Carbohydrate Metabolism

Nov 3, 2011

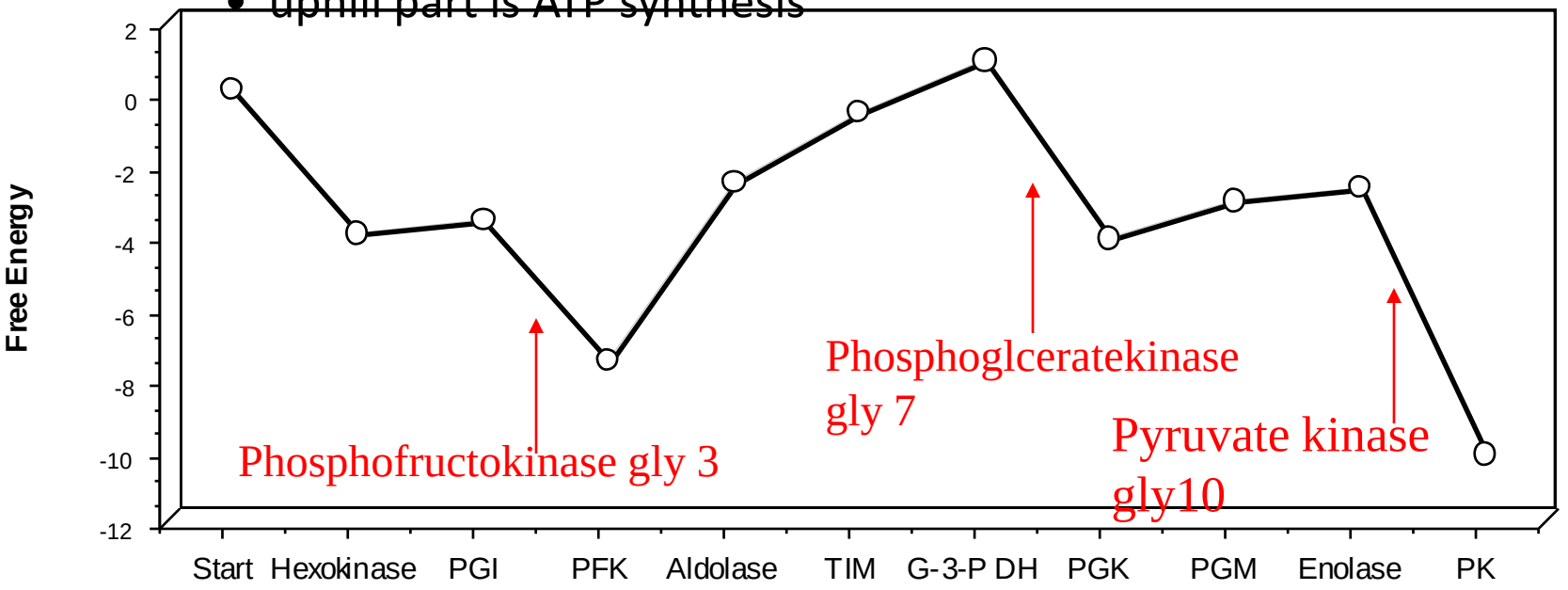
- Intro to Metabolism
  - –ATP, the energy currency of the cell
  - –sugar structure
- Glycolysis Phase I
  - – gly 1-5
- Glycolysis Phase II
  - – gly 6-10
- Control in Glycolysis

Control of Glycolysis

- Energy Coupling (review phosphoryllation potential slide 5)
  - Gly 3? driven by ATP hydrolysis
    - uphill part is phosphorylation of sugar
  - Gly 7? driven by BPG hydrolysis
    - uphill part is ATP synthesis
  - Gly 10? driven by PEP hydrolysis
    - uphill part is ATP synthesis

Major control point

Not likely control since it is the last step in glycolysis



Free Energy of Hydrolysis

Ann. Rev. Physiol. 1985. 47:707-25

RESPIRATORY CONTROL AND THE INTEGRATION OF HEART HIGH-ENERGY PHOSPHATE METABOLISM BY MITOCHONDRIAL CREATINE KINASE

William E. Jacobus

20 mM in heart..Phosophocreatine

10 mM in heart… ATP

Table 13-2. Standard Free Energies of Phosphate Hydrolysis of Some Compounds of Biological Interest

