*Glycolysis

1. Phospho-rylation of Glucose

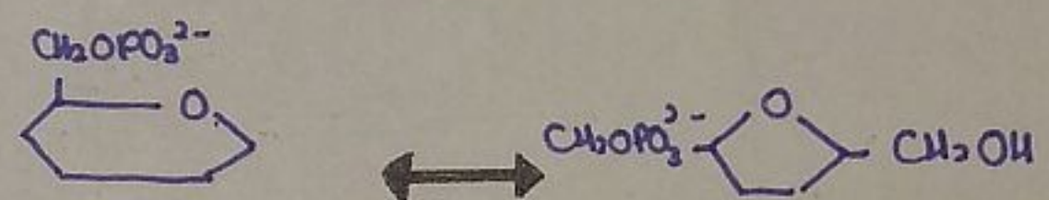
Glucose → Glucose-6-Phosphate

* Regulation:
- This step is irreversible ⇒ rate determining
- Hk: inhibited by glu-6-P, deoxyglucose
- Gk: • inhibited by fru-6-P • stimulated by insulin, glucose (indirectly) (inc. Gk transcription)
* GkRP in liver reversibly binds to Gk in the presence of Fru-6-P
  - Gk translocated into nucleus → binds to RP → inactivates enzyme
  - when glucose levels ↑ → Gk is released from RP → reenters cytosol → active

* Diseases:
- inactivating mutations of Gk ⇒ maturity onset of type 2 diabetes (rare) → impaired insulin secretion

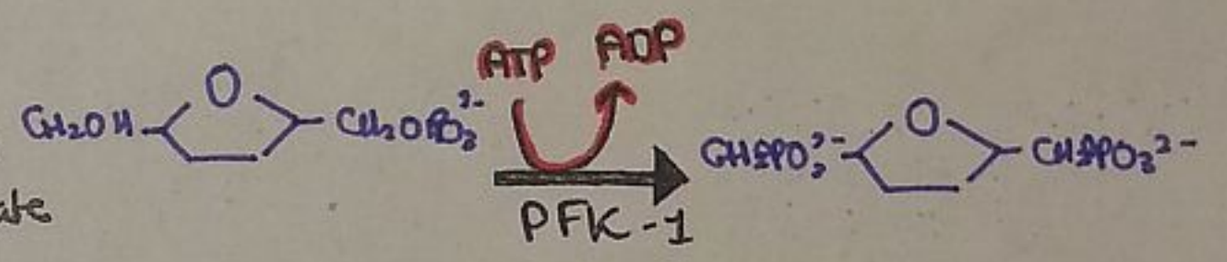
* glu-6-P could be used for: glycogen syn., pentose phosphate pathway.

2. Isomer-ization of glu-6-P



Glucose-6-Phosphate (aldose) → Fructose-6-Phosphate (ketose)

3. Phosphorylation of fru-6-Phosphate

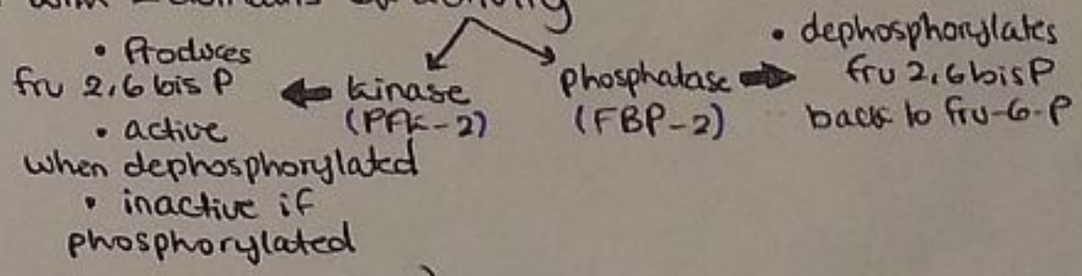


Fructose-6-Phosphate → Fructose 1,6-bisphosphate

- This is the most imp. reg. rxn and the rate-limiting and committed step of glycolysis.

