Glycolysis

The Glycolytic Pathway

The Reactions of Glycolysis

Fermentation: The Anaerobic Fate of Pyruvate

Control of Metabolic Flux

Metabolism of Hexoses Other Than Glucose

The Glycolytic Pathway
(Embden-Meyerhof-Parnas Pathway)

Glycolysis converts one C_6 unit (glucose) to two C_3 units (pyruvate) of lower energy in a process that harnesses the released free energy to synthesize ATP from ADP and P_i

Overall reaction -

$$\text{Glucose} + 2NAD^+ + 2ATP + 2P_i \rightarrow$$
$$2NADH + 2\text{pyruvate} + 2ATP + 2H_2O + 4H^+$$

Stage I - Investment of 2ATP to split hexose glucose into 2 molecules of triose glyceraldehyde-3-phosphate

Stage II - Generation of 4ATP from the conversion of glyceraldehyde-3-phosphate into pyruvate

Glycolytic enzymes located in cytosol, loosely associated, no organized complexes

Oxidizing power of NAD^+ must be recycled

1. Anaerobic muscle - homolactic fermentation
2. Anaerobic yeast - alcohol fermentation
3. Aerobic conditions - mitochondrial oxidation

Reactions of Glycolysis

Hexokinase (glucokinase in liver)

phosphoryl group transfer - first ATP investment

Random Bi Bi mechanism

ternary complex with glucose-Mg^{2+}-ATP (catalysis by proximity effects)

Reactions of Glycolysis

Phosphoglucose isomerase (glucose-6-phosphate isomerase)

isomerization (aldose to ketose) reaction

pH dependent, pK = 6.7 (Glu) and pK = 9.3 (Lys)

absolute stereospecificity

Reactions of Glycolysis

Phosphofructokinase

phosphoryl group transfer - second ATP investment

one pathway rate-determining reaction

regulated enzyme

Reactions of Glycolysis

Aldolase

retro aldol condensation

Uni Bi kinetics

stereospecificity

two mechanistic classes:

- Class I - Schiff base formation-enamine stabilization
- Class II - Divalent cation stabilization of enolate

The Reactions of Glycolysis

Triose phosphate isomerase

isomerization reaction

concerted general acid-base catalysis involving low-barrier H-bonds

pH dependent - pK = 6.5 (Glu, His) and pK = 9.5 (Lys)

loop structure gives stereoelectronic control

diffusion-controlled reaction (catalytic perfection)

Reactions of Glycolysis

Glyceraldehyde-3-phosphate dehydrogenase

aldehyde oxidation drives acyl-phosphate synthesis - first high-energy intermediate

NAD^+ reduction

nucleophilic SH group forms thioester bond

Reactions of Glycolysis

Phosphoglycerate kinase

phosphoryl transfer - first ATP generation

sequential kinetic mechanism

two-domain enzyme (catalysis by proximity effects)

driving force of reaction is phosphoryl group transfer

The Reactions of Glycolysis

Phosphoglycerate mutase

transfer of functional group from one position to another in a molecule

phosphoenzyme (His phosphorylated)

formation of bisphospho intermediate (2,3-bisphosphoglycerate)

detour pathway in erythrocytes (Hb allostery)

Reactions of Glycolysis

Enolase

dehydration reaction - second high-energy intermediate

divalent cation required (Mg^{2+})

Reactions of Glycolysis

Pyruvate kinase

phosphoryl transfer reaction - second ATP generation
K^+ and Mg^{2+} required

Fermentation: The Anaerobic Fate of Pyruvate

Need to recycle NAD^+

Homolactic fermentation

$$\text{Glucose} + 2ADP + 2P_i \rightarrow 2\text{lactate} + 2ATP + 2H_2O + 2H^+$$

$$\Delta G^{\circ\prime} = -196\ kJ \cdot mol^{-1}$$

Lactate dehydrogenase

$$\text{pyruvate} + NADH \rightarrow \text{lactate} + NAD^+$$

stereospecificity in hydride transfer

mammalian isozymes:

two subunits (M and H) - five tetrameric forms

H_4 LDH has low K_m for pyruvate and is allosterically inhibited by pyruvate

M_4 LDH has higher K_m for pyruvate is not inhibited

Fermentation: The Anaerobic Fate of Pyruvate

Need to recycle NAD^+

Alcoholic fermentation

Glucose + 2ADP +2P_i

$\rightarrow$ 2ethanol + 2CO_2 + 2ATP + 2H^+

$\Delta G^{\circ\prime} = -235\ kJ \cdot mol^{-1}$

Pyruvate decarboxylase - thiamine pyrophosphate coenzyme (not present in animals)

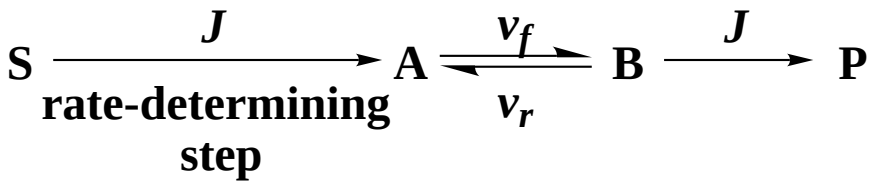
Alcohol dehydrogenase - Zn^{2+} and NADH dependent

Control of Metabolic Flux

Rate of flow (flux = J) of intermediates through a metabolic pathway is constant and is set by the rate-determining step(s)