Compound	$\Delta G^{\circ\prime}$ ($kJ \cdot mol^{-1}$)
Phosphoenolpyruvate	−61.9
1,3-Bisphosphoglycerate	−49.4
Acetyl phosphate	−43.1
Phosphocreatine	−43.1
PP_i	−33.5
ATP (→ AMP + PP_i)	−32.2
ATP (→ ADP + P_i)	−30.5
Glucose-1-phosphate	−20.9
Fructose-6-phosphate	−13.8
Glucose-6-phosphate	−13.8
Glycerol-3-phosphate	−9.2

Source: Jencks, W.P., in Fasman, G.D. (Ed.), *Handbook of Biochemistry and Molecular Biology* (3rd ed.), Physical and Chemical Data, Vol. 1, pp. 296–304, CRC Press (1976).

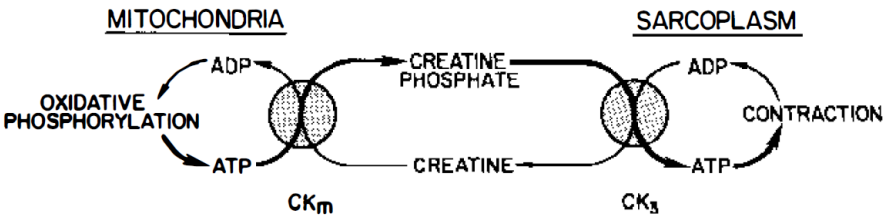


Figure 6 Model for the integration of heart high-energy phosphate metabolism. CK_m and CK_3 are abbreviations for the mitochondrial and sarcoplasmic isozymes of creatine kinase. The flux of high-energy phosphate is indicated by the *dark arrows*.

