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Mini Review

ATP-citrate lyase: A mini-review

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ABSTRACT

ATP-citrate lyase (ACLY) is a cytosolic enzyme that catalyzes generation of acetyl-CoA from citrate. Acetyl-CoA is a vital building block for the endogenous biosynthesis of fatty acids and cholesterol and is involved in isoprenoid-based protein modifications. Acetyl-CoA is also required for acetylation reactions that modify proteins such as histone acetylation. In the present review some of the known features of ACLY such as tissue distribution, subcellular localization, enzymatic properties, gene regulation and associated physiological conditions are highlighted.

1. Introduction

ATP citrate lyase (ACLY) is a cytosolic enzyme responsible for the synthesis of acetyl-CoA [1]. It catalyzes the formation of acetyl-CoA and oxaloacetate from citrate and CoA with a simultaneous hydrolysis of ATP to ADP and phosphate. The product, acetyl-CoA, is involved in several important biosynthetic pathways, including lipogenesis and cholesterogenesis. Acetyl-CoA is also required for acetylation reactions that modify proteins such as histone acetylation.

The current review summarizes some of the known features of ACLY such as tissue distribution, sub-cellular localization, enzymatic properties, gene regulation and physiological roles associated with this enzyme.

2. Tissue distribution and subcellular localization

ACLY is most abundantly expressed in the liver and white adipose tissue [2,3] while it exhibits low expression levels in brain, heart, small intestine and muscles [2,4]. ACLY is also expressed and active in pancreatic beta cells [3,5]. Additionally, over-expression of ACLY is associated with certain pathological conditions that will be discussed later in this article.

ACLY is mainly a cytosolic enzyme. It is reported that ACLY is bound to endoplasmic reticulum in mammalian cells [6]. However, recently it was showed that, in addition to cytoplasm, ACLY is also detected in nuclei of different mammalian cells [7]. ACLY was identified in both nucleus and cytoplasm of human glioblastoma cells, mouse embryonic fibroblasts, murine pro-B-cell lymphoid cells and human colon carcinoma cells [7]. Citrate is a small molecule that can diffuse freely through the nuclear pore complex [8] hence, ACLY-mediated acetyl-CoA production may occur in both cytoplasmic and nuclear compartments of mammalian cells [7].

3. Crystal structure

ACLY protein is a homotetramer of four identical subunits [9]. Each polypeptide chain contains 1101 amino-acid residues [10]. The crystal structure of full-length ACLY protein is yet unresolved. However, Sun et al. recently succeeded in crystallizing chymotrypsin-truncated human ACLY [11,12]. X-ray crystallography data obtained from these studies revealed about two-third of the structure of ACLY. The binding sites for both citrate and ATP have also been identified [11]. More recently the structure for the amino-terminal portion of the enzyme containing 1–817 amino acid residues was crystallized in the presence of tartrate, ATP and magnesium ions [12].

4. Enzymatic properties

4.1. Reaction

ACLY catalyzes the formation of acetyl-CoA and oxaloacetate from citrate and coenzyme A in the presence of ATP. The simple version of the reaction could be expressed in the following equation;

Abbreviations: ACLY, ATP-citrate lyase; ACSS2, acyl-CoA synthetase short-chain family member 2; DPNH, reduced diphosphopyridine nucleotide.

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Citrate + ATP → acetyl-CoA + oxaloacetate + ADP + Pi

The detailed mechanism involves several steps. The enzyme catalysis is initiated by autophosphorylation of a histidine residue generating an unstable citryl-phosphate within the active site. Then, a covalent citryl–enzyme complex is formed and attacked by CoA to generate citryl-CoA. Finally, the enzyme catalyzes the cleavage of citryl-CoA into acetyl-CoA and oxaloacetate [13].

4.2. Assays

ACLY activity is usually measured by coupling to enzymes such as malate dehydrogenase or chloramphenicol acetyltransferase [14,15]. Oxaloacetate production is measured by reaction with DPNH/NADH. The change in absorbance in the presence of exogenous ATP allows determination of specific ACLY activity. This assay can measure ACLY activity in a broad range of samples differing in protein concentrations. However, this approach is an indirect measure of ACLY activity. More recently Ma et al. reported a direct homogenous assay for the measurement of ACLY activity [16]. They added radioactively labeled citrate into ACLY-reaction mixture and its incorporation into acetyl CoA was measured.

