Induced Fit and the Catalytic Mechanism of Isocitrate Dehydrogenase

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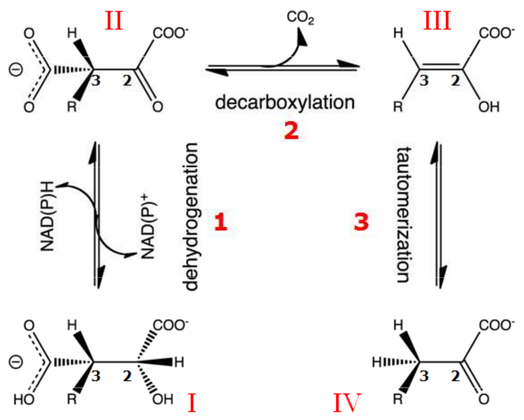
1. SUMMARY

NADP$^+$ dependent isocitrate dehydrogenase isoform 1 (IDH1; EC 1.1.1.42) belongs to a large family of α-hydroxyacid oxidative β-decarboxylases that catalyze similar three-step reactions, with dehydrogenation to an oxaloacid intermediate preceding β-decarboxylation to an enol intermediate followed by tautomerization to the final α-ketone product.

IDH1 is involved in the citric acid (Krebs) cycle and converts 2*R*,3*S*-isocitrate into α-ketoglutarate. A comprehensive view of the **induced fit** needed for catalysis is revealed on comparing the first **fully closed** conformations of *Escherichia coli* IDH1 in crystal structures of a pseudo-Michaelis complex of wild-type *E. coli* IDH1 and of a reaction product complex of the K100M mutant with previously obtained **quasi-closed** and **open** conformations. As previously predicted by Aktas and Cook (2009), **Lys230*** is positioned to **deprotonate/reprotonate** the α-hydroxyl in two of the reaction steps and **Tyr160** moves into position to **protonate** C3 following β-decarboxylation. A proton relay from the catalytic triad Tyr160-Asp307-Lys230* connects the α-hydroxyl of isocitrate to the bulk solvent to complete the picture of the catalytic mechanism.

2. INTRODUCTION

The 3-step mechanism of IDH1: (**R** = CH_2COO^-)

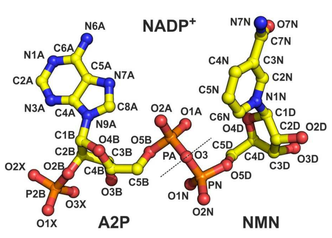


This mechanism requires a **Catalytic base** for proton abstraction from C2 and a **Catalytic acid** for protonation of C3 after decarboxylation.

Conflicting hypotheses have been made regarding their identities.

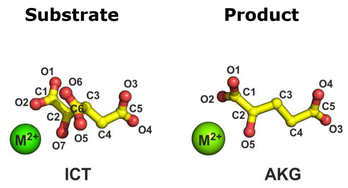
Cofactor

NADP$^+$ – β-Nicotinamide Adenine Dinucleotide Phosphate



A2P – Adenosine 2',5'-diphosphate
NMN – Nicotinamide mononucleotide

Substrate **Product**



ICT – 2*R*,3*S*-isocitrate
AKG – α-ketoglutarate
M^{2+} – co-catalytic metal ion

E. Coli IDH1 (416 aa) is very easy to purify and crystallize.

In *wt E. coli* IDH, using **Ca^{2+}** as co-catalytic metal ion lowers K_{cat} by more than 2500-fold. This was used in attempts to obtain 3D structures of a **pseudo-Michaelis complex**: **wtIDH:NADP$^+$:ICT:Ca^{2+}** by soaking and co-crystallization.