But the pathway must be able to respond to specific biological energy needs (i.e., communicate with other steps)

$$J = v_f - v_r$$



$$\frac{\Delta J}{J} = \frac{\Delta[\mathrm{A}]}{[\mathrm{A}]} \cdot \frac{v_f}{(v_f - v_r)}$$

Two cases:

1. Irreversible reaction - v_r approaches 0, v_f (v_f - v_r) approaches 1, nearly equal increase in Δ[A] to respond to increase ΔJ

2. Approaching equilibrium - $v_r \sim v_f$, $v_f/(v_f - v_r)$ approaches infinity, much smaller increase in Δ[A] to respond to increase ΔJ

Control of Metabolic Flux

Rate determining step functions far from equilibrium and has a large negative free energy

Substrate control is only one way to rationalize control of rate-determining step of a metabolic pathway

Other flux-controlling mechanisms:

1. Allosteric control - regulated by effector molecules (substrates, products, coenzymes in the pathway) that change enzyme activity
2. Covalent modification - regulated by modifications (phosphorylation, dephosphorylation) that change enzyme activity
3. Substrate cycles - vf and vr of nonequilibrium reactions are catalyzed by different enzymes and thus may be independently varied
4. Genetic control - enzyme concentration may be altered by protein synthesis in response to metabolic needs

Mechanisms 1-3 respond rapidly (seconds to minutes) and denoted short-term control
Mechanism 4 responds more slowly (hours to days) and denoted long-term control

Control of Metabolic Flux

Control of glycolysis in muscle

Look for large negative ΔG under physiological conditions:

hexokinase	$\Delta G = -27\ kJ \cdot mol^{-1}$
phosphofructokinase	$\Delta G = -26\ kJ \cdot mol^{-1}$
pyruvate kinase	$\Delta G = -14\ kJ \cdot mol^{-1}$

Phosphofructokinase (PFK-1):

Tetrameric enzyme (R and T states)
ATP is substrate and allosteric inhibitor
Two ATP binding sites per subunit (substrate site and inhibitor site)
ATP binds well to substrate site in either R or T state
ATP binds to inhibitor site in T state
Fructose-6-phosphate binds to R state

At high [ATP], ATP acts as allosteric inhibitor and decreases affinity of PFK-1 for F6P

More important allosteric effector is fructose-2,6-bisphosphate

Control of Metabolic Flux

Control of glycolysis in muscle

Metabolic flux through glycolysis can vary 100-fold but ATP varies only 10%
Adenylate kinase - 10% decrease in [ATP] translates into a 4-fold increase in [AMP]

Consider substrate cycling:

Two enzymes are involved in establishing equilibrium-like conditions:

1. Phosphofructokinase-1 (PFK-1)

fructose-6-phosphate + ATP
$\rightarrow$ fructose-1,6-bisphosphate + ADP
$\Delta G = -26\ kJ \cdot mol^{-1}$

2. Fructose-1,6-bisphosphatase (FBPase)

fructose-1,6-bisphosphate + H_2O
$\rightarrow$ fructose-6-phosphate + P_i
$\Delta G = -9\ kJ \cdot mol^{-1}$

Net reaction is ATP + $H_2O \Leftrightarrow$ ADP + P_i (futile cycle)

Control of Metabolic Flux

Control of glycolysis in muscle

Assume 4-fold increase in [AMP] causes PFK-1 activity (v_f) to increase from 10 to 90% of its maximum and FBPase activity (v_r) to decrease from 90 to 10% of its maximum

Maximum activity of PFK-1 is 10-fold > maximum activity of FBPase

Assume PFK-1 activity = 100 units (v_f)
FBPase activity = 10 units (v_r)

At low [AMP]:

$$J_{low} = v_f(low) - v_r(low) = 10 - 9 = 1$$

At high [AMP]:

$$J_{high} = v_f(high) - v_r(high) = 90 - 1 = 89$$

Therefore:

$$J_{high}/J_{low} = 89/1 = 90!$$

Laws of thermodynamics are not violated!

(Cannot favor both forward and reverse reactions of a single enzyme)

Metabolism of Hexoses Other Than Glucose

Fructose, galactose, and mannose are converted to glycolytic intermediates and then processed as described previously

Fructose - fruit and hydrolysis of sucrose

In liver:

Fructokinase - phosphoryl transfer to form fructose-1-phosphate

Fructose-1-phosphate aldolase (type B) - aldole cleavage to form dihydroxyacetone phosphate and glyceraldehyde

Glyceraldehyde kinase - phosphoryl transfer to form glyceraldehyde-3-phosphate

or

Alcohol dehydrogenase, glycerol kinase, glycerol phosphate dehydrogenase

Excess fructose depletes liver P_i (activating glycolysis $\rightarrow$ lactate buildup)

Metabolism of Hexoses Other Than Glucose

Galactose - hydrolysis of milk sugar
(not recognized by glycolytic enzymes)

Galactokinase - phosphoryl transfers to form galactose-1-phosphate

Galactose-1-phosphate uridylyl transferase - uridylyl transfer from UDP-glucose to galactose-1-phosphate

UDP-galactose-4-epimerase - epimerization converts UDP-galactose to UDP-glucose

Phosphoglucomutase - isomerization reaction to form glucose-6-phosphate

Galactosemia - increased [galactose] and [galactose-1-phosphate] $\rightarrow$ galactitol in lens of eye

Metabolism of Hexoses Other Than Glucose

Mannose - digestion of polysaccharides and glycoproteins

Hexokinase - phosphoryl transfer to form mannose-6-phosphate

Phosphomannose isomerase - isomerization to form fructose-6-phosphate (mechanism similar to phosphoglucose isomerase)