4.3. Inhibitors

Several ACLY inhibitors have been evaluated for their ability to block fatty acid synthesis and/or cholesterol biosynthesis. Citrate analogs are one of the most widely studied ACLY inhibitors. These included the (+) and (−)-2,2-difluorocitrate, both of which demonstrated activity against rat-liver ACLY [17]. Benzenesulfonamides also inhibited ACLY activity at low micromolar range [17]. In addition to the synthetic inhibitors a naturally occurring citrate analog namely (−)-hydroxycitrate is found to be a potent inhibitor of ACLY [17]. Treatment of (−)-hydroxycitrate results in decreased cholesterol and fatty acid synthesis in HepG2 cells. However, this inhibitor has certain limitations. It is inefficiently transported across the cell membrane and very high concentrations are required to achieve complete inhibition of ACLY activity [17]. Moreover, (−)-hydroxycitrate also inhibits isocitrate dehydrogenase (IDH) at similar concentrations that are required to inhibit ACLY. Another ACLY inhibitor SB-204990 is shown to be effective in both *in vivo* and *in vitro* models [14]. Radicicol, a naturally occurring antifungal macrolide, noncompetitively inhibits rat liver ACLY. However, it has been much more widely studied for its ability to bind to and inhibit heat shock protein 90 (Hsp90). Although several ACLY-inhibitors have been described, at this point they only have a heuristic value. The therapeutic potential of ACLY-inhibitors needs further clarification. Additional studies are required to verify the sensitivity and specificity of existing inhibitors and to identify more potent and specific ACLY-inhibitors.

5. Regulation of ACLY expression and activity

ACLY expression is mainly regulated by the transcription factor SREBP-1 (sterol regulatory element binding protein-1) [18]. SREBP-1 up-regulates ACLY at mRNA level *via* Akt signaling [19]. However, ACLY protein levels are independent of SREBP-1 [20]. It has been suggested that PI3K/Akt pathway stimulates ACLY activity predominantly through phosphorylation of ACLY rather than transcriptional up-regulation. The phosphorylation of ACLY contributes to its protein stabilization [20]. Thr446, Ser450 and Ser454 residues of ACLY are shown to be phosphorylated *in vitro* [21]. It has also been shown that treatment with PI3K inhibitors does not have a dramatic effect on dephosphorylation and inactivation of ACLY in lung cancer cells. Therefore, it has been suggested that ACLY activity is also regulated by some other pathways [20]. ACLY is reported to be phosphorylated at different sites by other kinases such as nucleoside diphosphate kinase [22] and cyclic AMP dependent protein kinase [23]. Phosphorylation of ACLY is enhanced by glucagon, insulin, vasopressin and transforming growth factor β1 [4].

6. Pathways served by ACLY

ACLY is a cross-link between glucose metabolism and fatty acid synthesis/mevalonate pathways (Fig. 1). In cytoplasm, glucose-derived citrate is transformed into acetyl-CoA by ACLY. Acetyl CoA is an essential substrate for mevalonate and FA synthesis pathways [14]. In the fatty acid synthesis pathway, acetyl-CoA is carboxylated into malonyl-CoA by acetyl-CoA carboxylase (ACACA). Next, the main lipogenic enzyme fatty-acid-synthase (FASN) performs condensation of acetyl-CoA and malonyl-CoA to produce the long-chain fatty acid palmitate [24]. Acetyl CoA is also a precursor for the mevalonate pathway. This pathway leads to the synthesis of farnesyl-pyrophosphate (FPP). FPP is involved in cholesterol biosynthesis but can also lead to synthesis of geranylgeranyl-pyrophosphate (GG-PP). Both FPP and GG-PP are respectively involved in farnesylation and geranylgeranylation of a variety of proteins [25]. Moreover, acetyl-CoA is required for acetylation reactions, for instance histone acetylation, that modify proteins having critical roles in regulating global chromatin architecture and gene transcription [7].

Recently, it was reported that in the tumor cells with defective mitochondria or in proliferating cells under hypoxic conditions, reductive carboxylation of glutamine-derived α-Ketoglutarate (α-KG) is responsible for supplying citrate for *de novo* lipogenesis [26,27].

7. Physiological roles associated with ACLY

Alterations in expression or activity of ACLY have been observed in different pathological conditions (Fig. 2). Moreover, variations in expression patterns of ACLY have been noticed during different

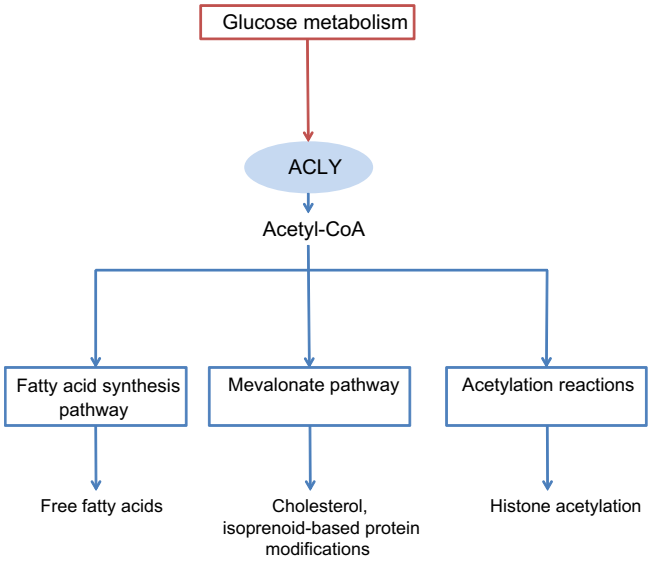

Fig. 1. ATP-citrate lyase is at the crossroads of several pathways. Glucose-derived citrate is converted by the action of ATP-citrate lyase (ACLY) to acetyl-CoA that is used as a precursor in fatty acids and mevalonate synthesis pathways. Acetyl-CoA is also required for acetylation reactions that modify proteins such as histone acetylation.

