Water Soluble Vitamins Problem Set 2015

1. What type of reactions use thiamin as a necessary cofactor? Does the pyrimidine ring or thiazole ring play a greater part in the reaction mechanism?

Thiamin is used to facilitate the oxidative decarboxylation of α-keto acids as well as the transfer of α-ketols between sugar molecules. The thiazole ring plays a greater part in the reaction mechanism due to the fact that C2 of the thiazole ring forms a covalent bond with the carbonyl carbon in both cases to facilitate transfer or decarboxylation.

2. Provide three reasons that alcoholics may be deficient in thiamine. What disease might result from chronic deficiency state?

Alcoholics generally have poor diets so do not take in much thiamine to begin with. Additionally, alcohol has been shown to prevent the conversion to the active cofactor form. Low amounts of coenzyme storage as well as loss via urinary excretion can lead to a deficiency state. Chronic deficiency can lead to Wernicke-Korsakoff syndrome or possibly Beriberi.

3. Cytochrome P450 reductase (CPR) is an enzyme responsible for the reduction of a large portion of cytochrome P450 (CYP) enzymes. What color would you expect a purified solution of CPR to be, assuming all enzyme was in the oxidized state? Do you predict any change in the color of the CPR when CYP3A4 and excess NADPH are added to the solution?

Assuming complete oxidation of the CPR FAD and FMN, the purified solution should look yellow due to the oxidized flavin cofactors. Upon addition of CYP3A4 and excess NADPH, the cofactors should be converted to their reduced forms which are colorless. The solution should become clearer, not taking into account the added color from CYP3A4.

4. What is the coenzyme form of Vitamin B₆ and why is it contraindicated with levo-DOPA therapy?

The active form of Vitamin B₆ is pyridoxal-5-phosphate. B₆ enhances decarboxylation of levo-DOPA to dopamine in the periphery, which should happen after crossing the blood brain barrier. Dopamine will not cross the blood brain barrier so increase in periphery decarboxylation will result in a decrease in the therapeutic effect of an identical dose of levo-DOPA in the presence of high B₆.
5. How does PLP control carbon atom reactivity in PLP-dependent reactions?

By switching the structure of the substrate-PLP complex between an external and internal imine, either the PLP or substrate carbon will be more electrophilic. This will help control the site of nucleophilic attack by water.

6. How is low Vitamin B₆ diagnosed?

Low plasma PLP and low transaminase activity in the presence and absence of supplemented PLP. If transaminase activity increases significantly upon addition of extra PLP, the patient can probably stand to take some extra.

7. What three forms of tetrahydrofolate are essential for the synthesis of nucleic acids? What is their functional role?

N₁₀ formyl THFA, N₅N₁₀ methenyl THFA and N₅N₁₀ methylene THFA. THFA is used for methyl transfer reactions.

8. Describe the therapeutic strategy of “Leucovorin rescue”.

Methotrexate reduces the levels of DHFA reductase, which can be lethal. By supplementing the patient with leucovorin (N₅ formyl THFA), a higher than “lethal” dose can be administered.

9. Why is folate an especially important vitamin in women that are considering becoming pregnant or that are pregnant?

Folate deficiency is associated with birth defects such as orofacial clefts and neural tube deficits.

10. How does Vitamin B₁₂ deficiency promote folate deficiency, in biochemical terms?

Vitamin B₁₂ is crucial for the recycling of folate. B₁₂ helps convert N₅ Methyl THFA back to THFA. Therefore, if someone is B₁₂ deficient they will not be able to maintain healthy levels of TFHA because it will be sequestered as N₅ Methyl THFA.

11. Why do alcoholics frequently suffer from folate deficiency?

Alcohol interferes with the reabsorption of tetrahydrofolate from the bile.
12. What is the chemical difference between the folic acid in vitamin supplements and dietary sources?

The form of folic acid used in supplements is the fully oxidized form.

13. What two classes of enzymes use Vitamin B\textsubscript{12} as a cofactor? What are their main function?

Vitamin B\textsubscript{12} is used in THFA recycling which is used for methyl transfer reactions. B\textsubscript{12} is also a cofactor in CoA reactions which metabolize fatty acids. The two types of reaction are methyl transfers and “mutase” reactions.

14. What is the role of “intrinsic factor” in Vitamin B\textsubscript{12} physiology? Why do elderly patients often need B\textsubscript{12} supplementation?

The “intrinsic factor” is a glycoprotein excreted by the stomach that helps transport B\textsubscript{12} across the ilium. Elderly patients often need B\textsubscript{12} supplements because they have lower levels of gastric acid and the “intrinsic factor” which results in the requirement for high initial intake.

15. What are the vitamer forms of B\textsubscript{12}? Which one participates in the classes of B\textsubscript{12}-dependent enzymes referred to in question 13?

Cobalamins (cyanocobalamin, hydroxocobalamin, methylcobalamin and 5-deoxyadenosylcobalamin). The THFA recycling cycle uses cobalamin and transforms it to methylcobalamin.

16. What test is done to check for cobalamin deficiency?

Schilling’s Test, or more commonly determination of methyl malonic acid in urine.

17. What type of chemical reactions is Coenzyme A used for and what vitamin is converted to Coenzyme A?

Vitamin B\textsubscript{5} is converted to CoA where it is used in acetyl-group transfer reactions.

18. Biotin deficiency has been shown to cause loss of hair. Therefore, everyone should use shampoo and conditioner full of biotin to promote hair health. True or false.

False. This has not been shown to help hair health in the slightest and is a marketing trick to help sell more shampoo.
19. Please show how NAD or NADP can be used to desaturate a carbon-carbon bond.

\[ \text{NAD(P)} \rightarrow \text{NAD(P)H} \]

20. What disease commonly occurred in the “corn belt” of the US in the 1900’s? What could have remedied this situation?

The lack of nicotinic acid and tryptophan in corn, due to the storage process, caused a large amount of pellagra. This could have been avoided by supplementation of the diet with meat, fish, whole grain cereals or peanuts.

21. What is a major function of Vitamin C in the cell?

Vitamin C acts as a free radical scavenger as well as a coenzyme for a number of reactions.

22. Bonus Question: What Nobel Prize winner claimed that mega-dosing (multiple grams/day) of Vitamin C was “How to Live Longer and Feel Better”? Do you agree or disagree with the efficacy of high intake of Vitamin C in prevention of disease?

Linus Pauling. There is no truly correct answer to the second question however, prevailing research has not shown a benefit of mega-dosing Vitamin C in reduction of cancer risk. Additionally, there is an upper limit to the amount of Vitamin C able to be absorbed per day so additional intake would mostly be excreted in the urine.