PFK--Committed Step: Allosteric Control

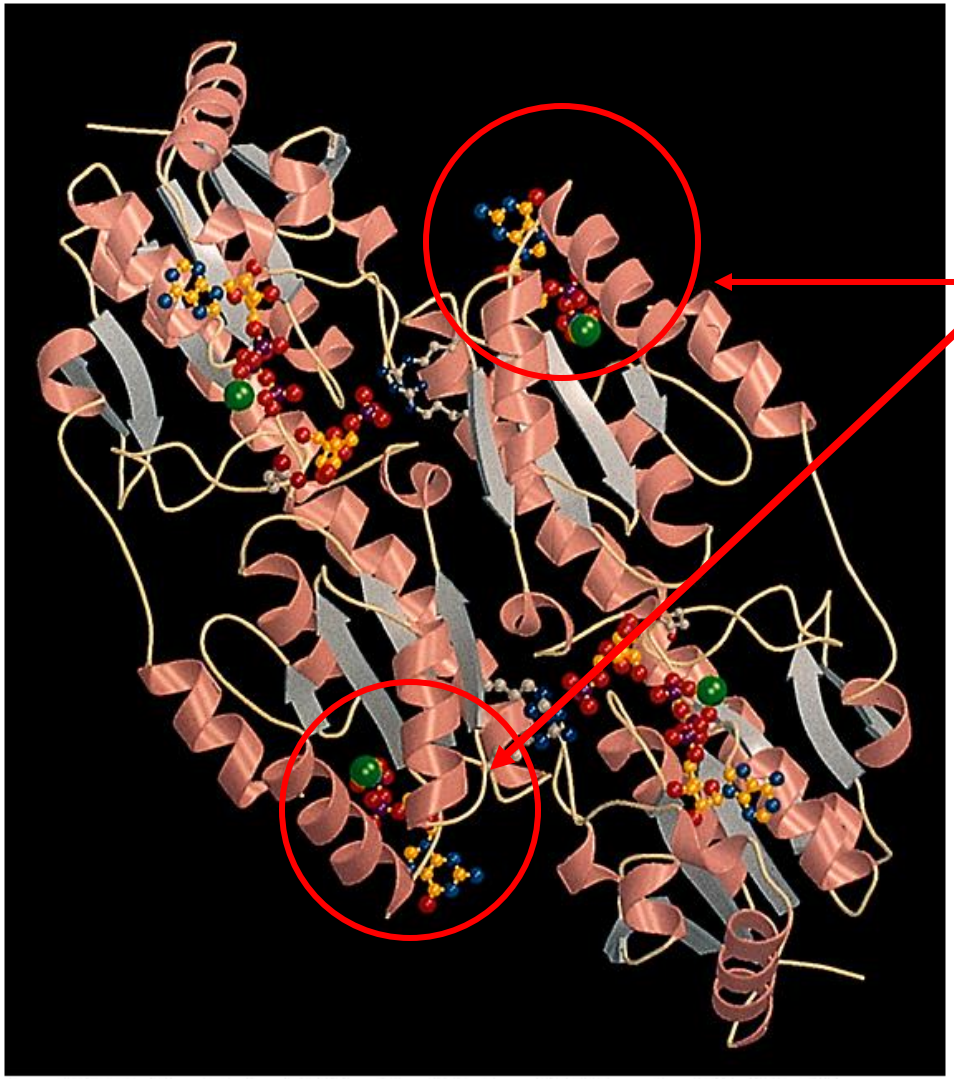
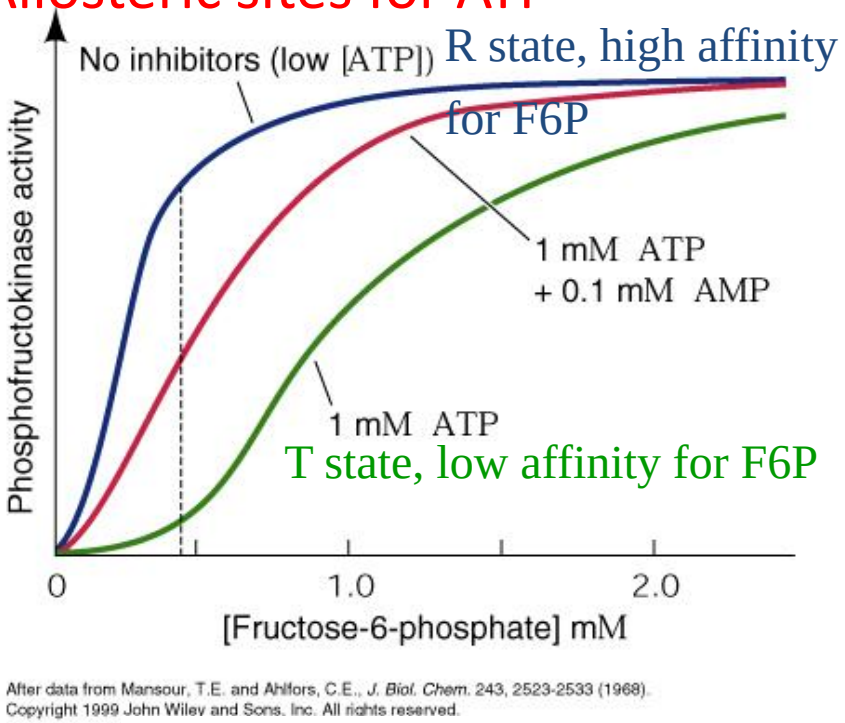


Figure 14-21. The X-ray structure of PFK from *E. coli*. [Courtesy of Philip Evans, Cambridge University.]