* PFK-1 is inhibited by:
  - ATP → allosterically
  - Citrate (also favors use of glucose for glycogen synthesis)
  * AMP activates PFK-1 → allosterically

* regulation of PFK-1 by fructose 2,6-bisphosphate:
- most potent activator of PFK1, even if ATP levels are high.
- PFK-2 converts Fructose-6-Phosphate ⇒ Fructose 2,6-bisphosphate
  - it is a bifunctional protein with 2 domains of activity



* Fru 2,6 BP is under hormonal regulation (in the liver.)

- During well-fed state: Blood sugar high, decreased levels of glucagon, elevated insulin levels
  → insulin binds to receptor → decreased cAMP → Protein kinase A levels ↓
  → decreased PkA activity → dephosphorylation of PFK-2/FBP-2 complex
  → dephosphorylated PFK-2 is active, FBP-2 is inactive → favors formation of fructose 2,6 bisphosphate from fructose 6-phosphate
  → elevated conc. of fruc 2,6 BP → activate PFK-1 → glycolysis rate ↑

- During fasting: elevated levels of glucagon, low insulin levels
  → glucagon binds to receptor → activates adenylyl cyclase → increased cAMP → PkA ↑
  → increased PkA activity → phosphorylation of PFK-2 → inactive
  * This results in inhibition of glycolysis and activation of gluconeogenesis.

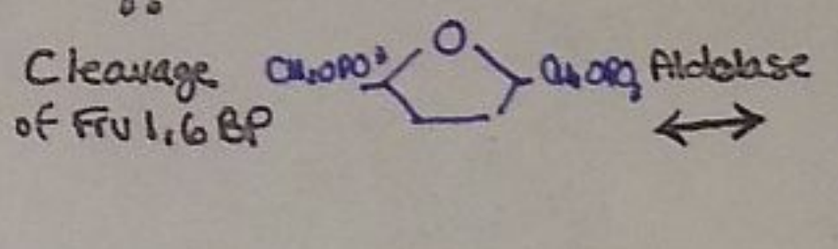
***end of Phase I: preparative phase (Phosphorylated forms of intermediates are synthesized at the expense of ATP). Now the ATP-generating Phase (II) will begin.

notes:

- The irreversible phosphorylation of glucose traps the sugar as cytosolic glu-6-P.
- catalyzed by Hexokinases (I-III) in tissues and Hexokinase IV (glucokinase) in liver.
- Hk: low Km, high affinity → can pick up glucose even when it is present in low conc.
  Km < 0.02 mM, low Vmax
  - inhibited by its reaction product
- Gk: high Km, low affinity → ∴ liver will not use up glucose when it's levels are normal / slight hypoglycemia, it supplies glucose to maintain normal levels instead.
  Km 10-20 mM ∴ will not phosphorylate glucose when its conc. is low (Fasting = 4mM)
  - induced by ↑ insulin, glucose
  - only works if glu level > 100 mg/dl
  - High Vmax → removes glu flood delivered by portal circ. → prevents hyperglycemia
  - glucose sensor: determines insulin secretion threshold.
  - fascilitates glucose phosphorylation during hyperglycemia ⇒ sequesters (traps) cellular phosphate in the form of phosphorylated hexoses. (high Vmax)

4.

