Identification of the Inhibition Mechanism of Hydroxycitrate on Human ATP Citrate Lyase

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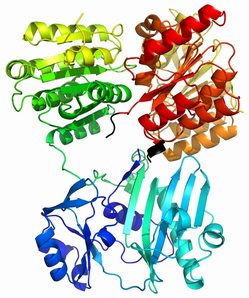
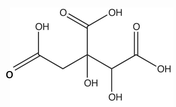
Introduction

ATP citrate lyase (ACLY) is responsible for catalysing the reaction: citrate + ATP + CoA → acetyl-CoA + oxaloacetate + ADP. Since acetyl-CoA is a precursor for *de novo* lipogenesis, this enzyme is related to multiple types of cancer and diabetes. Understanding the catalytic mechanism as well as how to inhibit this enzyme can give insight into the rational design of drugs to target the enzyme and treat these diseases. In this study, a structural approach was used to understand part of the catalytic mechanism as well as the inhibition mechanism of one of the enzyme's inhibitors, hydroxycitrate. The reaction is thought to occur through four steps:

1) ATP + E → E-P + ADP
2) E-P + citrate → E·citryl-P
3) E·citryl-P + CoA → E·citryl-CoA + P_i
4) E·citryl-CoA → E + acetyl-CoA + oxaloacetate



3MWD



Hydroxycitric Acid

Aim

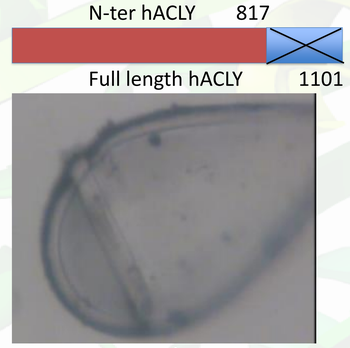
First, we aim to obtain the structure of the N-terminal protein (N-ter hACLY) with all key amino acid residues that are responsible for the first two steps of the reaction. Then we aim to have the inhibitor, hydroxycitrate, bind to N-ter hACLY, to determine the structure of the complex. Last, we aim to phosphorylate N-ter hACLY and bind hydroxycitrate to determine this structure so as to understand the inhibition mechanism.

Methods

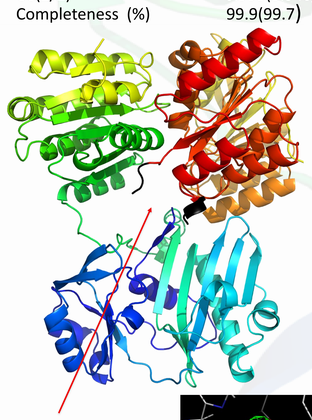
Major steps are: site-directed mutagenesis to introduce mutations, protein expression and purification to obtain pure protein, crystallization to obtain crystals, exposure of crystals to X-rays to obtain diffraction data, data processing and structure solution. Other experiments, e.g western blots, kinetic assays, were used to guide experimental design.

Results

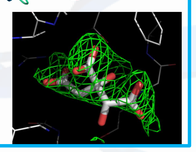
Crystals of N-ter hACLY (residues 1-817) with hydroxycitrate diffracted to 2.4 Å (left) but showed no electron density for the catalytic loop. Two mutations were done to obtain a different crystal form, where the catalytic loop was seen but these crystals were hard to grow and diffracted anisotropically to 2.0 Å (middle). The linker was removed by TEV protease to give a third crystal form that grows easily and diffracts isotopically to 1.7 Å (right).

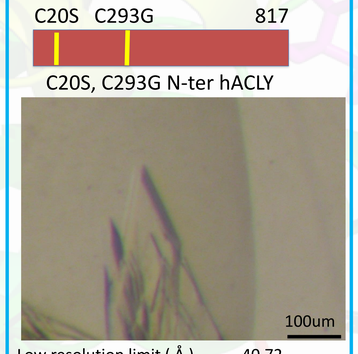


Low resolution limit (Å)	55.96
High resolution limit (Å)	2.40
Space Group	C2
R_{merge}	0.103(0.829)
Mean((I)/σ(I))	8.6(1.5)
CC(1/2)	0.995(0.540)
Completeness (%)	99.9(99.7)