Dimer of PFK shown

Substrate binding sites in center

Allosteric sites for ATP



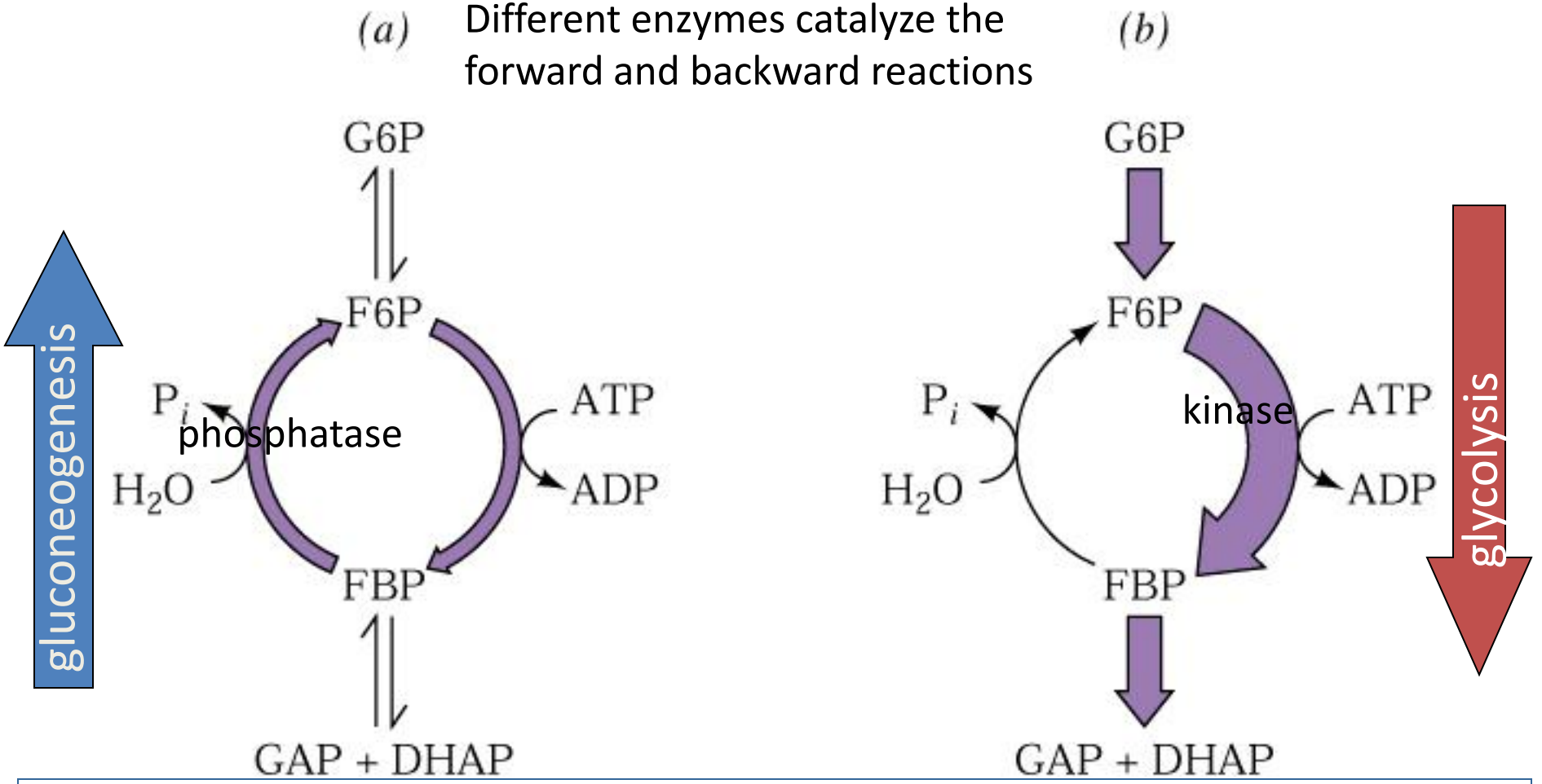
After data from Mansour, T.E. and Ahlfors, C.E., *J. Biol. Chem.* 243, 2523-2533 (1968).

$PFK_T \leftrightarrow PFK_R$

Only T state conformation binds ATP at inhibitor site, high ATP, shift to T, low affinity for f6P

Substrate Cycling

Different enzymes catalyze the forward and backward reactions



Reciprocal Regulation: Fructose 2,6 bisphosphate stimulates kinase and inhibits phosphatase

Biochem. J. (1987) 245, 313–324 (Printed in Great Britain)

REVIEW ARTICLE

Role of fructose 2,6-bisphosphate in the control of glycolysis in mammalian tissues

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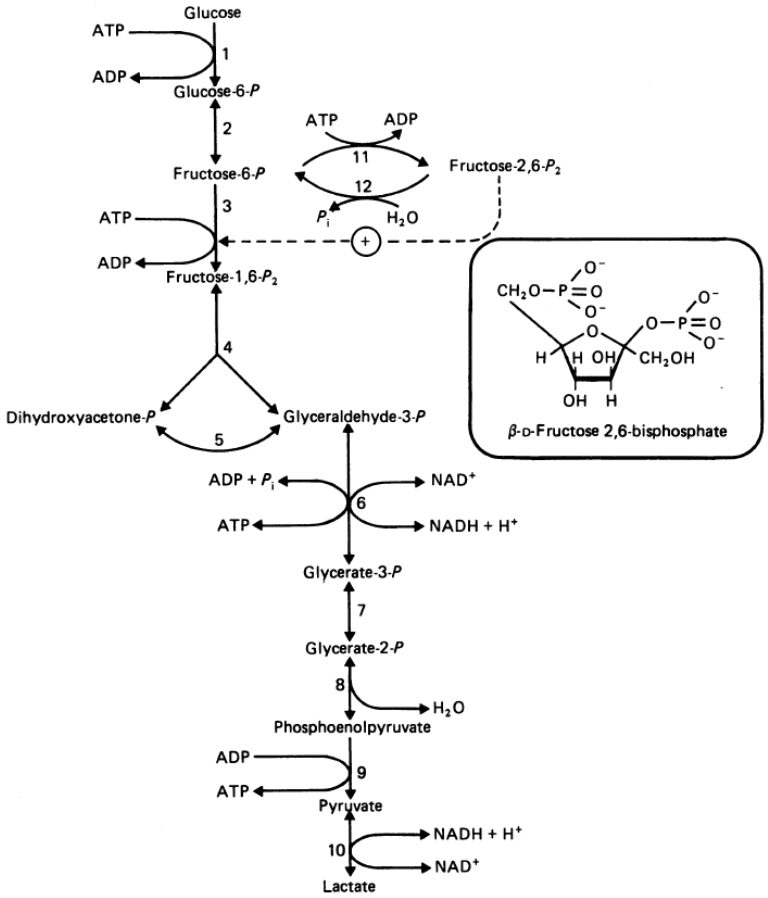


