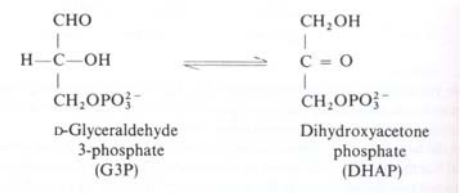
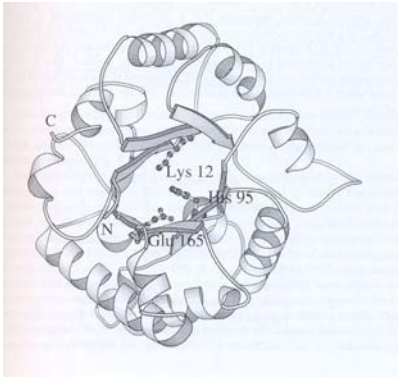
5.5.2 Triosephosphate isomerase



- Two identical subunits
- One of the most perfect enzyme
- X-ray crystallography, affinity labeling, site-directed mutagenesis were used to understand this enzyme

5.5.2 Triosephosphate isomerase

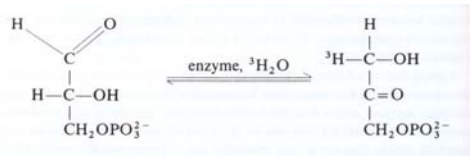
- X-ray crystallographic studies
  - Diameter : 3.5nm
  - TIM barrel structure (α/β type fold)
  - Asn14 and Asn78 important for interaction b/n subunits (proven by site-directed mutagenesis)
  - Mobile loop (166-176 residues) undergoes major change when bound with transition state analogue
  - Glu165, His95, Lys12

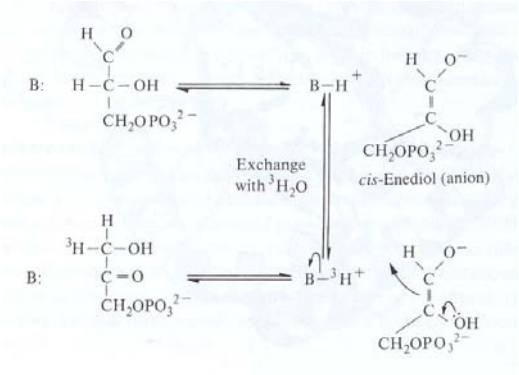


5.5.2 Triosephosphate isomerase

- Isotope labeling

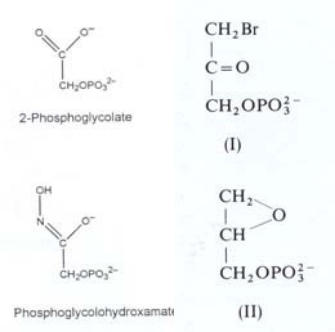
→ cis-enediol is proposed as an intermediate



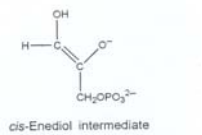


5.5.2 Triosephosphate isomerase

- Inhibitiors support the cis enediol intermediates
- Affinity Labeling
  - Two affinity labeling compounds to inactivate the enzyme were developed
  - Modification on Glu165 or Try164 (migration)
  - Glu165 was confirmed by site-directed mutagenesis
  - pKa study showed that His 95 is acid
  - Mutation study showed that positive charge on Lys12 is important : neutralizing Phospho group



