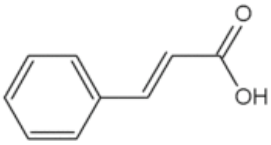
1. **Drugs.** In class several weeks ago, we looked at a figure from a recent paper describing the mode of action of the anti-CML drug candidate ON012380, and discussed some similarities between Abl tyrosine kinase and Tyrosinase. I'd like you to extend that analysis by sketching out how you think a similar dataset would look for two Tyrosinase inhibitors. Specifically, please consider thiourea (which many of you found to act as a non-competitive inhibitor in the assays you performed; its formula is H_2NCSNH_2), and cinnamic acid (a close structural relative of phenylalanine, shown below). Thiourea is commonly thought to inhibit tyrosinase by displacing the molecular oxygen from the active site.

cinnamic acid: 

Please **sketch two graphs** (you may choose either Michaelis-Menton plots or Lineweaver-Burke plots):

- one showing the effect on tyrosinase activity of increasing amounts of L-DOPA in the presence of a constant amount of oxygen
- one showing the effect on tyrosinase activity of increasing amounts of molecular oxygen in the presence of a constant amount of L-DOPA

Each graph should contain three lines:

- one for tyrosinase alone
- one for tyrosinase plus cinnamic acid
- one for tyrosinase plus thiourea

Key point here:
- cinnamic acid should behave as a competitive inhibitor with DOPA, and non-competitive with oxygen
- thiourea should behave as a competitive inhibitor with oxygen, and non-competitive with DOPA

Thus on the graph where [S] refers to DOPA, cinnamic acid should increase K_M (with little change in V_{max}) and thiourea should decrease V_{max} (with little change in K_M).
On the graph where [S] refers to oxygen, cinnamic acid should decrease V_{max} (with little change in K_M) and thiourea should increase K_M (with little change in V_{max}).

2. **Regulation of protein action by phosphorylation**. The enzyme Pyruvate Kinase catalyses the interconversion of phosphoenol-pyruvate and pyruvate in the final step of glycolysis. It is thus a key site of regulation in the main "thoroughfare" of reactions that convert cellular stores of glucose into ATP for use in the cell. The enzymatic activity of pyruvate kinase is modulated in multiple ways, including:

- regulation by high levels of fructose-1,6,-bisphosphate, a glycolytic intermediate
- regulation by high levels of ATP, the effective end-product of glycolysis
- regulation by low blood-sugar levels, which endanger brain function

Please consider the biochemical logic regarding regulation of this enzyme. It may help to think of this enzyme as a valve in the "pipeline" connecting stored glucose in the cell with ATP:

glucose stores --> --> --> fructose-1,6-bisphosphate --> --> --> || --> --> ATP
|
pyruvate kinase

Please **speculate about what impact each of the stimuli listed above would likely have on pyruvate kinase**. For each stimulus, please indicate:

- whether you expect the regulator to increase or decrease enzyme activity
- whether you expect the regulation to act directly by (non-covalent) allosteric modulation or indirectly, via phophorylation (e.g., the stimulus activates a kinase that phosphorylates pyruvate kinase)

high level of fructose-1,6-bisphosphate:
- should stimulate pyruvate kinase because it indicates that glycolytic intermediates are building up and the cell needs to process them into energy more quickly
- should stimulate pyruvate kinase directly -- it is indicative of an INTRAcellular need

high level of ATP:
- should inhibit pyruvate kinase because it indicates that the cell has plenty of energy currency, and glucose could probably be better used for other things
- should inhibit pyruvate kinase directly -- it is indicative of INTRAcellular energy status

low blood-sugar levels:
- should inhibit pyruvate kinase because it indicates that the organism needs sugar in the blood, thus over-riding any cellular need for ATP derived from the glucose
- should inhibit pyruvate kinase via phosphorylation -- it is indicative of EXTRAcellular energy status

3. **Protein regulation and metabolism basics.**

a) We have studied four main mechanisms for the regulation of protein activity, listed below. **Please circle all of these you believe to be reversible:**

Competitive inhibition **Allosteric effectors**

Selective proteolysis **Phosphorylation**

b) We have seen examples of control of key metabolic enzymes by both phosphorylation and allosteric effectors. **Which of these two forms of control would you expect to be associated primarily with response to organism-level conditions, and which would you expect to be associated primarily with cell-level controls** (or is there no such link)?

cell-level: allosteric
organism-level: phosphorylation

c) Please briefly **explain the biochemical logic underlying your answer to part (b).**

allosteric effectors are diffusable indicators of local conditions
phosphorylation is controlled by enzymes that are activated by cascades of cell signalling events in response to external stimuli

d) **Please explain** in a sentence or two **how a multi-subunit, cooperative enzyme can be more responsive to small changes in substrate concentration than can an enzyme that obeys classical Michaelis-Menton kinetics.** (Figures are welcome!)

Because the slope of the sigmoidal response curve of a cooperative enzyme is variable (very low at first, very steep in the middle), and because the position of the curve can be affected by various modulators of enzyme activity (think pH and BPG with Hb), it can be "tuned" so that the steepest part of the curve falls at the cellular [S]. This leads to big changes in velocity with small changes in [S].

Question #4, continued...

e) In a few sentences, **please explain what end-product inhibition of metabolic pathways is, and how it works.** (Diagrams are useful here, too.)

end-product inhibition refers to the sensitivity of metabolic pathways to a buildup of the ultimate product of the pathway. If the pathway is producing more of the end-product than the cell can use, it makes sense to shut down the pathway.

f) **Why are serine, threonine, and tyrosine the only three amino acids that are commonly phosphorylated?**

They are the only three hydroxylated amino acids

5. Metabolic logic.

a) The utilization of glucose in the cell, either for breakdown or for storage, requires that it first be phosphorylated. Mammals have two different enzymes that perform this function in different tissues.

- The **brain and muscles** are major glucose consumers. In these cells, the phosphorylation of glucose to form glucose-6-phosphate is accomplished by **hexokinase**. Hexokinase binds glucose with a *low* K_M and is inhibited by the product of the phosphorylation reaction.

- In the **liver**, the organ responsible for storing excess blood glucose in the form of glycogen, the dominant catalyst is **glucokinase**. Glucokinase binds glucose with a *high* K_M and is *not* inhibited by high glucose-6-phosphate levels.

Please explain the metabolic logic of these two differences (K_M and feedback inhibition) **between hexokinase and glucokinase, in light of the different primary fates of glucose in the tissues in which these enzymes are found.** Please ensure that your answer is explicit, for example in distinguishing between catabolic vs. anabolic fates, the meaning of high vs. low K_M, inhibition vs. no inhibition.

At low concentrations of glucose in the blood, glucose should go the consumer organs. Thus, cells in these organs bind and phosphorylate glucose effectively (that is, they need to use kinases with low K_M for glucose). At these concentrations of glucose, the storage organ should take in little glucose (and so it uses a kinase with high K_M for glucose). At low levels of glucose in the blood, catabolism is king.

At high concentrations of glucose in the blood, muscle and brain cells with have plenty of glucose-6-phosphate, and don't need more. Thus hexokinase is inhibited, and glucose levels in the blood rise. This allows glucokinase to bind its substrate well, and the excess glucose is taken into liver cells where it can be stored in the form of glycogen. As long as blood glucose levels are high, it should continue to be stored, so glucokinase keeps working and is not inhibited by glucose-6-phosphate. At these high levels of blood glucose, there is plentiful material for generating cellular energy, and so anabolic processes kick in.