PRE-ACTIVITY ASSIGNMENT

- What are [1-^{14}C]-Glc, [3,4-^{14}C]-Glc, [6-^{14}C]-Glc, and [U-^{14}C]-Glc?

  These refer to the specific numbered carbons in glucose that have ^{14}C instead of C-12. The U-^{14}C is uniformly labeled.

IN-CLASS ACTIVITY

Critical Thinking Questions

1.
   - Group of noncovalently associated enzymes that catalyze two or more sequential steps
   - 3 enzymes: pyruvate dehydrogenase, dihydrolipoyl transacetylase, dihydrolipoyl dehydrogenase
   - Advantages are 1) enhance reaction rate by eliminating need for enzymes to find each other 2) channeling of intermediates reduces chance that they will react with something else 3) coordinated control of reactions
2.
   - Coenzymes needed: TPP, lipoamide, conenzyme A, FAD, NAD^+
   - Coenzymes: small organic molecules required for the catalytic activity of an enzyme. Coenzyme must be regenerated in order to complete the catalytic cycle.
3. Hydrolysis of thioester is more energetically favorable than hydrolysis of simple ester (thioester is stabilized by resonance due to large radius of S)
4. 2,5 are the carboxyl carbon, 1,6 are the methyl carbon
5. No
6. Carboxyl carbons of acetyl CoA, which are carbons 2, 5 of glucose
7. Carbons 3, 4 from glucose
8. Glycolysis. Carbon 3 of glucose is lost in the PDH-catalyzed conversion of pyruvate to acetyl-CoA. Any carbon going through this reaction had to have come from glycolysis and not yet entered TCA.
9. No because this carbon is lost in the PDH reaction before any carbons from glucose enter TCA.
10.
    - Carbons 3, 4 from glucose are removed from pyruvate by PDH and never enter TCA. Therefore, generation of $^{14}CO_2$ from 3,4- ^{14}C-glucose is a good measure of flux through glycolysis.
    - Carbon 6 from glucose is the methyl carbon in acetyl CoA and enters TCA. Therefore, generation of $^{14}CO_2$ from 6-14C-glucose is a good measure of flux through TCA.
11. Need to divide 21.3 ÷ 2 to account for fact that 3,4 labels twice as many carbons as 6.
12. ^{14}C-CO_2 could be formed from radiolabeled carbon 1 either by PPS or TCA. ^{14}C-CO_2 could be formed from radiolabeled carbon 6 only through going through TCA. Carbons 1 and 6 both go into TCA and ultimately get lost as CO_2 after two turns of the cycle. Once they enter TCA, 1 and 6 are indistinguishable, so carbon 6 is equal to carbon 1 going through TCA.

1 glucose → 2 lactate, therefore 740µmol glucose would be converted to 1480µmol pyruvate if conversion 100%. 90% conversion = 1332, which is very close to the value 1320 given in the table.

Less than 1% to CO_2 is reflected in the CO_2 production resulting from uniformly labeled U-^{14}C glucose. 36µmol of CO_2 produced from U-^{14}C glucose divided by total glucose carbons 6 × 760µmol or 4560µmol is 36µmol ÷ 4560µmol = 0.79 %.