eme 1. Glycolytic pathway and structure of β-D-fructose 2,6-bisphosphate

'he numbers in the Scheme refer to enzymes: 1, hexokinase; 2, phosphoglucose isomerase; 3, 6-phosphofructo-1-kinase; 4, ldolase; 5, triosephosphate isomerase; 6, glyceraldehyde-3-phosphate dehydrogenase; 7, phosphoglycerate mutase; 8, enolase; , pyruvate kinase; 10, lactate dehydrogenase; 11, 6-phosphofructo-2-kinase; 12, fructose-2,6-bisphosphatase. The inset shows he structure of β-D-fructose 2,6-bisphosphate which is the natural anomer.

F2,6BP production

F2,6 BP is made from F6P by PFK-2, a different enzyme that ALSO has a phosphatase activity associated with it.

In the liver, the PFK-2 system (11 and 12 in image at left) is under the control of **glucagon**, a major hormone that signals when glucose is low and glycogen needs to be made (glycolysis inhibited). Glycogen causes (eventually) the phosporyllation of the PFK-2 system and shuts down the production of F2,6BP which shuts down glycolysis.

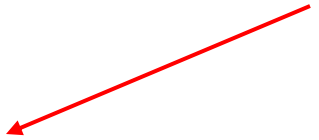
Control of Glycolysis (1)

- Velocity = $\frac{V_{max}[S_t]}{K_m + [S_t]} = \frac{k_{cat}[E_t][S_t]}{K_m + [S_t]}$

- Typical enzyme concentrations, pM-μM
- How can Enzyme levels be controlled?
  - Sequestered storage, triggered release
  - Zymogens (inactive precursors)
    - *quick inefficient*
  - Transcriptional activation (small molecule metabolites or hormones bind to the genes)
    - *slow, efficient*
  - mRNA processing activation; (small molecules bind to untranslated nascent mRNA and affect translation) riboswitches
    - *quick efficient*

Control of Glycolysis (2)

- Velocity = $\frac{V_{max}[S_t]}{K_m + [S_t]} = \frac{k_{cat}[E_t][S_t]}{K_m + [S_t]}$



- Typical substrate concentrations, 10μM-10 mM
- How can substrate levels be controlled?
  - Sequestered storage (glycogen stores in muscle/liver), hormone triggered release (glucagon)
  - Conversion of related molecule (lactate to pyruvate)
  - Hunger signal to organism (hormone)

Control of Glycolysis (3)