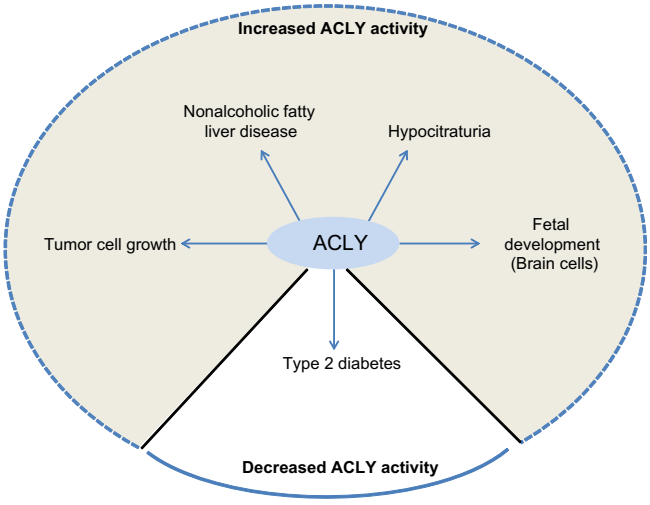

Fig. 2. Alterations in expression patterns of ACLY have been associated with several pathological conditions. Additionally, over-expression of ACLY is observed in murine fetal brain cells.

stages of embryonic development. In this section we will describe the physiological roles associated with ACLY in light of these observations.

7.1. Fetal growth and development

Beigneux et al. attempted to generate homozygous ACLY-knockdown mice by intercrossing heterozygous ACLY+/− mice. However, genotyping of more than 60 litters did not yield any homozygous ACLY-knockdown mouse. It showed that ACLY is essentially required for embryonic development and that the alternate ACSS2-dependent acetyl CoA production pathway is not sufficient to support embryonic development. The heterozygous ACLY+/− mice however showed normal lipid synthesis, suggesting that half-normal ACLY levels have no or little impact on cellular lipid homeostasis. Strikingly, ACLY expression in mice was found to be much higher in developing brain in comparison to the adult brain [28].

7.2. Tumor cell growth and survival

ACLY expression and activity has been reported to be up-regulated in lung, prostate, bladder, breast, liver, stomach, and colon tumors [20,29–34]. In human lung adenocarcinoma the expression of phosphorylated-ACLY has been shown to correlate with stage, differentiation grade and poor prognosis [20]. Inhibition of ACLY by either RNA*i* or pharmacological inhibitors results in growth-arrest in tumor cells, both *in vitro* and *in vivo* [14,20]. It has been observed that the phosphorylation of ACLY in cancer cells is directly regulated by the PI3K/Akt pathway [20]. Moreover, ACLY knockdown significantly impairs Akt-mediated tumorigenesis *in vivo*.

7.3. Metabolic disorders

Initially, it was observed that ACLY knockdown or inhibition leads to a decrease in glucose-induced insulin secretion [35]. This observation indicated potential involvement of ACLY in diabetes pathophysiology. Later, it was shown that expression and activity of ACLY are decreased by 55% in pancreatic islets of patients with type-2 diabetes in comparison with non-diabetic donors [36]. Recently, it has also been reported that elevated levels of circulating fatty acids suppress ACLY activity leading to pancreatic cell stress and apoptosis [37]. As cytosolic acetyl-CoA is required for both fatty acid and cholesterol biosynthesis, targeting ACLY could also have an impact on obesity. It has indeed been shown that plasma cholesterol and triglycerides levels are reduced in presence of ACLY inhibitors [38].

Additionally, over-expression of ACLY is observed in nonalcoholic fatty liver disease which leads to accumulation of triglycerides and hepatic steatosis [39]. Lastly, ACLY is also shown to be involved in citrate metabolism. Increased ACLY activity has been observed in rats with hypocitraturia [40].

The present review describes some of the known features of ACLY such as tissue distribution, subcellular localization, enzymatic properties, gene regulation and associated physiological conditions. ACLY plays a central role in energy metabolism as it synthesizes acetyl-CoA, a precursor for both fatty acid and cholesterol synthesis. ACLY expression is essentially required for fetal growth and development. Additionally, there is an increasing evidence supporting the importance of ACLY in many pathophysiological events such as tumor cell growth and metabolic disorders.

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