CHEM-643 Biochemistry Name ________________________________
Final Examination
7:00– 10:00 PM, Monday, 9 December 2013
Dr. H. White – Instructor

- There are 12 pages to this examination **plus two reference sheets** at the end that can be removed.
- **Write your name** on each new page.
- **Read every question** so that you understand what is being asked. If you feel any question is unclear or ambiguous, **clearly explain your answer or interpretation**.
- Please call my attention to any suspected errors you encounter.
- This is examination is closed book until 9:00PM when you may refer to your assignments and your lecture notes. Textbooks or electronic devises cannot be used. However, you may bring and use printouts refer of the metabolic pathway sheets available on the course website.
- This examination will assess your learning, problem-solving skills, and ability to communicate clearly. It is intended to be challenging even to the best students in the class. Some of the questions will deal with material you have not seen before and is not in your text; however, the questions can be answered by applying basic principles discussed in the course. Remember, ***Problem-solving is what you do when you don't know the answer. If you know the answer, it is not a problem.***
- Do not expose your answers to the scrutiny of your neighbors. Please fold under each page before you go on to the next. You may use the backs of pages, if you need more space.
- The maximum possible score is 135. Graded Exams can be picked up starting Thursday afternoon and will be held until Spring Semester.

Have a Safe and Happy Holiday!

Exam Statistics:

Total points possible 135

Class Range ________________

Class Mean _________________

Your Score _______

Your Rank in Class _____ out of 29

Course Grade _____

Part I - Basic Vocabulary and Working Knowledge "Bloom's Basement" (1 point each)

______________________________ 1. Vitamin lacking in vegan diets.

______________________________ 2. Phenylalanine can be converted to this amino acid but not the reverse.

______________________________ 3. Methionine donates methyl groups to this cofactor.

______________________________4. An amino acid that is a source of carbon that becomes the methyl group of methionine.

______________________________5. Purine compound used by birds to excrete nitrogen.

______________________________6. Supplementation with this vitamin reduces the incidence of spina bifida

______________________________7. Group of plants that harbor symbiotic nitrogen-fixing bacteria.

______________________________ 8. Number of phosphorus atoms in NADP

______________________________ 9. Cofactor required to shuttle fatty acids into the mitochondria

______________________________ 10. Cofactor associated with many reactions involving amino acids.

______________________________ 11. Respiratory quotient for a person using triglycerides as a sole energy source.

______________________________ 12. Respiratory quotient for a person using carbohydrate as a sole energy source.

______________________________ 13. Lipid soluble redox coenzyme that can accept one or two electrons,

______________________________ 14. Water soluble redox coenzyme that can accept one or two electrons,

______________________________ 15. Bicyclic ring system found in folic acid.

______________________________ 16. Enzyme target of statin drugs.

______________________________ 17. Number of cis double bonds in arachidonic acid

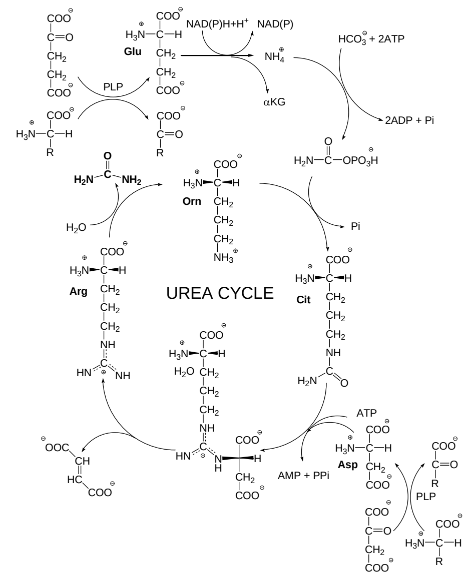
______________________________ 18. Another name for inositol hexaphosphate.

______________________________ 19. Ketone bodies are generated from the catabolism of _____.

______________________________ 20. Dodecameric enzyme that incorporates ammonia into an amino acid.

Part II Multiple Choice. For each of the following four questions select the best answer for 3 points and explain your answer for 2 additional points.

The figure to the right depicts the urea cycle in mammals. During the catabolism of proteins for energy, nitrogen from amino acids is channeled into the urea cycle to form urea, a non-toxic compound that is excreted.