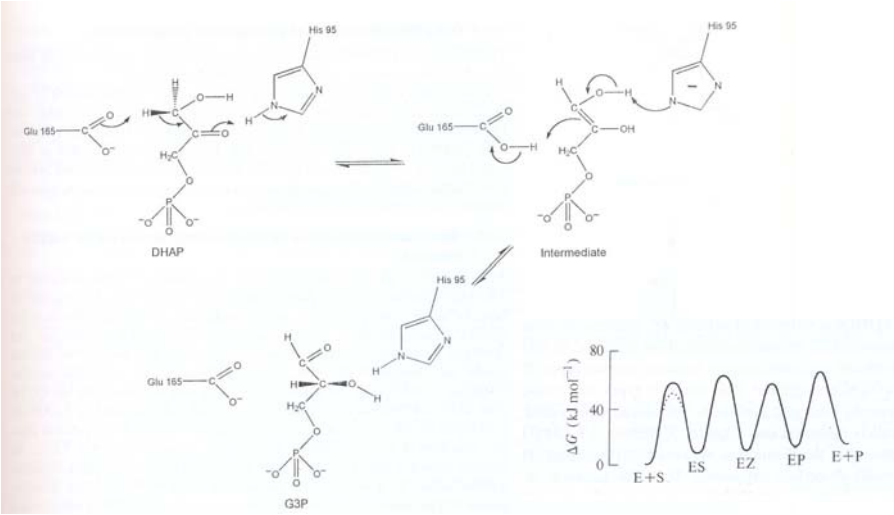
Affinity labeling



Inhibitors

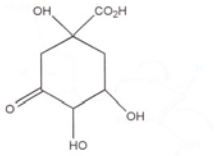
5.5.2 Triosephosphate isomerase

- The proposed mechanism

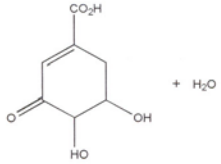


5.5.3 Dehydroquinase

- Important step to make aromatic amino acids
- Two types of enzymes (biosynthetic DHQase type I and catabolic DHQase type II) : No similarity in amino acid sequences
- Two enzymes were evolved from distinct ancestors
- Type I : dimeric with subunit Mr 27,000
- Type I : Easily deactivated by heat and denatured by guanidinium chloride



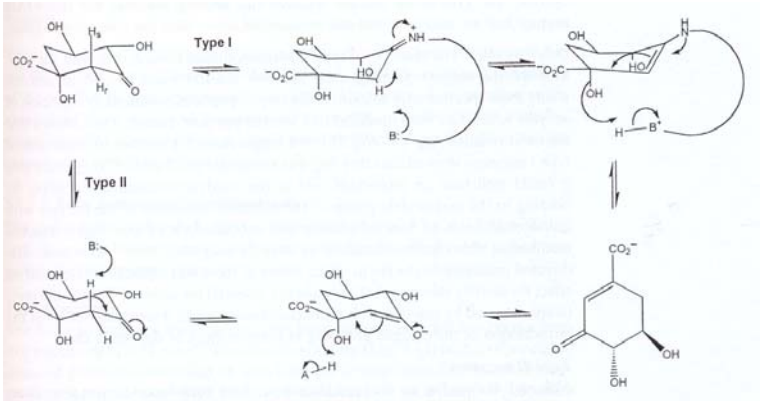
3-Dehydroquinate



3-Dehydroshikimate

5.5.3 Dehydroquinase

- Type II: dodecameric with subunit of Mr 16,000 and much more stable
- Type I catalyzes cis-elimination while type II does trans-elimination



