

CH461/561 Exam 3 KEY

1. $k_{\text{cat}} = 8 \times 10^5 \text{ s}^{-1}$
 $K_m (\text{ATP}) = 42 - 48 \text{ mM}$ (depending upon how you calculated it)
 $k_{\text{cat}}/K_m = 1.7 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$

It is not quite catalytically perfect, but not too far from being there.

The MUI-1 inhibitor is a **mixed** inhibitor, but it binds somewhat tighter to the ES complex.

$$K_i = 36 \text{ nM}$$

$$K_i' = 6 \text{ nM}$$

2. This enzyme makes a ternary complex using a random pathway.

$$K_m (\text{GP}) = 4.2 \mu\text{M} \quad K_d (\text{GP}) = 11 \mu\text{M}$$

$$K_m (\text{ATP}) = 1.8 \text{ mM} \quad K_d (\text{ATP}) = 4.7 \text{ mM}$$

$$k_{\text{cat}} = 185 \text{ s}^{-1}$$

3. 8 pts to follow directions and fill in the information at top of form. 5 pts for each description and 6 pts for the compare/contrast.

4.

- a) Briefly describe the overall tertiary and quaternary structure of ICDH.
 What kind of domains does it have (*e.g.* what class)?
 Briefly describe the dimerization interface (seen in the 1XKD structure).

The overall tertiary structure of one subunit of ICDH is a single α/β domain. It consists most of β - α - β motifs to give a central β -sheet (twisted saddle), consisting of parallel β -strands (6 parallel & 4 anti-parallel pairings). The strands in one end of the saddle point one way, while the strands in the other end point the other way (mostly). There are two strands in the center that alternate (which gives it an overall pattern of $\uparrow\uparrow\uparrow\downarrow\uparrow\downarrow\downarrow\downarrow\downarrow\uparrow$, starting from the N-terminal part). The overall appearance is of a β -twisted saddle surrounded on both sides by α -helices, but with 3 exceptions: (1) at the N-terminus is a short loop with a small 2-

stranded sheet before the major sheet begins; (2) between strands 7 & 9 of the sheet (5th and 6th in primary structure) there is a long loop (residues ~162-205) consisting of two extended chains (with short paired strands in them) flanking an α -helix all by itself (not really – this will be part of dimerization domain); (3) at the C-terminus, after the last β -strand in the 1^o structure (actually #4 of the sheet), there are 3 α -helices (repeated α -loop- α motifs) that stack with the first α -helix to make a 4-helix bundle.

The quaternary structure is a **homodimer** with C2 symmetry. The internal extension (“exception” #2 above) is a critical part of the interaction domain. The 2 strands in the extended chains interact in an anti-parallel fashion to make a single 4-stranded anti-parallel β -sheet, and the 2 α -helices pack onto each other in a right-handed sense (like a coiled-coil, but shorter). The other significant interaction surface is between 2 α -helices that pack onto the β -sheet: a shorter helix (residues ~285-296) and longer one (residues ~306-322). The longer helix in one subunit primarily interacts with the shorter helix in the other subunit (as dictated by the C2 symmetry).

- b) Examine the active site. Which amino acids are part of the sites binding the substrates? Fill out the tables on the next pages for the most important residues you identify for ICDH’s function. For the column in which you report distances, indicate which atoms you used to measure the distance (of the amino acid and the substrate or effector).

(1) Residues important for binding isocitrate

Residue (amino acid & #)	Type of secondary structure element	Likely role	Distance to nearest atom of substrate
Arg 153	β -strand	Ionic interaction/H-bond donor to 2-carboxylate (central CO ₂ ⁻)	N – O = 2.8 Å
Arg 119	α -helix	Ionic interaction with 2-carboxylate (central)	N – O = 3.2 Å (maybe H-bond donor)
Tyr 160	α -helix	H-bond donor to 2-carboxylate	-OH – O = 2.6 Å
Arg 129	β -strand	Ionic interaction with 1-carboxylate	N – O = 3.1 Å (maybe H-bond donor)
Ser 113	Coil (helix)	H-bond donor to 3-carboxylate	-OH – O = 2.6 Å
Asn 115	α -helix	H-bond donor to 3-carboxylate	Amide N – O = 2.5 Å
Asp 307	α -helix	H-bond acceptor from isocitrate hydroxyl group	O – O = 2.9 Å