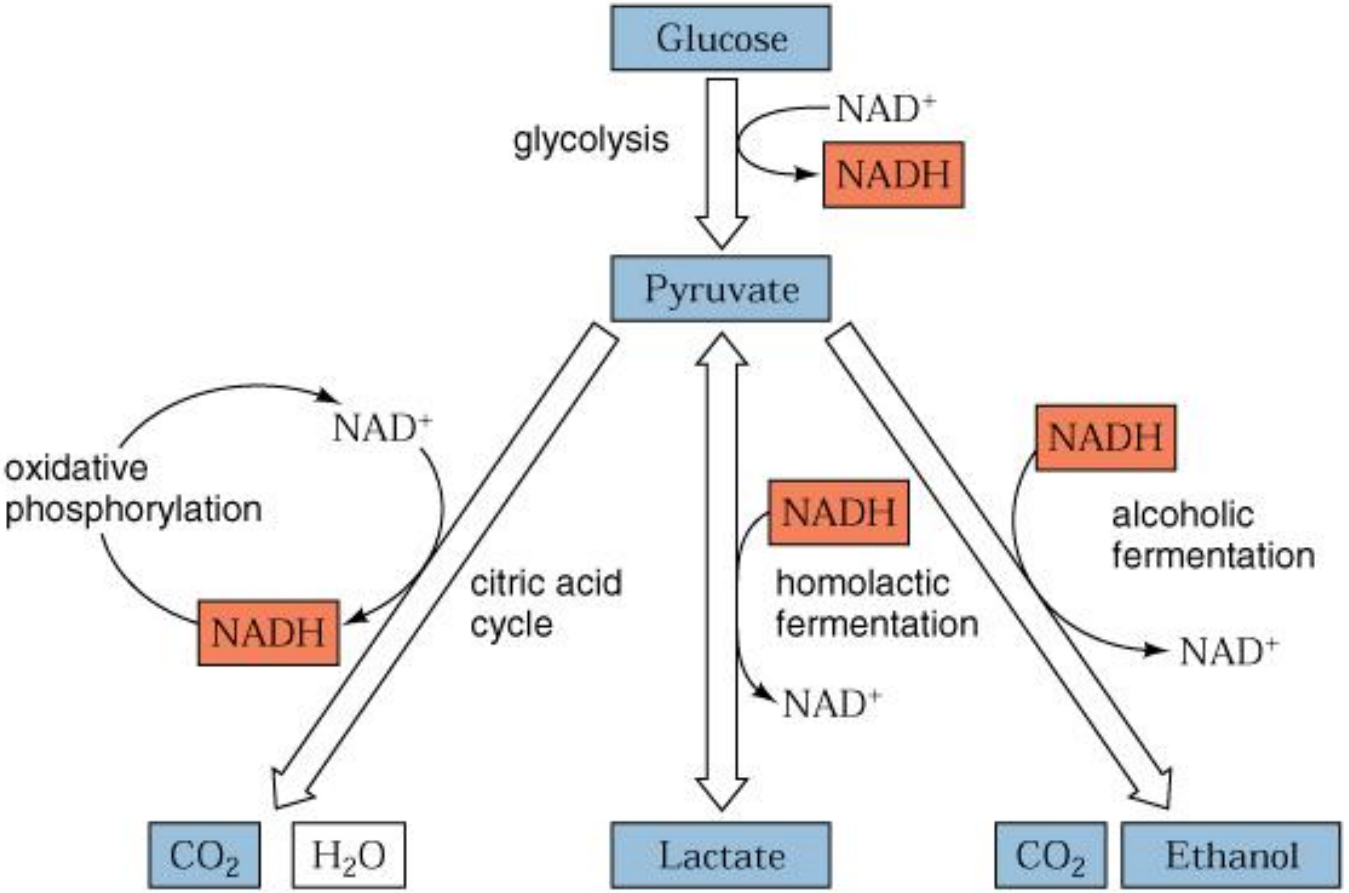
- Velocity = $\frac{V_{max}[S_t]}{K_m + [S_t]} = \frac{k_{cat}[E_t][S_t]}{K_m + [S_t]}$

- Typical k_{cat}, 10^2-10^6 s^{-1}
- How can k_{cat} be increased/decreased?
  - Allosteric effectors (example PFK-gly3)
  - Reversible covalent modification
    - Phosphoryllation, adenylation, methylation, acetyllation, others (example pyruvate dehydrogenase)