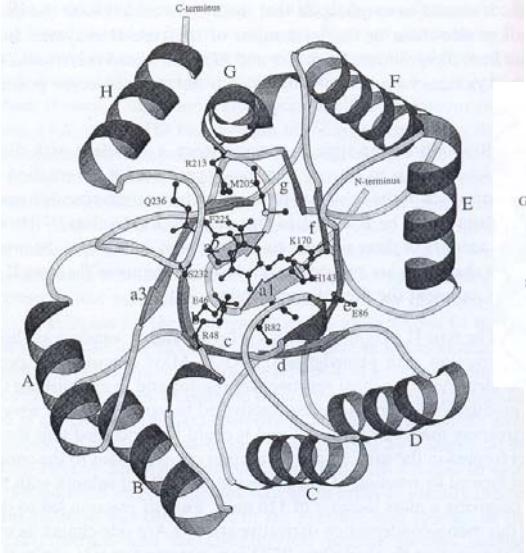
5.5.3 Dehydroquinase

- Chemical modification
  - Existence of Schiff base in Type I enzyme was proved by sodium borohydride
  - Adding radioactive NaB3H4 and identifying the modified position using CNBr and trypsin → Lys170 was identified
  - After modification, enzyme was more stabilized with guadinium chloride ; Caused by increase of flexibility
  - Lys170Ala mutant is 106-fold less active than wild type although the binding with substrate changed three fold
  - Adding diethylpyrocarbonate lead enzyme inactivation → His is also involved in the reaction
  - Among 6 His, His143 was identified as an important residue

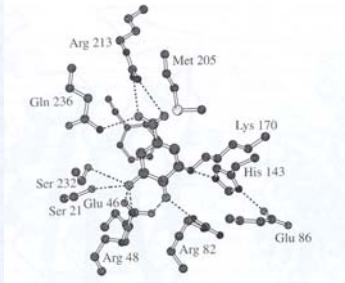
5.5.3 Dehydroquinase

- Arg 213 was also conserved in all type I enzymes and identified as an important one
- Type II enzyme was poorly characterized
  - No evidence that Lys is involved in the reaction
  - His may be involved in the reaction, but the identity of the side chain is not determined
  - Arg23 was identified with site-directed mutagenesis
  - Tyrosine specific modification also inactivate the enzyme → Tyr 28
- X-ray crystallography with type I
  - The structure is similar to Class I fructose bisphosphate aldolase, which use Schiff base

5.5.3 Dehydroquinase



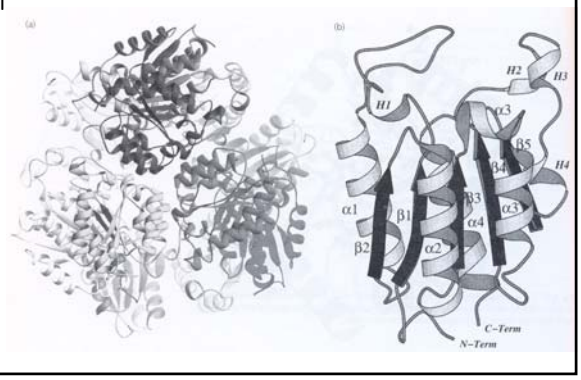
Type I Enzyme



5.5.3 Dehydroquinase

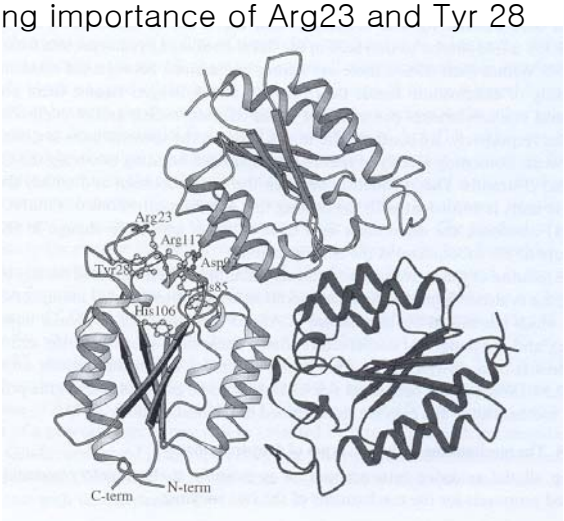
- Structural study showed that Type II enzyme is a tetramer of trimeric units
- Strong salt bridge and H-bond within trimeric unit, but weaker bond between trimeric unit
- At low GdnHCl