These are maybe OK...			
Val 351	Turn/loop	Van der Waals with adenine ring	3.3 Å
These are NOT good ones!			
Leu 114	Leu 103	Asp 283	Glu 203

(2) Residues important for binding NADP

Residue (amino acid & #)	Type of secondary structure element	Likely role	Distance to nearest atom of substrate
Arg 395	turn	Ion bridge with 3'-P _i (probably makes it specific for NADP vs. NAD)	Between guanidinium N's and P _i O's the distance is 2.5–3 Å
Asn 352	coil	H-bond acceptor from exocyclic amino group of adenine	C=O to exocyclic amino N = 2.8 Å
Tyr 345	turn	H-bond donor to 3'-P _i	-OH to Pi O = 2.6 Å
Tyr 391	α-helix	H-bond donor to 3'-P _i	-OH to Pi O = 2.7 Å
Lys 344	turn	Ionic interaction with linking phosphates of dinucleotide	Lys N to P's = 6.8, 7.3 Å (5.4Å to nearest O)
Thr 104	coil	Possible H-bonding with 2'-OH of nicotinamide-linked ribose	O to O = 3.1 Å
Thr 341	coil	H-bond donor to ring O of nicotinamide-linked ribose	Amide N-H to O = 2.5Å
Lys 100	β-strand	Charge-dipole interaction between amino group of Lys and amide O of nicotinamide	N to O = 3.7Å (too far for H-bond)
These are maybe OK...			
Thr 338	coil	Possible H-bond donor to amide N of nicotinamide	N – O = 2.9 Å
His 339	coil	Possible H-bonding to cyclic	N – N = 2.8 Å

		N of adenine	
Gly 340	coil	Van der Waals interaction with nicotinamide	~3.3 Å
Lys 344	Turn/loop	Ionic interaction with 3'-P _i and β-P _i	5 & 4.5 Å
These are NOT good ones!			
Gly 321	Asp 392		

(3) Residues important for binding Mg²⁺

Residue (amino acid & #)	Type of secondary structure element	Likely role	Distance to nearest atom of substrate
Asp 307	α-helix	Bind Mg ²⁺	O – Mg = 2.1 Å
Asp 311	α-helix	Bind Mg ²⁺	O – Mg = 3.5 Å
There are also 2 waters (29, 66) that are also coordinated by these Asp's that are within coordination sphere of Mg (water O – Mg = 1.9, 2.1 Å)			
The other major interaction of Mg is with the hydroxyl and 1-carboxylate of isocitrate			

- c) Write below a proposed mechanism for the ICDH-catalyzed reaction. Include at least 3 residues that would be involved in such a mechanism, and briefly explain what each is doing (*e.g.* acting as acid/base at a specific step, stabilizing a specific intermediate state, *etc.*)

Step1:

NADP⁺ abstracts hydride (H:) from C-2 of isocitrate, and Asp 307 abstracts a proton from the hydroxyl oxygen (at C-2), as it is converted to a ketone. (The proton abstract could also be water #29, but seems less likely, based on the distances).

Step2:

Decarboxylation at C-3 is accompanied by formation of enolate, which is stabilized by Mg²⁺ (Mn²⁺). (Asp 311 is likely involved in accommodation of the ketone as it continues to interact with Mg²⁺.)

Step3:

Protonation at C-3 finishes the reaction. Likely proton donors: Asp 307 (protonated in step 1, 4.5 Å away), Arg 119 (5 Å away), Tyr 160 (4.5 Å away),

- d) Examine the structure of the phosphorylated enzyme (4ICD). It might also be helpful to look at 2 mutants that were made at position 113: Ser to Asp (6ICD) or Glu (7ICD). Such mutations are often made to mimic the phosphorylated Ser residue. Briefly speculate about how phosphorylation of Ser113 inhibits activity of ICDH. Also explain briefly why the mutants (Ser 113→Asp/Glu) would mimic the phosphorylated enzyme.

Obviously, if it were phosphorylated, it would block the binding of citrate.

3 reasons (in order of increasing importance):

1. Would lose H-bonding to isocitrate
2. Steric hindrance for isocitrate binding
3. Charge-charge repulsion between negatively charge phosphate and carboxylates of isocitrate.

There are also conformational changes that may inhibit isocitrate binding.

5. Lets keep *some* mystery in life, eh?...