Cleavage of Fru 1,6 BP



Fru 1,6 BP

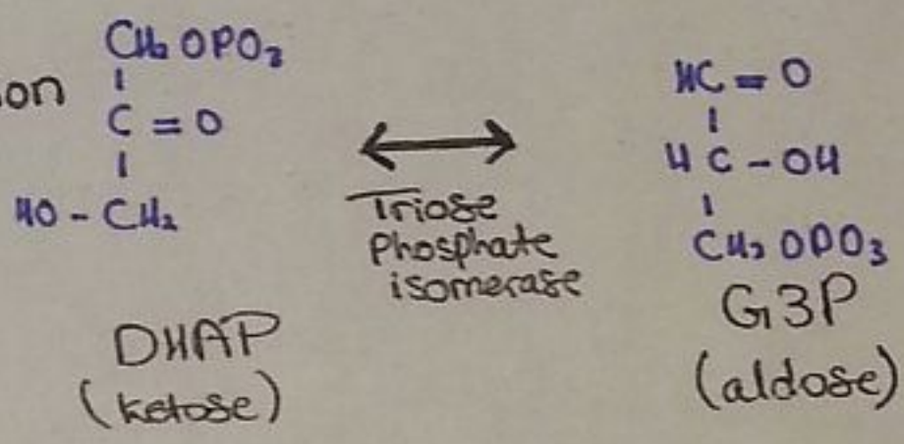
Dihydroxyacetone Phosphate

Glyceraldehyde 3-Phosphate

- Aldolase cleaves Fru 1,6 BP
- reaction is reversible, not regulated.

5.

Isomerization of DHAP



DHAP (ketose)

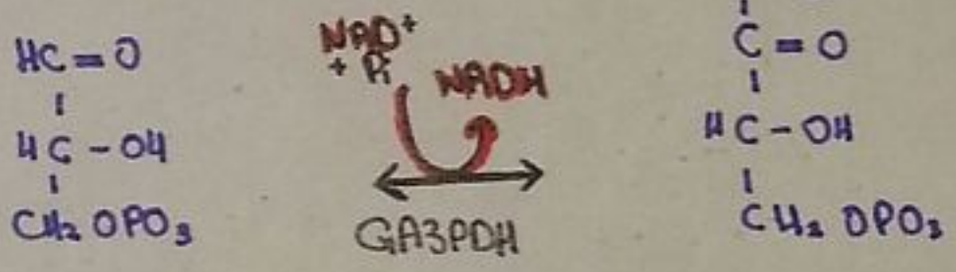
G3P (aldose)

- This results in the net production of two G3P molecules from cleavage products of fru 1,6 BP

*reactions 6-10 are x 2.

6.

Oxidation of G3P



G3P

1,3BPG

- This oxidation is coupled to the attachment of Pi to the carboxyl group.
- this is a very high energy Pi group
- there is limited NAD+ in cells, ∴ must be reoxidized by: 1) conv. of pyruvate → lactate
2) ETC → requires shuttles

7.

Synthesis of 3-Phospho glycerate

1,3BPG

3PG

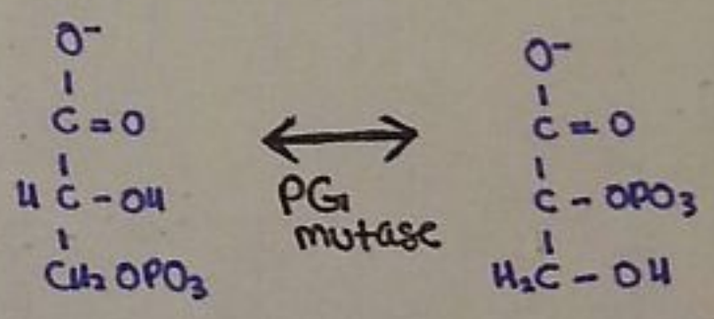
- this kinase rxn replaces 2 ATPs consumed earlier.
- this rxn is an example of substrate level phosphorylation.

*6) some 1,3 BPG is converted to 2,3 BPG by: BPG mutase
- this is present in high amounts in RBCs → increases O_2 delivery.
→ it is hydrolyzed by a Phosphatase to 3PG (an intermediate in glycolysis)
- this is a short in glycolysis

*6) Arsenic Poisoning:
- pentavalent arsenic (arsenate)
- prevents net ATP and NADH production:
- competes with Pi as a substrate for GA3PDH → forms a complex → hydrolyzes → 3PG
∴ bypasses synthesis of 1,3 BPG → no Pi transfer.

8.

