

Plant Metabolism

Final Exam

April 30, 2007

Name: _____

Please limit your answers to the space available for each question.

1. (5 pts) Where can nitrate typically be stored at the whole plant level, (3) and where inside the cells (2)?

Generally in the leaves and roots, or stated another way, in the roots and shoots. In the leaves, higher levels of nitrate can be found in the midrib. At the subcellular level, nitrate is only accumulated (stored) in the vacuoles.

2. (5 pts) Where is nitrate reduced to NH_3 inside plants (2), and in what subcellular compartment(s) and in what organs (3)?

Nitrate to NH_3 predominately occurs in the leaves, but can also occur in the roots. Nitrate reductase is found in the cytosol and nitrite reductase if found in the stroma of chloroplasts (plastids).

3. (6 pts) Describe **two characteristics** of the enzyme itself (2) and the **general reaction catalyzed** by nitrate reductase (2). In addition, what major posttranslational mechanism acts to control nitrate reductase activity (2)?



Of course, nitrate is reduced to nitrite, and is NADPH dependent.

Has a mini-electron transport chain where NADPH is the electron donor and electron acceptor/donors include FAD, MoCo cofactor, Heme and NO_3^- is the final electron acceptor.

Phosphorylation/dephosphorylation or allostery is suitable

4. (4 pts) What is chlorate (ClO_3^-) used for (2)? What kind of loss-of-function mutants can it reveal (2)?

Chlorate is an analog of nitrate and can be used to screen for loss of function nitrate uptake transporters in the roots or loss of function nitrate reductase mutants. In the absence of functional uptake transporter for nitrate or nitrate reductase, wt plants grown on chlorate will die, while the mutants will survive longer.

5. (4 pts) How does sulfate enter into the cells of roots (2)? What drives the uptake of sulfate in roots (2)?

Sulfate transporters in the roots use the energy derived from a proton electrochemical gradient across the cell membrane to drive the transport of sulfate into the cytosol by a symport mechanism. Proton gradient is created by proton pump ATPases where the proton concentration is high on the outside and lower in the cytosol. Generally the ratio of protons to sulfate transported is about 3.

6. (6 pts) Compare and contrast the major characteristics of nitrate and sulfate transporters.

<u>Nitrate Transporter</u>	<u>Sulfate Transporter</u>
Nitrate normally limiting	Sulfate normally not limiting
Mediated by H^+ electrochemical gradient	Mediated by H^+ electrochemical gradient
$2 H^+/NO_3^-$	$3 H^+/SO_4^{2-}$
Encoded by gene family	Encoded by gene family
High and/or low NO_3^- affinity	High SO_4^{2-} affinities in roots, low affinity in leaves
Soil nitrate presence inducible	Inducible by plant SO_4^{2-} starvation; low in soil
Nitrate proton symporter	Sulfate proton symporter

7. (4 pts) SO_4^{2-} to S^{2-} is a more energy intensive reductive process than either nitrate or carbon dioxide fixation. How is this energy and reducing equivalent demand met in plants?

The ATP and reducing potential is largely provided by photosynthetic electron transport in the chloroplast in the form of Fdx_{red} or NADPH although reduction can occur in the roots in plastids. In the latter case, then the reducing potential come from PPP produced NADPH, and ATP from respiration.

8. (3 pts) Briefly list or describe each of the three general steps of sulfate assimilation into a terminal organic form.

Activation of sulfate: requires ATP and forms 5'-adenylylsulfate (APS) or PAPS
 Reduction to sulfide: reduction requiring reduced thioredoxin or Fdx_{red} , glutathione_{red}
 Incorporation of sulfide to form cysteine: requires *O*-Acetylserine to form cysteine

9. (5 pts) OAS(thiol)lyase is located where in the plant cell (3)? What does it do (2)?

Chloroplast/plastid (high)
Cytosol (low)
Mitochondrion (intermediate)

This enzyme catalyzes the incorporation of sulfide in OAS to form cysteine and acetate.

10. (4 pts) What is significant about the compound *ent*-kaurene in phytohormone metabolism (2)? Where is *ent*-kaurene synthesized in plant cells (2)?

ent-kaurene is an intermediate derived from the terpenoid biosynthetic pathway precursor geranylgeranyl diphosphate and it is the product of the first committed step of GA biosynthesis.

Chloroplast/plastid

11. (5 pts) What is one important form of GA biosynthetic regulation?

Feed-back inhibition by GA_1 that reduces the levels of mRNAs by a repressor of pre-mRNA synthesis that reduces the oxidase and hydroxylase enzymatic activity earlier in the pathway from GA_{53} to GA_1 .

