

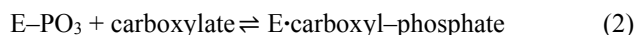
## Conservation of a glutamate residue in ATP-citrate lyase and succinyl-CoA synthetase

M.E. Fraser, J. Huang

Department of Biological Sciences, University of Calgary, 2500 University Dr. NW, Calgary, Alberta, T2N 1N4, Canada

frasm@ucalgary.ca

Succinyl-CoA synthetase (SCS), the enzyme that catalyzes the only substrate-level phosphorylation in the citrate cycle, is the prototype for a family of ADP- or GDP-forming acyl-CoA synthetases that includes ATP-citrate lyase (ACLY) [1]. These enzymes catalyze the formation of a thioester bond between an organic acid and CoA, using the energy of nucleotide triphosphate (NTP) and in the presence of magnesium ions. A histidine residue is transiently phosphorylated during catalysis [2], leading to the proposed catalytic mechanism:



where E represents the enzyme; –, a covalent bond; and ·, noncovalent interactions. For SCS, the carboxylate is succinate; for ACLY, it is citrate and there is fourth step in which citryl-CoA is cleaved to form acetyl-CoA and oxaloacetate.

A glutamate residue of ACLY, E599, was proposed to play a role in the cleavage of citryl-CoA [3]. This glutamate residue is conserved not only in ACLYs but also in SCSs (Fig. 1). The structures of SCSs and ACLYs found in the Protein Data Bank [4] are used to investigate the role of this conserved glutamate residue.

Human ACLY	IRTIAIIAEGIPEALTRKLIKKA-DQKGVTTIIGPATVGGIKPGCFKIGNTGGMLDNLASKLYR
<i>Chlorobium limicola</i> ACLY	IQLVSMITEGVPEKDAKRLKLA-QKLGKMLNGPSSIGIMSAGECRLGVIGGEFKNLKCNLRYR
Human GTPSCS $\alpha$ -subunit	IPLVVCITEGIPQDMVRVKKLLRQEKTRLIGPNCPGVINPGECKIGIMPG-----HIHK
<i>Escherichia coli</i> $\alpha$ -subunit	IKLIITITEGIPTLDMVTKVVKL-DEAGVRMIGPNCPGVITPGECKIGIQPG-----HIHK
<i>Thermus aquaticus</i> $\alpha$ -subunit	IPLIVLITEGIPTLDMVRAVEEI-KALGSRLIGGNCPGIISAEETKIGIMPG-----HVFK

**Figure 1.** Alignment of portions of the sequences of ACLYs and SCSs. The alignment shows conservation of a glutamate residue, E599 in human ACLY, E112 in the A-subunit of *Chlorobium limicola* ACLY, E105 $\alpha$  of human GTPSCS, E98 $\alpha$  of *E. coli* SCS, and E97 $\alpha$  of *Thermus aquaticus* GTPSCS.

[1] Sánchez, L. B., Galperin, M. Y. & Müller, M. (2000). *J. Biol. Chem.* **275**, 5794.

[2] Kreil, G. & Boyer, P. D. (1964). *Biochem. Biophys. Res. Commun.* **16**, 551.

[3] Wei, X., Schultz, K., Bazilevsky, G. A., Vogt, A. & Marmorstein, R. (2020). *Nat. Struct. Mol. Biol.* **27**, 33.

[4] Berman, H. M. *et al.* (2000). *Nucleic Acids Res.* **28**, 235.

**Keywords:** ATP-grasp fold; phosphohistidine; magnesium ions; nucleotide