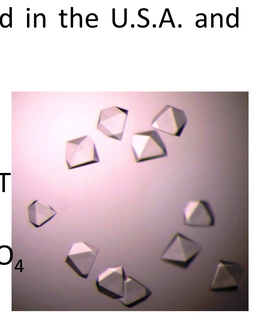
The **K100M** mutation in *E. coli* IDH reduces K_{cat} by a factor of 20,000. This mutant was also used in attempts to obtain a 3D structure of a **pseudo-Michaelis complex**: **K100M IDH:NADP$^+$:ICT:Mg^{2+}** by soaking and co-crystallization.

Prior to this work, there were **27 crystal structures** of *E. Coli* IDH1 in the PDB:
- None are representative of a true **pseudo-Michaelis complex**
- None are representative of a true **product complex**

3. CRYSTALLIZATION

E. Coli IDH1 (wt and K100M) was produced in the U.S.A. and shipped to Portugal for crystallization.

Crystallization buffer for *wt* IDH:
1.85 M $(NH4)_2SO_4$, 50 mM citric acid/Na_2HPO_4 pH 5.8, 0.1 M NaCl, 0.2 M DTT
Crystallization buffer for K100M IDH:
1.85 M $(NH_4)_2SO_4$, 50 mM citric acid/Na_2HPO_4 pH 5.2, 0.1 M NaCl, 0.2 M DTT



4. IDH1 CRYSTAL SOAKS

Several crystals of ***wt* IDH1** were soaked in solutions containing 52 mM Ca^{2+}, 300 mM isocitrate and 400 mM NADP$^+$ or thio-NADP$^+$ for 1/2 ~ 3 hrs.

Several crystals of **K100M IDH1** were soaked in solutions containing 52 mM Mg^{2+} or Ca^{2+}, 300 mM isocitrate or 77 mM α-ketoglutarate, and 10 mM NADPH or 400-500 mM NADP$^+$ or thio-NADP$^+$ for 1/2 ~ 3 hrs.

5. DATA COLLECTION AND STRUCTURE REFINEMENTS

A total of **27 datasets** were collected from IDH crystal soaks:
- 18 *in-house* (11 at room temperature)
- 6 at the ESRF (Grenoble, France)
- 2 at the SLS (Villigen, Switzerland)
- 1 at Diamond (Didcot, U.K.)

E. coli IDH1 crystalizes in the tetragonal space group $P4_32_12$, with cell parameters a ≈ 105 Å and c ≈ 150 Å

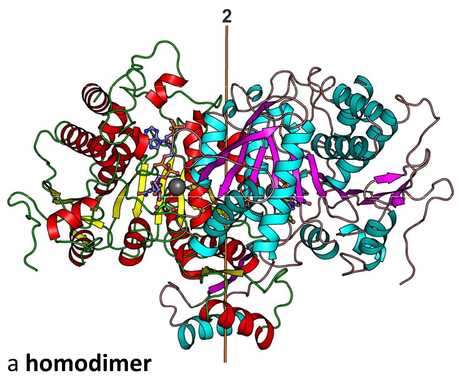
Data resolution: between **2.7** and **1.65** Å.
Data processing: XDS/CCP4 (synchrotron) and Proteum Suite (in-house)
Structure determination: Molecular Replacement with PHASER using PDB entry 1ai2 (Mesecar *et al.*, 1997) as the search model
Preliminary refinement: REFMAC5 in the CCP4 suite

Several **problems** were faced and solved:

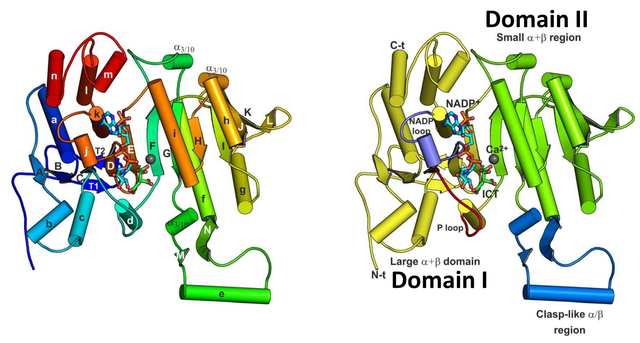
1. difficulty in finding proper **cryoprotecting conditions**
2. extreme **radiation damage** at RT (typical crystal lifetime ~ 2hrs)
3. no NADP$^+$ **binding**
4. **hydrolyzed** NADP$^+$ at the active site

