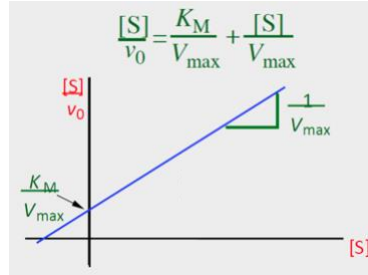
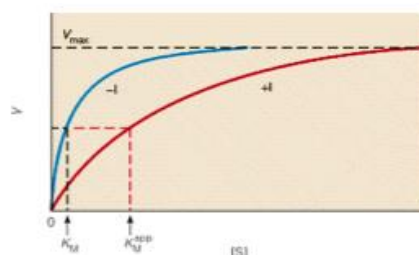


- **Hanes-Woolf plot** – slope is $1/V_{\max}$, y-axis intercept is $K_M/V_{\max}$, even weighting of the data.



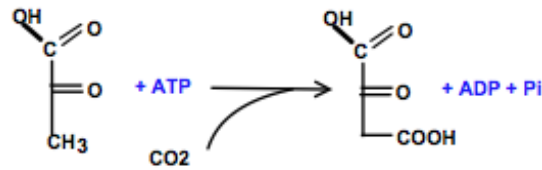
Enzyme Regulation

- **pH**
 - Usually only active in a **narrow pH range** (usually 5-9) due to pH sensitivity of substrate binding, reduced catalytic efficiency of the enzyme, ionisation of substrate, protein structural changes (usually at pH extremes).
 - Kinetic analysis as a function of pH provides information about the nature of functionally (catalytically) important residues.
 - Tend to get a bell-shaped curve when looking at pH and reaction rate, different enzymes have **different optimum pH** suited to environments where they work.
 - **Denaturing** is normally only at extreme changes in pH.
 - pH affects the state of the groups in the active site by protonation, as well as the substrate.
- **Temperature**
 - Increase in temperature gradually increases the rate to a maximum, increasing enzyme activity by making more flexible, then begins to affect active site (reversible) before fairly rapid decrease in rate until enzyme becomes too flexible and eventually denatures.
 - Relatively small impact from increased number of collisions as temperature increases.
- **Types of regulation**
 - Enzyme flexibility provides opportunity for regulation.
 - Can be positive (activation) or negative (inhibition).
 - Can involve covalent binding or non-covalent (ionic, hydrophobic, van der Waals forces) interactions with the regulatory molecule.
 - Can be reversible (covalent and non-covalent) or irreversible (post translational modifications, protein cleavage, irreversible regulatory molecule binding), from point of view of cell economy it doesn't make sense to have many reactions completely irreversible.
 - **Non-covalent regulation** – highly specific so only the target enzyme is affected, two main types are allosteric regulation and simple inhibitors, can bind to E (competitive), E and ES (non-competitive), and ES (uncompetitive) which is more complicated because this is usually in multisubstrate reactions.
- **Competitive Inhibition** – structure similar to substrate, occupies active site, inhibitor (I) and substrate (S) compete, binding of I and S is mutually exclusive, effect is reversed by increasing substrate concentration.
 - **Kinetics of competitive inhibition** – if $[S]$ increases to infinity then all I is displaced so $V_{\max}$ remains unchanged, at all $[S]$ I will move equilibrium from E to ES so $[E][S]/[ES]$ will increase so K_M will appear to increase to K_{app} and apparent affinity for substrate is decreased.

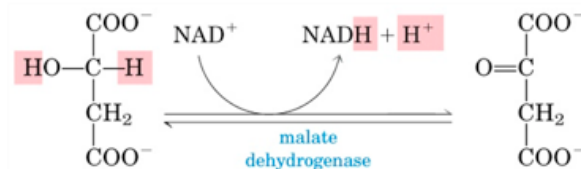


It is assumed that I binds reversibly to the enzyme and is in rapid equilibrium with it so that $K_I = [E][I]/[EI]$, then $v_o = V_{\max}[S] / K_M(1 + [I]/K_I) + [S]$, and $V_{\max} = k_2[E]_T$.

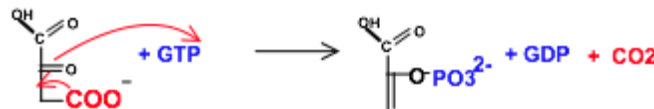
- **Pyruvate carboxylase** – converts pyruvate to oxaloacetate in the mitochondria by carboxylating it (biotin is carboxylated in the biotin carboxylase domain, using ATP, then biotin carrier domain swings CO_2 to the carboxyl transferase domain where pyruvate is carboxylated), Mg^{2+} coordinates.



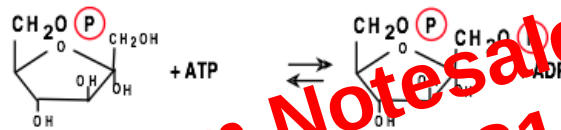
- **Malase** turns oxaloacetate to **malate** to get it out of the mitochondria via the malate shuttle using **malate dehydrogenase** with NAD then it is converted back with NADH and H^+ .