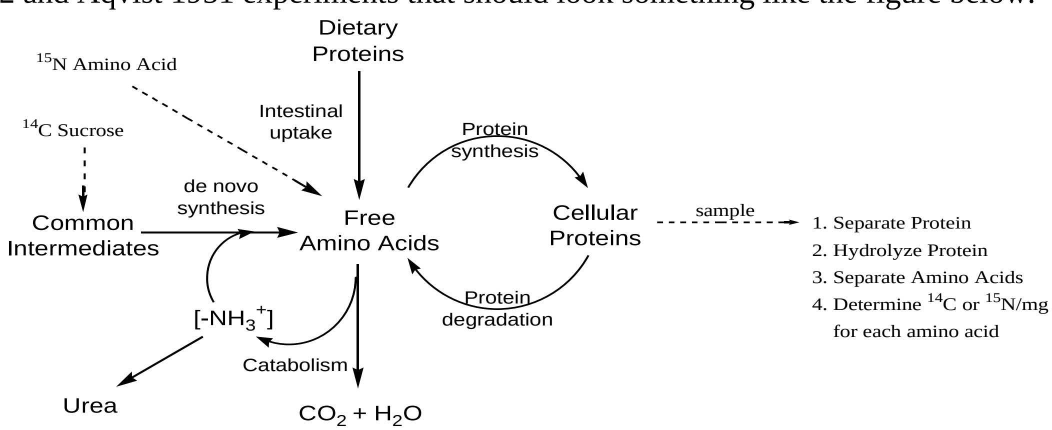


____ 1. In a mouse, ^{14}C from dietary sucrose, shows up three days later in arginine found in proteins. Which of the following metabolic precursors of arginine would contain the ***least*** amount of ^{14}C from dietary sucrose?

A. Ornithine B. Citrulline C. Pyruvate D. Bicarbonate

Explanation:

____ 2. By now you should have a conceptual overview of amino acid metabolism and the Steele 1952 and Aqvist 1951 experiments that should look something like the figure below.

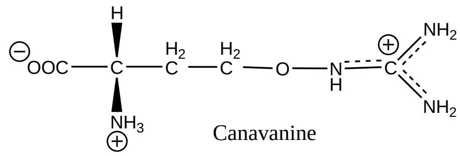


The amount of ^{14}C and ^{15}N in an amino acid will depend on the relative importance of the processes represented by arrows. Consider the results presented in Tables 1 and 3 for threonine (Provided as tear off sheet at end of test). From these results one can conclude that:

A. Threonine synthesis and degradation are much greater than for other amino acids.
B. Threonine synthesis and degradation are much less than for other amino acids.
C. Threonine synthesis is much greater, but degradation is much less than for other amino acids.
D. Threonine synthesis is much less but, degradation is much greater than for other amino acids.

Explanation:

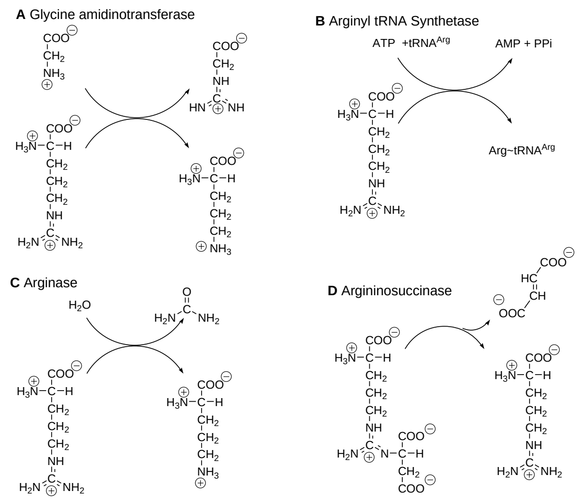
_____ 3. Canavanine (below) is a toxic analog of arginine found in the seeds of wild lima beans and alfalfa sprouts..



Canavanine

It would make us sick because our enzymes cannot discriminate between canavanine and arginine. Canavanine is not toxic to germinating legume seeds because their enzymes can discriminate between arginine and canavanine. Several enzyme reactions involving arginine are shown at the right. Which one would be most likely to lead to the toxicity associated with canavanine?