12. (5 pts) What is the biosynthetic pathway of origin of the ABA C_{40} precursor (3)? How is this particular pathway of ABA synthesis related to abiotic stresses like water deficits (2)?

Terpenoid pathway and violaxanthin or neoxanthin are ABA precursors.

Neoxanthin cleavage is the first committed step of ABA synthesis. This step is closely linked with abiotic stresses like water deficits as a number of the genes for this step are highly sensitive to stress conditions showing increased expression. Under water deficits, ABA and other signals act to close stomata which will lower internal CO_2 and carbon assimilation. Under high light and low carbon assimilation, reducing potential becomes excessive and the xanthophyll cycle must act to dissipate excess electron supply. A component of the xanthophyll cycle is violaxanthin which is the precursor for neoxanthin. Activation of the xanthophyll cycle causes a reduction in violaxanthin levels but not neoxanthin.

13. (5 pts) What is the biochemical or functional purpose for the ABA derived metabolites phaseic acid, dihydrophaseic acid and their glucosides?

Inactivation and degradation of ABA.

14. (5 pts) What two biosynthetic pathways are needed to synthesize zeatin (4)? What is the biosynthetic source of zeatin's glycosides (1)?

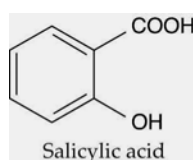
Terpenoid pathway and purine nucleotide pathway

UDPG or carbohydrate metabolism

15. (4 pts) What is the biochemical relationship of tRNAs and cytokinins?

Certain tRNAs have a modified base which can then be transformed into a cytokinin. However de novo biosynthesis of cytokinins from a basic nucleotide is the more important biosynthetic source.

16. (4 pts) What is the structure of salicylic acid? Draw it.



17. (4 pts) What is the starting precursor for JA synthesis (3)? *Be careful with your answer, don't be too general.* Jasmonic acid is structurally similar and derived from the same general class of cellular molecules as are what important signaling molecules in animal systems (1)?

A fatty acid from a membrane phospholipid, specifically α -linolenic acid

Any one of the three: prostaglandins, thromboxanes, leukotrienes

18. (5 pts) Why is it said that metabolically, that polyamine synthesis could compete with ethylene synthesis?

Because SAM is a precursor for both ethylene and polyamines spermine and spermidine.

19. (5 pts) What does it mean to be a "tryptophan auxotroph" (2)? What is the expected effect on IAA content of such plants (3)?

A tryptophan auxotroph is unable to make tryptophan because of a biosynthetic mutation.

A plant unable to make tryptophan is unable to make IAA via tryptophan dependent pathway. However, plants have a second tryptophan independent pathway using a precursor of tryptophan biosynthesis between chorismate and tryptophan. So depending on the enzyme that is defective in the Trp auxotroph, the mutant could have normal IAA levels or low IAA levels.

20. (4 pts) What effect on tomato fruit does effective antisensing ACC oxidase have (2)? What does ACC oxidase do metabolically (2)?

The formation of ethylene during ripening is reduced as is ACC oxidase enzymatic activity. If ethylene synthesis can be blocked, then ripening processes linked with the autocatalytic production of ethylene would be reduced, or delayed.

The oxygen-dependent ACC oxidase converts ACC to ethylene, CO₂ and HCN. Of course ethylene is the product of major biological activity.

21. (5 pts) Why is natural product and secondary metabolism so widely variable in the plant kingdom?

Secondary compounds and natural products are not universally essential for cell function and plant growth and development, and therefore are not highly conserved across the plant kingdom. However, these compounds invariably confer some level of selective advantage or benefit to the plants that produce them, so they are preserved and not genetically lost. Often the selective advantage conferred is unique and varies according to the need the compound(s) provide to the plant. The wide array of secondary compounds and natural products form networks of structurally related compounds, as a result of the activities of oxidases, hydroxylases, decarboxylases, isomerases, lyases, other enzymes that modify the basic structure of the different secondary and natural compound groups, and that can be modified over the course of evolution to improve plant fitness in its interactions with the environment and other organisms.

22. (4 pts) What is the major obstacle presently to creating designer plants producing a specific natural product of value in the desired concentrations, plant and tissue and at the desired time?

Lack of detailed knowledge and tools to work with to modify concentrations, transfer biosynthetic capabilities to other plants, and direct biosynthesis in the right cell, tissue, in the desired concentration and at the desired time.

23. (4 pts) Explain in what ways metabolism relates to your dissertation research project.

Best of luck in your professional and personal pursuits.