Shift of Pi group



3PG

2PG

- this dehydration redistributes energy within substrate and forms PEP
- PEP contains a high energy enol phosphate
- Flouride inhibits enolase

9.

Dehydration of 2PG

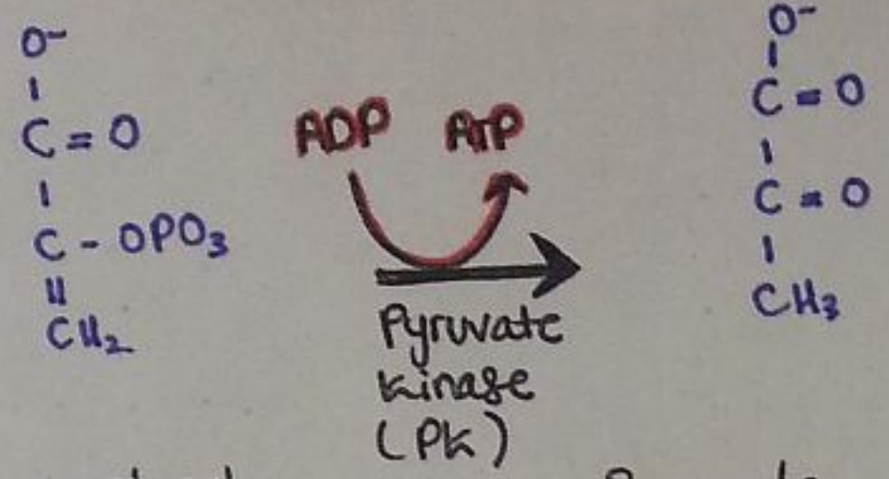
2PG

Phospho enol Pyruvate (PEP)

*notes on reaction 6:
- Alkalating compounds (e.g. iodoacetate), methyl mercuric chloride, and sulfhydryl reagents inhibit the action of GA3PDH.

*note on Arsenic Poisoning, reaction 6:
- Arsenite (trivalent) is more toxic, inhibits enzymes such as the pyruvate dehydrogenase complex, kG-D-etc. it is more toxic, and works by binding to both -SH groups of the cofactor lipoic acid.

10. Formation of Pyruvate



Phosphophenol Pyruvate (PEP)

Pyruvate

* Diseases - Pk deficiency:

- Hemolytic anemia : - reduced glycolysis rate → reduced ATP produced
  → alterations in RBC membrane → change cell shape
  → Phagocytosis
  - Pk def. is restricted to RBCs (lack mitochondria, completely dependent on glycolysis for ATP)
  - Severity depends on degree of enzyme def.
  - and on extent to which RBCs compensates by synthesizing increased 2,3 BPG levels
  - individuals heterozygous for this are resistant to malaria
  - severe deficiency requires blood transfusions
  - 90% of all glycolytic def. cases

*note: PGI def.: 4% of cases.

* Feedforward regulation:

- Pk is activated by Fru 1,6 BP → this links the two kinase activities (Pk and PFk-1): increased PFk-1 activity → elevated levels of Fru 1,6 BP → activates Pk

* covalent modulation of Pk:

- low glucose levels in blood → high glucagon → binds to receptor → adenylyl cyclase active → cAMP levels increase → activates PKA → phosphorylates Pk → inactive (in liver only)
- PEP is therefore unable to continue glycolysis ⇒ gluconeogenesis
- dephosphorylation of Pk by a phosphatase reactivates it.

*note: ATP fuels the ion pumps necessary for the maintanence of the flexible, biconcave shape that allows them to squeeze through capillaries.