6 datasets were selected for full structure refinement with PHENIX:

- **For the first time**, **fully-closed** enzyme conformations were obtained for one ***wt* IDH1 crystal soak** (**pseudo-Michaelis complex**) and one **K100M IDH1 crystal soak** (**product complex**)
- The results **confirmed** the details of the IDH1 catalytic mechanism proposed by Aktas and Cook (2009)



- *E. Coli* IDH is a **homodimer**
- In nearly all published crystal structures the dimer is crystallographic.
- Each active site is formed by residues from both monomers.



Domain structure of *E. Coli* IDH (1-416):
- Domain I, large α + β domain (1-124 and 318-416)
- Domain II, small α/β domain (125-317), includes a clasp-like α/β region

6. STRUCTURAL DYNAMICS AND INDUCED FIT

In a crystal structure, IDH can be in **3** different conformations:

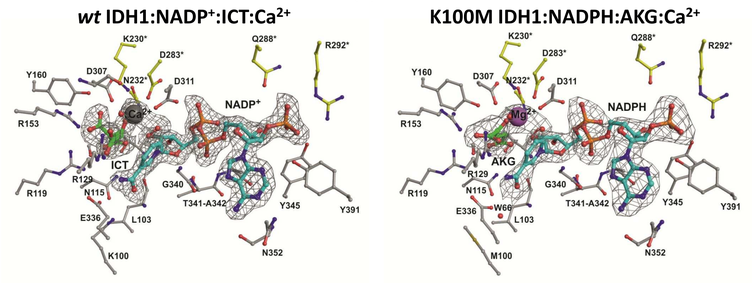
- **Open** (*E. coli* apo-isoform, PDB entry 1sjs, Finer-Moore et al, 1997)
- **Quasi-closed** (ALL other published *E. coli* IDH structures to date)
- **Fully closed** (4 IDH structures, 2 *E. coli* IDH structures from this work)

The enzyme conformation can be evaluated by the **relative orientation** between Domain I (large) and Domain II (small) with **DYNDOM**/**LSQKAB** in the CCP4 suite

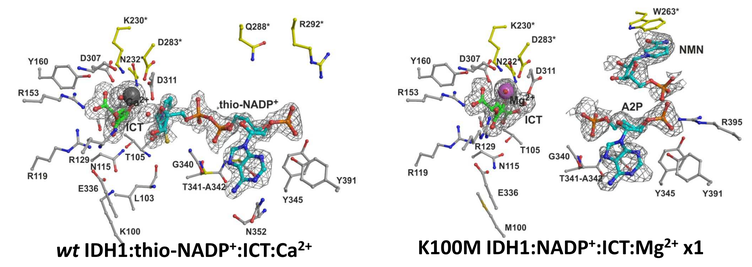
CCP4 DYNDOM also identifies **rotation axes** and **hinge residues**, but fails for small angles and non-identical structures: LSQKAB can be used instead after structural alignment in COOT.

The second monomer is represented with carbon atoms in a different color and with residue numbers labeled with an asterisk (*) .

***wt* IDH1:NADP$^+$:ICT:Ca^{2+}** **K100M IDH1:NADPH:AKG:Ca^{2+}**

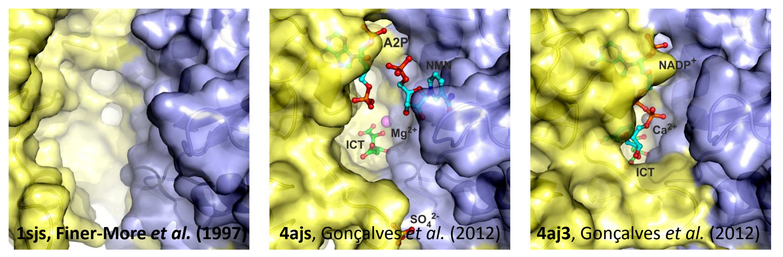


In the **fully closed conformation** (rotation angles larger than ~20°), the enzyme, co-factors and substrate can be in a **catalytically productive** conformation.