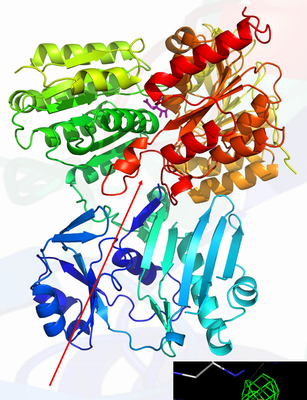


Missing catalytic loop

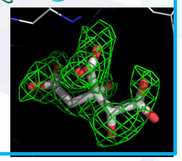


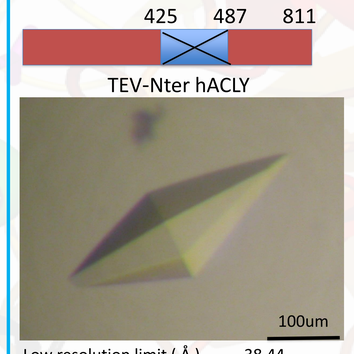


Low resolution limit (Å)	40.72
High resolution limit (Å)	2.00
Space Group	$P2_1$
R_{merge}	0.144(0.744)
Mean((I)/σ(I))	5.5(1.7)
CC(1/2)	0.975(0.486)
Completeness (%)	97.7(98.6)

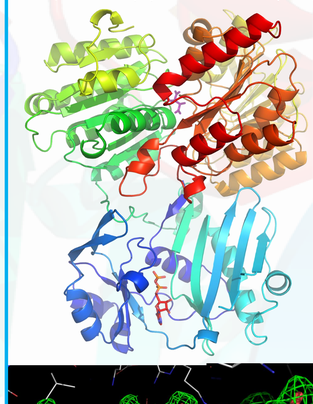


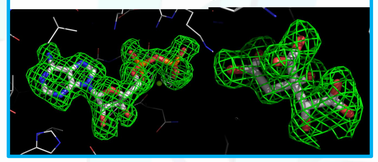
Obtain catalytic loop





Low resolution limit (Å)	38.44
High resolution limit (Å)	1.75
Space Group	$P2_12_12_1$
R_{merge}	0.102(0.629)
Mean((I)/σ(I))	7.1(1.8)
CC(1/2)	0.994(0.626)
Completeness (%)	99.2(99.4)





Kinetic Assays

Two-Step Assay:

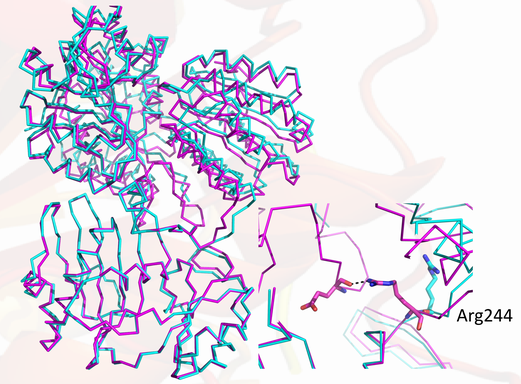
Citrate + ATP + CoA $\xrightarrow{ACLY}$ ADP + oxaloacetate + acetyl-CoA

Oxaloacetate + NADH $\xrightarrow{MDH}$ NAD^+ + malate

	K_m		K_i	
	Citrate	ATP	ADP	4*R*-hydroxycitrate
Full length hACLY	22.30 ± 4.28 µM	137.2 ± 18.15 µM	150 µM	3 µM

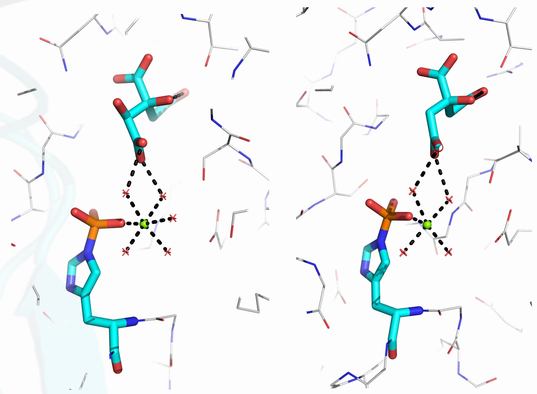
Structural Analysis

Catalytic Loop Stabilization



Superposition of two structures, magenta: with catalytic loop, cyan: without catalytic loop. With the catalytic loop, the conformation is more closed, with the citrate-binding domain potentially stabilizing the catalytic loop.

Binding Mechanism



4*S*-hydroxycitrate bound to TEV-Nter hACLY (left) and citrate bound to TEV-Nter hACLY (right) , with histidine phosphorylated and magnesium bound (green). The carboxyl group of hydroxycitrate interacts with water molecules that form octahedral coordination with magnesium.

Significance

This work demonstrates the success of rational design of the protein construct to obtain crystals that grow easily and diffract well. With this well-designed protein construct and high resolution diffraction, one can decipher the reaction mechanism in detail, as demonstrated by the hydroxycitrate inhibitor case. The structure with hydroxycitrate and ADP also sheds light on how this enzyme can be inhibited, potentially helping in the design of drugs to treat disease.