Explanation:



_____ 4. Pellagra is disease attributed to diets high in corn and lacking significant amounts of animal protein in the form of milk, eggs, or meat. Based on the codons in the corn gene encoding zein, a major protein component of corn seeds, its amino acid composition can be calculated [*Nuc. Acid. R*es. **9**:5163 (1981)]. The following table shows the amino acid composition of zein, minus its hydrophobic leader peptide.

AA	No.	AA	No.	AA	No.	AA	No.	AA	No.
Phe	13	Ile	9	Ser	15	Asp	0	Pro	23
Tyr	8	Val	5	Thr	5	Asn	10	Lys	0
Trp	0	Gly	5	Cys	2	Glu	1	Arg	2
Leu	43	Ala	29	Met	0	Gln	41	His	2

From a human nutritional perspective, zein would be considered an incomplete protein due to the:

A. Absence of Lys, Trp, and Met.
B. Low amounts of charged amino acids Glu, Asp, Arg, and Lys.
C. Unbalanced ratio of hydrophobic to hydrophilic amino acids.
D. The high amounts of Asn and Gln compared to Asp and Glu.

Explanation:

Part III – Problems and essays.

1. **Regulation of Amino Acid Metabolism by Attenuation.**

(36 Points) A bacterial cell must be sensitive to nutrients in its environment so that it does not waste energy synthesizing those compounds when they are available. For example, *Escherichia coli* can synthesize all of the amino acids found in proteins. If one or more of these amino acids becomes available, the biosynthetic pathways for them are shut down by end product inhibition and the synthesis of the enzymes in the pathways is repressed. As a result of DNA sequence and RNA sequence analysis combined with the physiologic response of various regulatory mutants, the fine control of the repression process is fairly well understood.

This question will focus on the exquisite fine control known as attenuation and its relation to amino acid metabolism. The following are some of the features of the attenuation mechanism quoted or paraphrased from Keller & Calvo *PNAS*, **76**, 6186-90 (1979).

a) Most transcription initiated at the relevant promotor terminates before the structural genes of the operon are reached, resulting in the synthesis of a leader RNA of about 150 nucleotides.

b) The site at which termination occurs **("attenuator")** is similar to previously identified transcription termination sites. It is a palindromic G-C rich region followed by a series of adenosines on the coding strand. The corresponding region of the leader RNA, which has a potential stem and loop structure followed by a series of uridines, is called the **"terminator."**

c) Each of the known leader RNAs contains a second potential stem-and-loop structure proximal to the terminator and overlapping with it in such a way that pairing with one region precludes pairing with the other.

d) Within each leader RNA, translational start and stop signals are positioned so that a peptide of 14-28 amino acids might be synthesized.

e) Each **leader peptide** contains in high frequency the amino acid corresponding to the particular operon.

f) The derepression of the operon requires the transcriptional read-through of the attenuator. This occurs only if a ribosome initiates the synthesis of the leader peptide and is retarded in its progress by lower than normal amounts of a specific amino acyl tRNA. This favors a different conformation of the leader RNA and signals transcriptional read through.

On the next page is given the ***non-coding*** strand of DNA corresponding to the control region of a particular amino acid operon [Gardner *PNAS* **76**, 1706-1710 (1979)]. Read the sequence as if it were the mRNA, T's rather than U's. **A Genetic Code sheet is at the back of the exam** and can be removed

5'...ACAGATAAAAATTACAGAGTACACAACATCCATGAAACGCATTAGCACCACCATTACC

ACCACCATCACCATTACCACAGGTAACGGTGCGGGCTGACGCGTACAGGAA

ACACAGAAAAAAGCCCGCACCTGACAGTGCGGGCTTTTTTTTTCGACCAAAG

GTAACGAGGTAACAACCATGGCGAGTGTTGAAGTTCGGCGGTACATCA...3'

A. (2 Point) **Underline** the transcriptional termination region.

B. (4 Points) This DNA sequence (sense strand) corresponds to that of the relevant leader RNA and mRNA. **Circle** the initiation and termination codons of the leader peptide.

C. (2 Point) **Put a box around** the initiation codon for the first structural gene.

D. (3 Points) **Write** the predicted amino acid sequence of the leader peptide above the appropriate codons. (2 point bonus if you can use the correct one-letter representations for **all** of the amino acids in the sequence.)