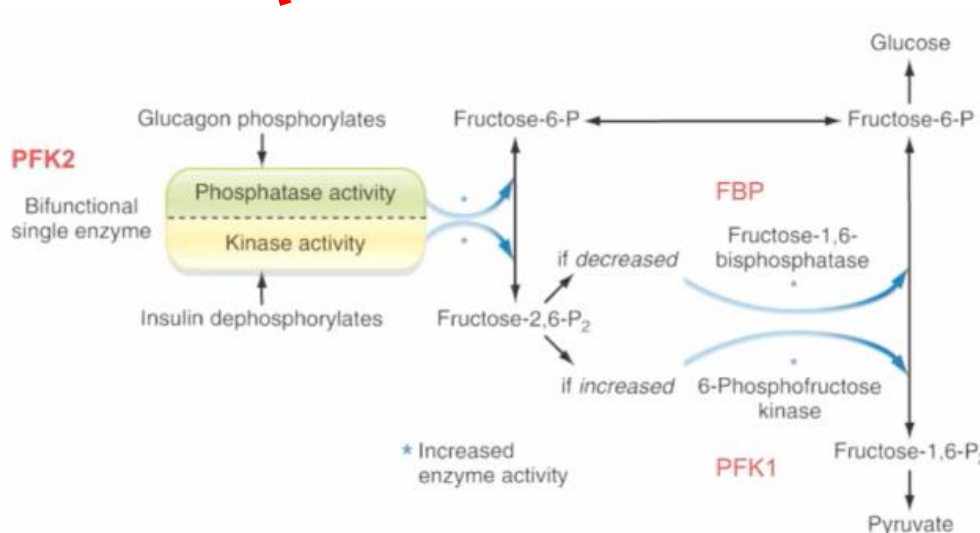
- **PEP carboxykinase** – converts oxaloacetate to PEP, requires GTP (made from ATP), requires Mg^{2+} for phosphoryl transfer, occurs outside the mitochondria.



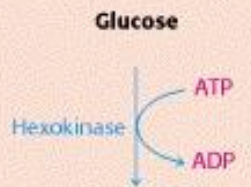
- **Fructose-1,6-bisphosphatase** – converts fructose-1,6-bisphosphate to fructose-6-phosphate (the reverse of the rate determining step in glycolysis), requires ATP, very highly regulated (reciprocal regulation).



- Regulatory steps have become important targets for treating diabetes and obesity – **glucagon** (increases blood glucose) and **insulin** (lowers blood glucose) are important regulators, both act on **phosphofructokinase**, insulin dephosphorylates and pushes to pyruvate, glucagon phosphorylates and pushes towards glucose.



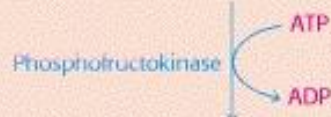
Stage 1



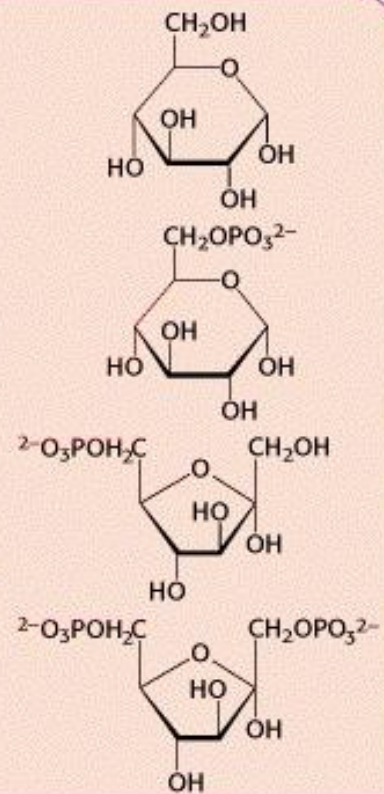
Glucose-6-phosphate



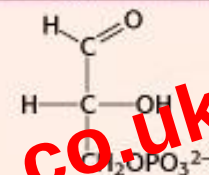
Fructose-6-phosphate



Fructose-1,6-bisphosphate



Stage 2



Stage 3



3-Phosphoglycerate



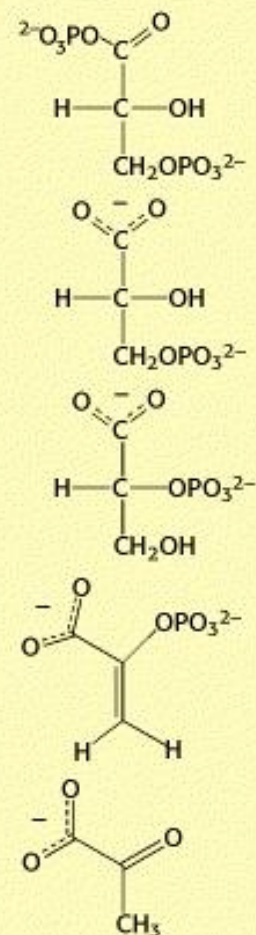
2-Phosphoglycerate



Phosphoenolpyruvate



Pyruvate



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