inhibitors compounds to *Candida albicans* and *Mycobacterium tuberculosis* have been investigated (Kim *et al*., 2012; Sriram *et al*., 2011). However, no inhibitor has been yet sought to *Pb*ICL and *Pb*MLS.

In the animal pathogen *Aspergillus fumigatus*, ICL expression was detected in hyphae and in conidia (Ebel *et al*., 2006). To evaluate if the glyoxylate cycle was involved in the virulence of *P. marneffei*, northern blot experiments were performed. These showed that after macrophage internalization of conidia the ICL-encoding gene (acuD) was highly expressed, suggesting a potential role for the cycle in the pathogen's adaptation inside macrophages (Thirach *et al*., 2008). Together, these data directly or indirectly support the relevance of the glyoxylate pathway in fungal virulence in plants, animals and humans.

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