E. (2 Points) This sequence is derived from the control region of the operon for **what amino acid**?

F. (4 Points) **Identify** the palindromic region corresponding to the terminator **with opposing arrows drawn above** the sequences. (Note that the palindromic properties refer to double-stranded DNA of which only one strand is shown. Each strand in these regions have the potential to form stem-loop structures where the loops are unpaired.)

G. (4 Points) **Identify** the second palindromic region which overlaps the terminator **with opposing arrows drawn under** the sequences.

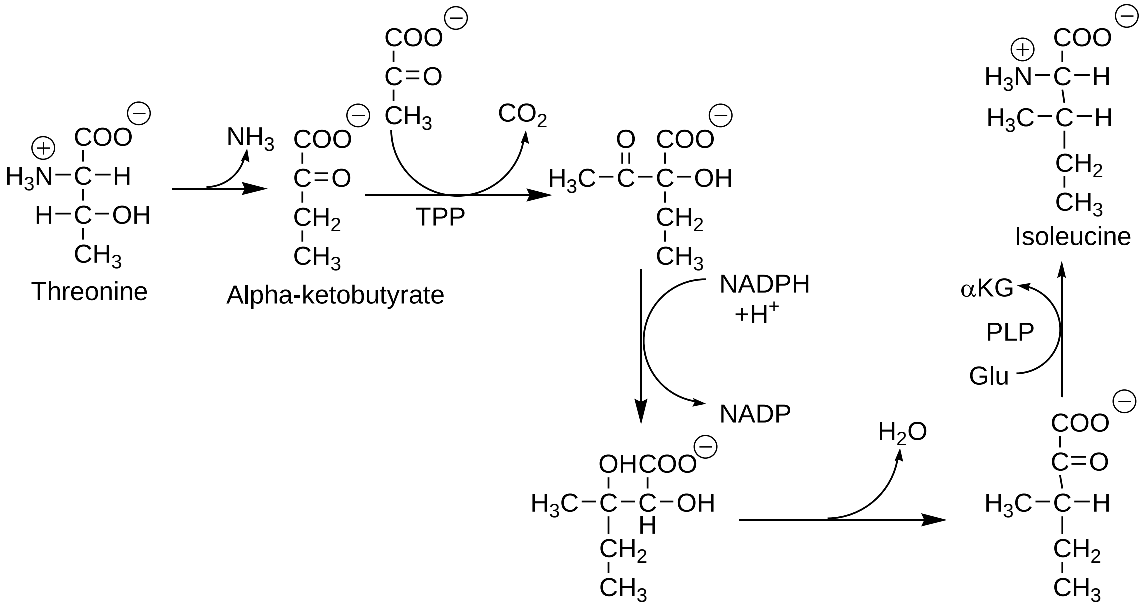
H. (5 Points) In the space below, **depict in a general way** the alternative base-paired structures possible for the leader RNA corresponding to the above sequence of DNA. **Which conformation would be favored** by low levels of the relevant amino acid?

I. (5 Points) Attenuation is an elegant and finely tuned mechanism for controlling the expression of amino acid operons. One must consider that even higher order regulation is involved such as that between operons. Consider the metabolism of the amino acid you have identified in part 5 and the sequence of the leader peptide from part 4. **What other amino acid** seems to be important in controlling the expression of this operon? **Does this make metabolic sense**? If so, **explain**.

J. (5 Points) Not all amino acid operons contain an attenuator region. Considering your group assignment to evaluate the hypothesis that enzymes involved in the biosynthesis of a particular amino acid will have less than expected amounts of that amino acid in their primary sequence. Would you **predict** whether an attenuator would enhance, diminish, or have little effect on the cognate amino acid composition of the enzymes in an amino acid operon? **Elaborate your reasoning.**

2) (14 Points) Threonine, an amino acid with two chiral centers, is the precursor of isoleucine, the only other common amino acid with two chiral centers, as is shown below.