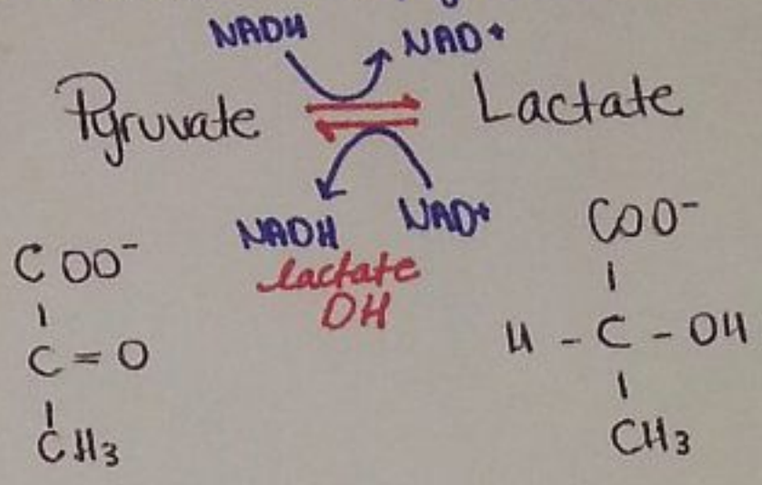
* Pk deficiency:

1) enzyme activity / stability may be altered / amount decreased
2) enzyme shows abnormal V_{max}/K_m for substrates / coenzymes (ADP / PEP)
3) it may show an abnormal response to the activator Fru 1,6 BP.

* Fate of Pyruvate:

1) Reduction to lactate
   - by lactate DH, the final product of anaerobic glycolysis.
   - in lens, cornea, kidney, testes, leukocytes, RBCs
2) Oxidative Decarboxylation to A CoA
   - by pyruvate DH complex to Acetyl CoA ⟶ TCA cycle / FA synthesis
   - in tissues with a high oxidative capacity; example: cardiac muscle
3) Carboxylation to Oxaloacetate
   - by pyruvate carboxylase : biotin dependent reaction
   - replenishes TCA cycle intermediates and provides substrate for gluconeogenesis
4) Reduction to ethanol
   - by pyruvate decarboxylase : in yeast / certain MOs, not humans.
   - enzyme requires thiamine pyrophosphate as a coenzyme

Reduction of pyruvate to lactate



Lactate is the final product of anaerobic glycolysis in lens/ cornea of eye, medulla, testes, leukocytes, RBCs (poorly vascularized, lack mitochondria)

Lactic Acidosis
- Lactate: > 5 mM (0.4 - 1.8 mM norm.)
- pH: < 7.2 (7.35 - 7.46 norm.)

* Notes:

- Direction of this rxn depends on NADH / NAD+ ratio, and the relative conc. of Pyruvate: Lactate.
- Liver + Heart: NADH / H+ ratio < muscles (exercising) → oxidize lactate to pyruvate.
- Liver: Pyruvate is then converted → glucose by gluconeogenesis or oxidized in the TCA cycle.
- Heart: Oxidizes Lactate → $CO_2 + H_2O$ in TCA cycle
- Muscle: To cope with increased energy demand during exercise
  - NADH production by GA3PDH and DH of TCA cycle exceeds oxidative capacity of the ETC.
  - Lactate levels ↑ 5 - 10 folds (when NADH/NAD+ ratio is elevated, it favors the reduction of pyruvate to lactate)
  - Lactate accumulates in muscles → drop in intracellular pH → cramps.
  - This eventually diffuses into the blood stream / gluconeogenesis.
- Lactate could be produced in the case of Hypoxia (brief episodes.)

* Lactic Acidosis

- most common cause of metabolic acidosis
  - increased production of lactic acid
  - decreased utilization of lactic acid
- Causes:

1) Collapse of Circ. System
   - MI: impaired O_2 transport
   - Resp. Failure: Pulmonary Embolism
2) Uncontrolled Hemorrhage / Shock
3) Direct inhibition of Oxidative Phosphorylation
4) Hypoxia
5) Alcohol intoxication
6) ↓ Gluconeogenesis
7) Pyruvate DH ↓ (inherited def. / thiamine def.)
8) TCA activity ↓
9) Pyruvate Carboxylase Deficiency
   - Oxygen debt: excess of O_2 required to recover from a period when the availability of oxygen has been inadequate.