the 12mer is separated into 4 trimers



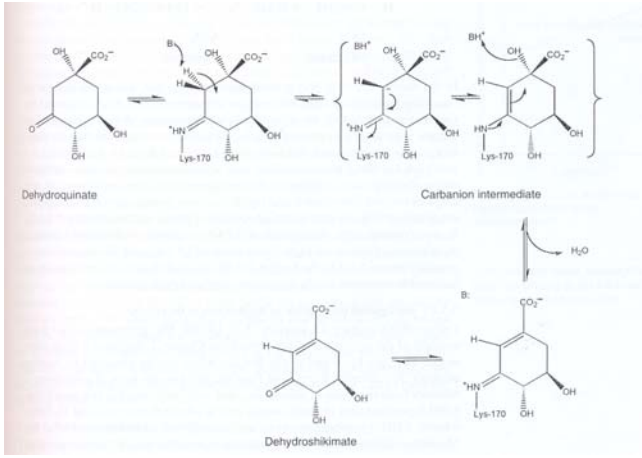
5.5.3 Dehydroquinase

- Showing importance of Arg23 and Tyr 28



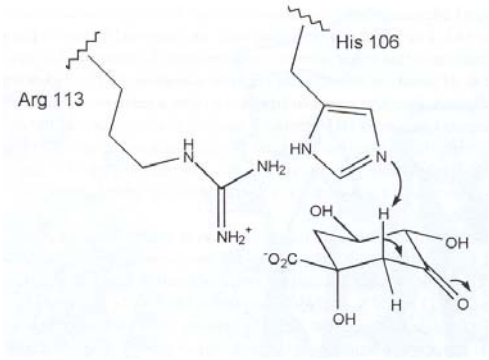
5.5.3 Type I Dehydroquinase

1. Lys170 forms a Schiff base
2. His143 acts as base and takes a proton from C2
3. –OH is detached from C1



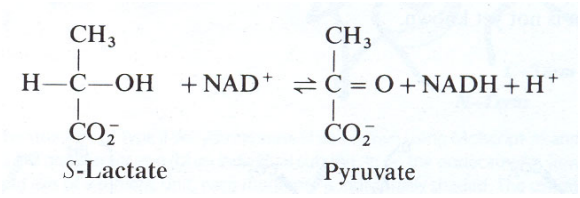
5.5.3 Type II Dehydroquinase

- Enolate intermediate
- Arg stabilizes the int.
- His acts as base with help of Glu104



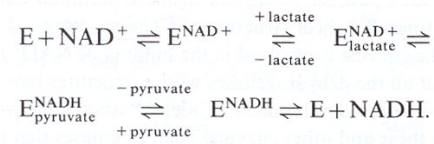
5.5.4 Lactate dehydrogenase

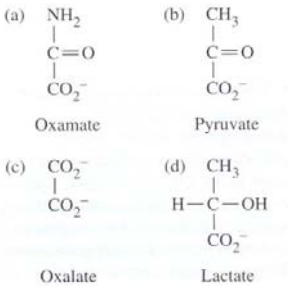
- Last step of anaerobic fermentation
- Generating NADH required for glycolysis
- Tetramers
- 5 isoforms of the enzyme from two subunits
- Isoform 1 (α4) dominates in heart, and 5 (β5) dominates in skeletal muscle



5.5.4 Lactate dehydrogenase

- Structural and kinetic studies with oxamate (competitive inhibitor of pyruvate) and oxalate (lactate)