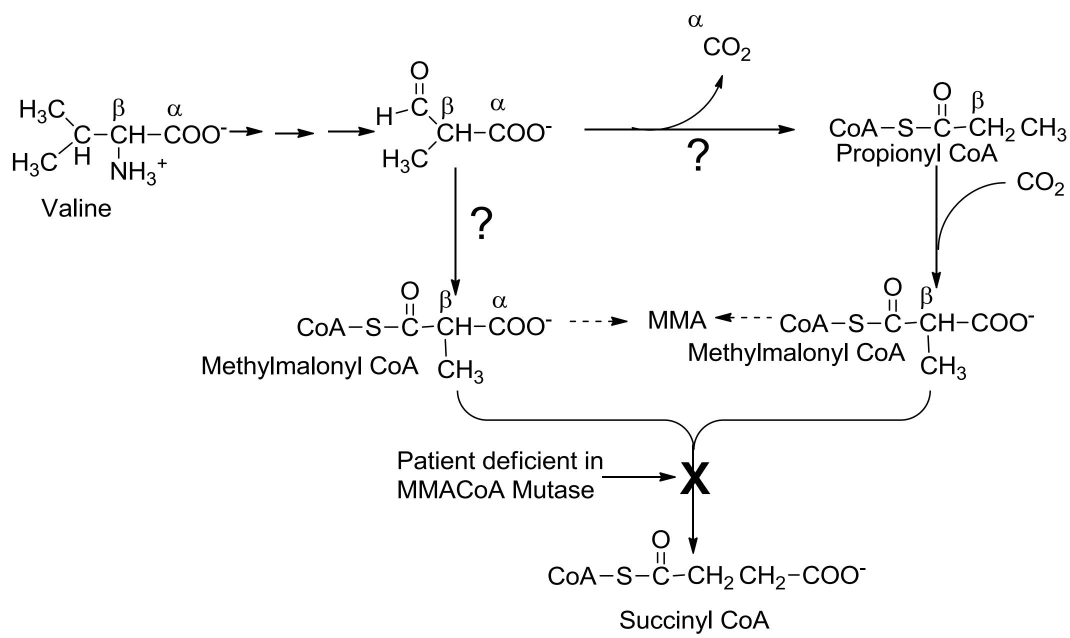
g) (4 points) **Put a circle around the carbon atoms** in isoleucine and its metabolic precursors that are derived from pyruvate.



h) (5 Points) Interestingly, each of the enzymes above has dual substrate specificity such that a methyl group can replace the ethyl group. Thus, in contrast to isoleucine, all of the carbon atoms of valine synthesized by this pathway come from pyruvate. Acetolactate synthase, the TPP-dependent enzyme, is the target for the potent sulfonylurea herbicides, Oust® and Glean®, manufactured by DuPont [*Trends in Biotech*. 2(6), 158-161 (1984)]. In cells inhibited by these compounds, α-ketobutyrate accumulates but pyruvate does not. How do you **explain this**?

i) (5 Points) While Oust® and Glean®, with K_i values in the nM range, are toxic to plants at a few grams per hectare, they have low toxicity to animals. What is a **reasonable explanation** for this large difference in toxicity?

3) (14 Points) Propionyl CoA is an intermediate in the catabolism of isoleucine, methionine, threonine, odd-chain fatty acids, and phytanic acid. The following experiment [Tanaka et al. *PNAS* **72**, 3692-3696 (1975)], involving a seven-year-old patient with methylmalonic aciduria, was designed to determine if valine should be added to this list. (See figure below.) Normally humans excrete less than 5 mg of methylmalonic acid (MMA) per day. The patient was excreting 2000-3000 mg of MMA/day, which was collected, purified, and analyzed by ^{13}C nmr which can detect ^{13}C in each carbon in a compound. Natural abundance of ^{13}C is about 1.1%.



Over a period of nine days, the patient consumed normal diet containing 2 mg of protein/kg/day except on day 3 and 7 when a high carbohydrate diet lacking protein was fed and α-^{13}C-valine or α and β-^{13}C-valine respectively were consumed with the meal. The table below shows the ratio of the ^{13}C nmr signal intensities between pairs of the indicated carbon atoms in MMA.

