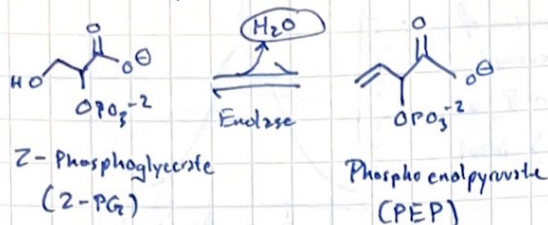


ENOLASE & METAL ION CATALYSIS

The reaction catalyzed by enolase:



→ The Enolase active site contains two Mg^{+2} ions, typically bound to other ionic side chain groups such as aspartate or glutamate.

- Note that 2-PG is a very negatively charged substrate. Mg^{+2} ions bind to 2-PG and also stabilizes the reaction intermediates of the reaction, containing a carbanion.

~~the better leaving group~~

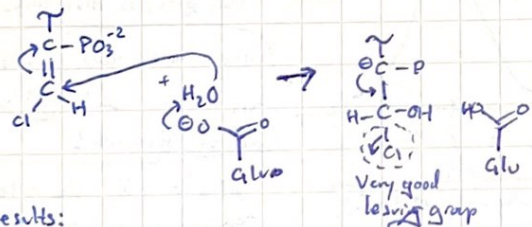
- Lysine-345 acts as the base to start the reaction (pulling the hydrogen off)

- Glu-211 acts as the acid to make

-OH a better leaving group.

~~Evidence~~ Evidence for Glu-211:

Researchers used an unnatural substrate, 3-Cl-PEP, and monitored the reverse reaction process:



Results:

a) with type:

3-chloro-PEP → 3-hydroxy-PEP

b) Alb-345, Glu211:

3-chloro-PEP → 3-hydroxy-PEP

c) Lys 345, Glu211:

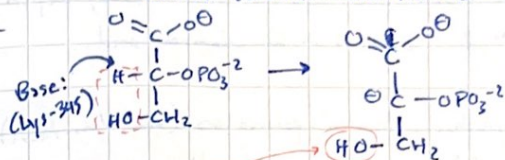
No reaction

- Provides evidence that glutamic acid indeed acts as the base, as this is the only plausible explanation of the post-reaction catalysis of 3-chloro-PEP.

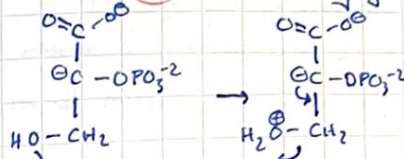
This reaction is part of the glycolysis pathway and is readily reversible.

Consider: How might an enzyme speed up a reaction?

→ Stabilize carbanion intermediate:



→ Make OH a better leaving group by donating proton:



E-AcidH

Q: How can we prove that this is indeed the mechanism?

A: Site directed-mutagenesis:

Mutant 1: Lys-345 → Ala-345

Mutant 2: Glu-211 → Gln-211

The rate of all the reactions (including wild type and mutants) were all measured:

a) Lys 345, Glu 211

$k_{\text{cat}} = 78 \text{ 1/sec}$

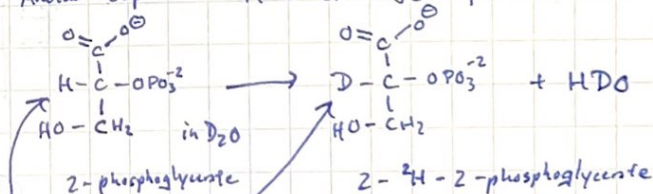
b) Ala 345, Glu 211

$k_{\text{cat}} = 6 \times 10^{-4} \text{ 1/sec}$

c) Lys 345, Gln 211

$k_{\text{cat}} = 7 \times 10^{-4} \text{ 1/sec}$

Another experiment: H-D exchange experiment:



We observe if the hydrogen is replaced by deuterium in a dissolved molecule when the molecule is dissolved in D_2O .

When both experiments are combined, we find that there is no H-D exchange in Ala345, Glu211 mutant.

The Alanine cannot act as a base, and therefore cannot pull off the H to exchange for deuterium.