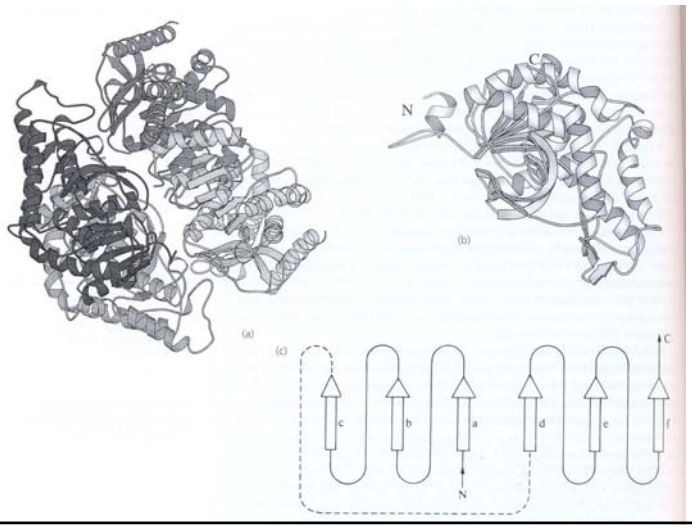




- Pre-steady state kinetics showed that dissociation step is slow. Indeed, it is not, but an additional slow conformational changing step exists

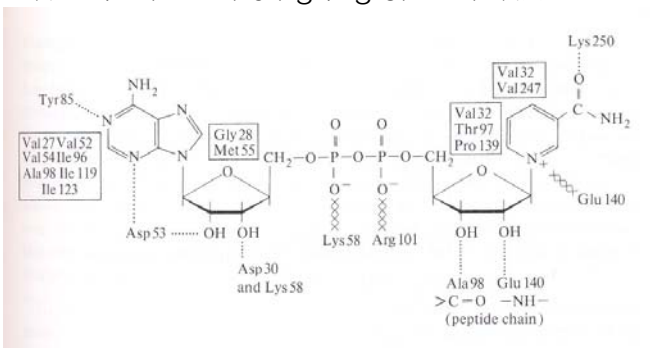
5.5.4 Lactate dehydrogenase

- 40% α-helix and 23% β-sheet



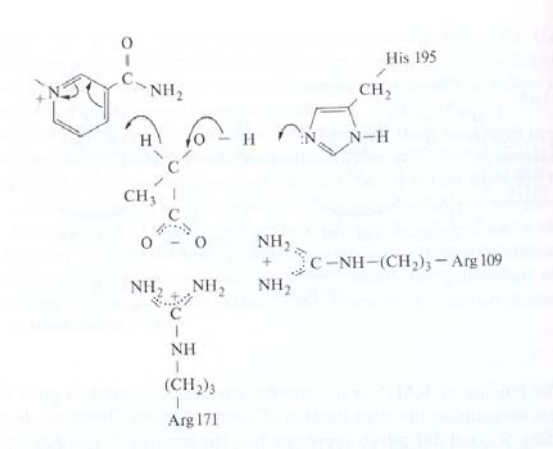
5.5.4 Lactate dehydrogenase

- N-terminal binding region with NAD



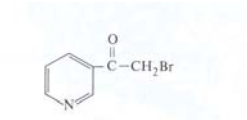
5.5.4 Lactate dehydrogenase

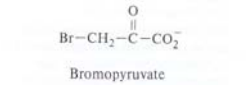
- Lactate locates between nicotinamide ring and His 195

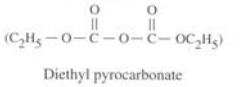


5.5.4 Lactate dehydrogenase

- Chemical modification
  - Histidine in the active site was proved by affinity labeling with 3-bromoacetylpyridine and bromopyruvate
  - Reactive His 195 was modified by diethylpyrocarbonate
  - Phenylglyoxal, specific to Arg, also deactivates the enzyme


3-Bromoacetylpyridine


Bromopyruvate


Diethyl pyrocarbonate

5.5.4 Lactate dehydrogenase

- Mutagenesis studies
  - Thr246Gly, Asp197Asn, Gln102Arg changed this enzyme to malate dehydrogenase
  - Changing at positions 102–105 and 236–237 maked the enzyme to react with even larger substrates
- Mechanism of dehydrogenases
  - All have binding sites with nucleotide
  - They are specific either to NAD+ or NADP+
  - His acts as base and abstracts proton from OH and hydride ion is transferred to NAD+