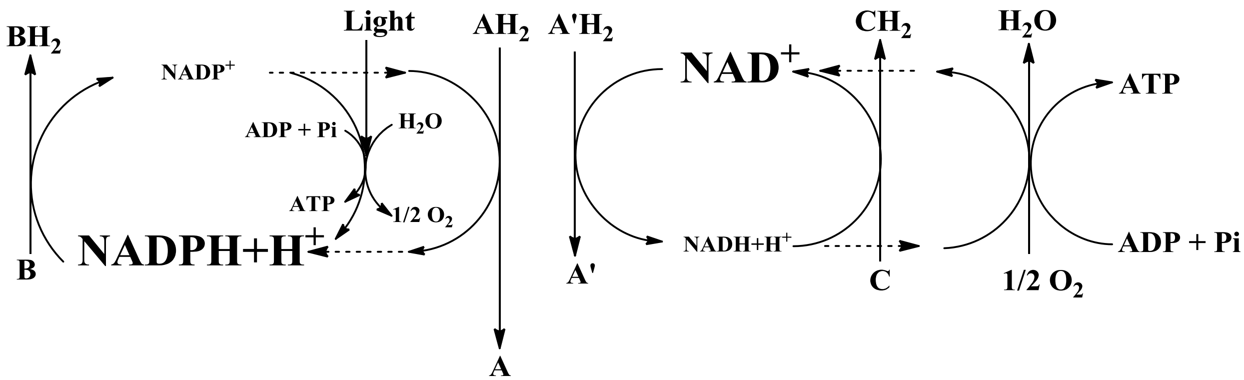
Day	**Source of valine**	**$^{13}COO^-/^{13}CH_3$**	**$^{13}CH/^{13}CH_3$**
1 & 2	Natural abundance dietary valine	0.4	0.77
3	451 mg α-^{13}C-valine		
3-6	Natural abundance dietary valine	0.44 ± 0.05	0.77 ± 0.03
7	392 mg α and β-^{13}C-valine		
7	Natural abundance dietary valine	0.49	1.66
8	Natural abundance dietary valine	0.33	0.86
9	Natural abundance dietary valine	0.36	0.77

a) (2 Points) **Draw** the structure of MMA.

b) (7 Points) **Based on these data**, is propionyl CoA an intermediate in valine catabolism? **Explain** the basis for your conclusion.

c) (5 Points) When the patient in this experiment was fed high amounts of vitamin B_{12}, the amount of MMA excreted was reduced almost to normal. **Propose a reasonable hypothesis** for the nature of his metabolic defect that would explain this observation?

4) (19 Points Total) The following is a figure used in the class with some text elements removed. Please **provide a title** for this figure **and a legend** that explains what the figure is intended to convey.



a) (3 Points) A title for the figure:

b) (8 Points) **Explanatory Figure Legend.** You may add back text to the figure to supplement your description. You may use bullet points to deal with different aspects of the figure. Use the back of this page, if you need more room.

c) (8 Points) Give one specific example for each or the four general reactions shown.