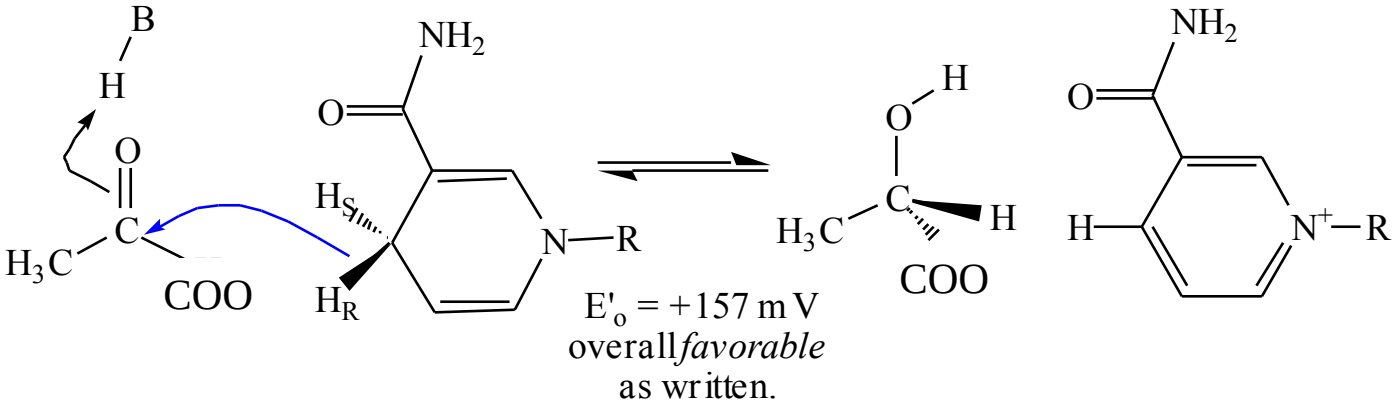
Control of Glycolysis (3b)

- Velocity = $\frac{V_{max}[S_t]}{K_m + [S_t]} = \frac{k_{cat}[E_t][S_t]}{K_m + [S_t]}$
- Typical K_m, 10-1000 uM
- How can K_m be increased/decreased?
  - Self Inhibition

Fates of Glucose: Fermentation



LDH Mechanism: NADH redox



•redox potential of NADH varies in different enzymes:

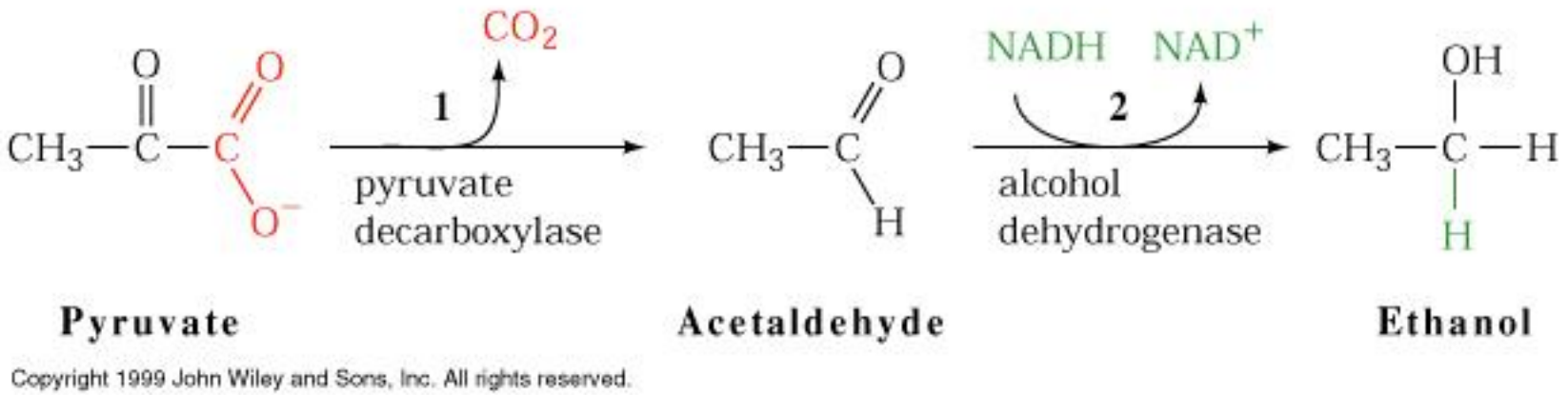
• transfer of the proH_R or proH_s hydride to substrate depends on enzyme class

• binding site selects conformation of the nicotinamide ring and only one stereoselected H is transferred (for reduction) or added (for oxidation).

•His 195 donates a proton to ketone, accepts a proton from alcohol

•Both His 195 and Arg 171 interact electrostatically to orient carboxyllic acid of pyruvate in enzyme active site

Fermentation: Alcohol



Voet, Voet, and Pratt Fundamentals of Biochemistry

* An example of decarboxylation followed by reduction to ethanol.
* On Tuesday, we will see how this system has been co-opted in pyruvate dehydrogenase to perform OXIDATIVE decarboxylation

Pyruvate Decarboxylase Mechanism

Thiamine pyrophosphate, coenzyme