

Seminar 3

Energetics

For these some of problems, the *eQuilibrator* tool is useful: equilibrator.weizmann.ac.il

1: Draw the ΔG diagrams (substrate, energy barrier, product) for (1) an irreversible reaction (2) a spontaneous reaction. What are the important feature of the energy diagram in each case?

2: What is the effect of enzyme catalysis on forward / reverse / net / exchange fluxes?

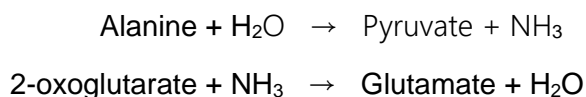
3: Find the ΔG for oxidation of glucose to lactate (glycolysis). Is the reaction favorable? How many ATP could you theoretically synthesize with this amount of energy? Compare with the actual ATP yield of glycolysis.

4: What is the ΔG for complete oxidation of glucose to CO_2 (balance the reaction with O_2 and H_2O) ? How does it compare to the glucose to lactate conversion? Compare this ΔG with the number of ATP typically obtained from complete oxidation of glucose; what fraction of the energy in glucose is captured as ATP?

5: Pyruvate kinase is one of the few reactions that is capable of “driving” ATP synthesis. Does this mean that the Gibb’s energy of phosphoenolpyruvate is higher than that of ATP?

6: Would you expect the ΔG of deamination reactions to be positive or negative, considering the redox chemistry? For example, consider the reaction $\text{Glutamine} + \text{H}_2\text{O} \rightarrow \text{Glutamate} + \text{NH}_3$. What does this reaction look like in the *eQuilibrator* database?

7: Transamination can be viewed as the coupling of an amination and deamination reaction. For example, a transaminase might couple



What is the resulting reaction and its ΔG ?

8: Are high-energy metabolites like ATP typically unstable in water? Why / why not?

9: If all cells run the metabolism towards lower G, how is energy-rich molecules ultimately synthesized (by primary producers) ?

Respiration

10: How many O_2 are required to oxidize one molecule of pyruvate? How many ATP are obtained? What factors are involved in determining these values? Are they exact?

11: For any substrate, the respiratory quotient R is defined as the molar ratio (CO_2 produced) / (O_2 consumed) for complete oxidation of the substrate. What chemical property does this ratio reflect? What values of R would you expect for glucose oxidation?

12: Can you calculate the respiratory quotient for palmitate (C16 fatty acid, sum formula $C_{16}H_{32}O_2$) without knowing the details of palmitate oxidation? Can you tell how many NADH (or equivalent carriers) will be used? Can you use this value to estimate the ATP production from palmitate?

13: Are there other functions of the respiratory chain besides ATP synthesis?

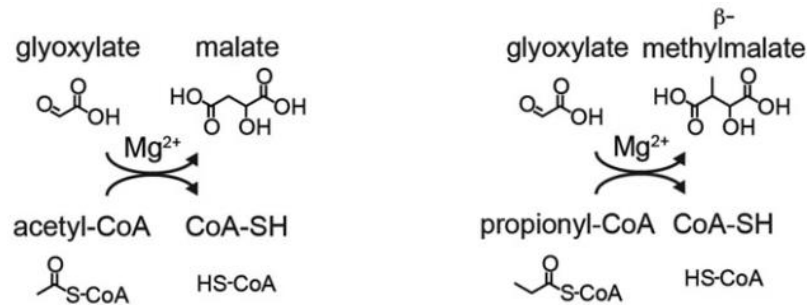
Enzyme classes

We have mentioned that there are only a few types of enzymes. Here we will examine them in some more detail. Knowing the enzyme classes helps you understand how enzymes are related, even across widely different species.

14: Which EC classes are used in the TCA cycle? Which ones are used in the non-oxidative arm of the pentose phosphate pathway (transaldolase, transketolase, etc) ? What are the major differences?

15: Look up the EC classification of ATP-citrate lyase. This case is a bit ambiguous. What EC classes are involved?

16: Knowing EC classification can be helpful for discovering the function of previously uncharacterized enzymes. For example, Strittmater et al (Hum Mol Genet 23:2313-2323, 2014) studied the function of the *CLYBL* gene product, which is sequence-related to ATP-citrate lyase (ALCY), and experimentally determined that it catalyzes the below reactions. Could you have predicted this from the EC classification of ATP-citrate lyase (*ACL*Y) ?

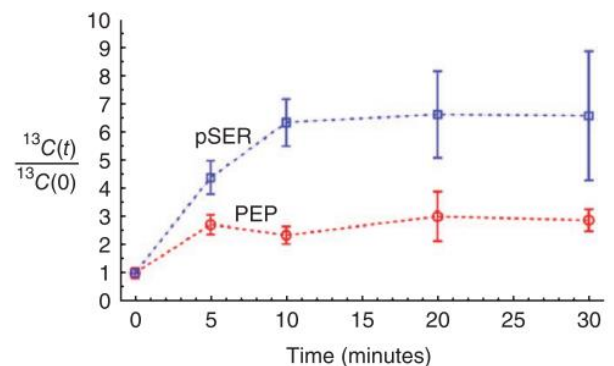


Amino acid metabolism & isotope tracing

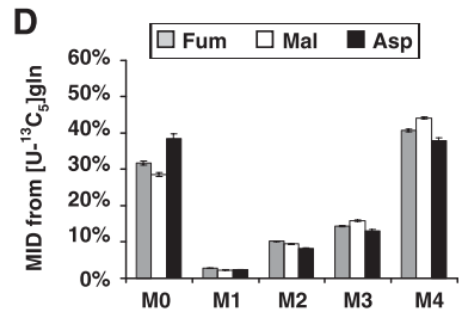
17: All amino acids except leucine and lysine can be used as substrates for gluconeogenesis in some way. Why are leucine and lysine exceptions? Can they still give useful products in case of glucose starvation?

18. During starvation, catabolism of muscle protein does not produce equal amounts of all protein-coding amino acids, but mainly glutamine and alanine. Why are these particular amino acids the major products?

19: The figure to the right is from Locosale et al. Nat Genet 43:868-874, 2011. The authors state that “¹³C incorporation into pSER (13C-pSER) reaches steady state at a time scale comparable to the time for phosphoenolpyruvate (PEP) to reach steady state, suggesting that the relative fluxes [of serine and pep synthesis] are comparable”. Do you agree with this conclusion?



20: The figure shows labeling of fumarate, malate, and aspartate from U-¹³C₅-glutamine in cells cultured in medium containing unlabeled aspartate (Gaglio et al, Mol Sys Bio 7:523, 2011). How is aspartate synthesized in these cells, and can you explain the mass isotopomers observed? Can you think of a reason why aspartate is being synthesized even though aspartate is available in the medium?



21: Some cells can engulf and digest protein from their surroundings to obtain amino acids. The figures are from Commisso et al, Nature 497:633-637, 2013, showing uptake of fluorescent labeled albumin (left), and the MID of alanine from ¹³C-labeled protein (right). Can we tell what fraction of alanine is obtained from protein consumption?