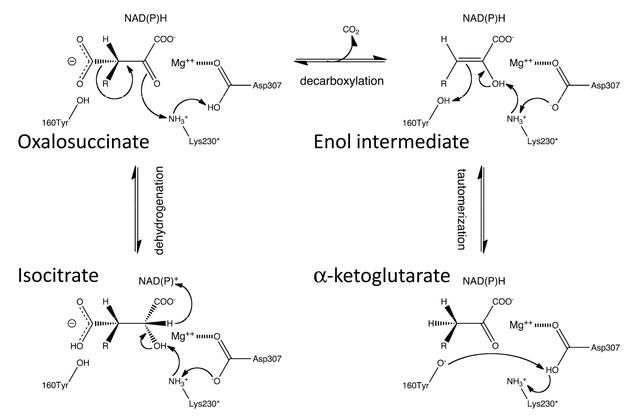
***wt* IDH1:thio-NADP$^+$:ICT:Ca^{2+}** **K100M IDH1:NADP$^+$:ICT:Mg^{2+} x1**

In the **quasi-closed conformation** (rotation angles ~20°), the enzyme, co-factors and substrate are in a conformation that is **not** catalytically productive (**left**) and NADP$^+$ may be partly **hydrolyzed** (**right**).



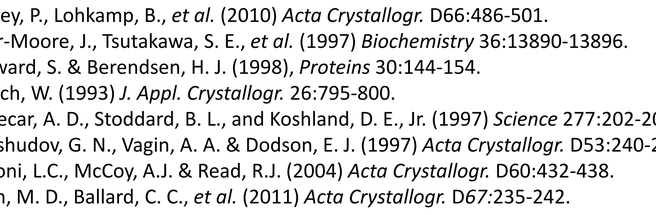
Closure of the active site in *E. coli wt* IDH1 as the large domain rotates from the **open** (left) to the **quasi-closed** (centre) and **fully-closed** (right) conformations. One IDH1 monomer is shown in yellow and the other in light blue.

7. THE CATALYTIC MECHANISM OF IDH1



The **fully-closed** enzyme conformations **confirmed the details** of the IDH1 catalytic mechanism proposed by Aktas and Cook (2009):

- **K 230*** is a **catalytic acid/base** active in all mechanism steps
- **Y160** is a **catalytic acid** essential for the enol tautomerization;
- **D307** or a **proton relay** from bulk solvent balance the proton flow.



Left - The pseudo-Michaelis complex ***wt* IDH:NADP$^+$:ICT:Ca^{2+}**
Right - The product complex **K100M IDH:NADPH:AKG:Ca^{2+}** was obtained by ICT turnover *in crystallum*

Reference: S. Gonçalves, S. P. Miller, M. A. Carrondo, A. M. Dean and P. M. Matias, "Induced Fit and the Catalytic Mechanism of Isocitrate Dehydrogenase" (2012) Biochemistry, 51:7098–7115.

Acknowledgements: This work is based in part on experiments performed at the ID14-4 and ID23-1 beamlines of the European Synchrotron Radiation Facility (ESRF) in Grenoble, France and at the PXIII beamline of the Swiss Light Source (SLS), Paul Scherrer Institute in Villigen, Switzerland. The research leading to these results received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement n.°226716, and Fundação para a Ciência e Tecnologia (Portugal) grants PEst-OE/EQB/LA0004/2011 and SFRH/BD/23222/2005 to SG. Funding was also provided by grant GM060611 from the NIH, and the 2009 Oeiras-Professor Doutor António Xavier Scientific Award and an FCT Visiting Professor Scholarship to AMD during his sabbatical in Portugal.

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