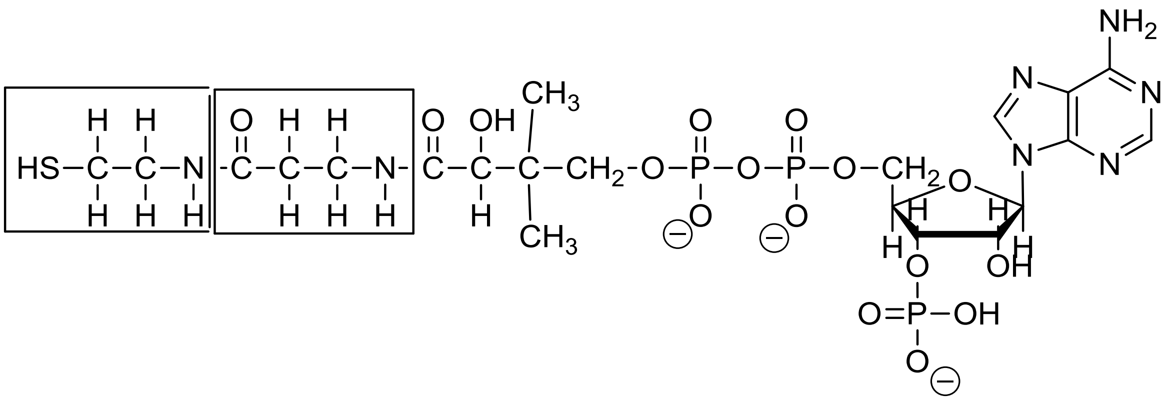
i) $AH_2 \rightarrow A$

ii) $A'H_2 \rightarrow A'$

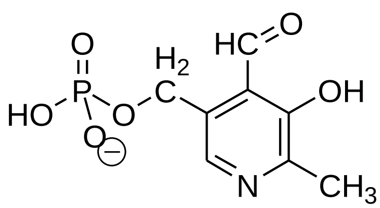
iii) $B \rightarrow BH_2$

iv) $C \rightarrow CH_2$

5) (12 Points) Coenzyme A below is assembled in bacteria from several separate precursor units. Two of the units, cysteamine and beta-alanine, are linked by amide bonds and boxed in the structure. Each is derived in separate, but similar, PLP-dependent reactions from readily available amino acids.



a) (4 Points) **What are the precursors** of cysteamine and beta alanine respectively?



b) (8 Points) **Pick one** of the two molecule from part a and show mechanistically how it is converted to cysteamine or beta alanine using PLP (at left).

Genetic Code Chart

UUU	PHE	UCU	SER	UAU	TYR	UGU	CYS
UUC		UCC		UAC		UGC	
UUA	LEU	UCA		UAA	End	UGA	End
UUG		UCG		UAG		UGG	TRP
CUU		CCU	PRO	CAU	HIS	CGU	ARG
CUC		CCC		CAC		CGC	
CUA		CCA		CAA	GLN	CGA	
CUG		CCG		CAG		CGG	
AUU	ILE	ACU	THR	AAU	ASN	AGU	SER
AUC		ACC		AAC		AGC	
AUA		ACA		AAA	LYS	AGA	ARG
AUG	MET	ACG		AAG		AGG	
GUU	VAL	GCU	ALA	GAU	ASP	GGU	GLY
GUC		GCC		GAC		GGC	
GUA		GCA		GAA	GLU	GGA	
GUG		GCG		GAG		GGG	

Name ______________________________

Table 1. Specific radioactivity of amino acids biosynthesized from ^{14}C sucrose in three days by a mouse (Steele, 1952).

Amino Acid	nCi/mgC	Amino Acid	nCi/mgC	Amino Acid	nCi/mgC	Amino Acid	nCi/mgC
Glutamate	19.0±1.9	Threonine	0.09±0.02	Valine	0.02±0.01	Lysine	0.0±0.02
Aspartate	15.8±0.9	Serine	8.4±0.1	Phenylalanine	0.02±0.07	Histidine	0.07±0.08
Alanine	26.5±3.3	Glycine	5.1±0.2	Tyrosine	0.0±0.07	Cystine	3.3±0.3
Proline	3.1±0.1	Isoleucine	0.06±0.05	Arginine	3.0±0.2	Methionine	1.03±0.06

Table 2. Growth of mouse L cells in media lacking the indicated amino acid (Eagle, 1955).

Amino Acid	Cell Growth	Amino Acid	Cell Growth	Amino Acid	Cell Growth	Amino Acid	Cell Growth
Glutamate	3.6 - 4.5	Threonine	0.2	Valine	0.06 - 0.2	Lysine	0.2 - 0.5
Aspartate	3.6 - 6.1	Serine	2.5 - 2.8	Phenylalanine	0.3 - 0.4	Histidine	0.3 - 0.4
Alanine	2.2 - 2.6	Glycine	3.6 - 3.7	Tyrosine	0.06 - 0.2	Cystine	0.1 - 0.3
Proline	2.4 - 6.8	Isoleucine	0.1 - 0.4	Arginine	0.4 - 0.9	Methionine	0.3 - 0.4
		Leucine	0.4 - 0.6	Tryptophan	0.3 - 0.4		

Table 3. Distribution of ^{15}N among the amino acids of liver proteins 8 hours after intravenous injection of various amino acid sources of ^{15}N. Values are normalized to the ^{15}N content of the source amino acid (100) incorporated into protein (Aqvist, 1951).

	Amino acids incorporated into rat liver proteins														
^{15}N-Amino Acid	Glu	Asp	Ala	Pro	Thr	Ser	Gly	Leu	Ile	Val	Phe	Tyr	Arg	Lys	His
Glutamate	100	50	74	12	3	46	19	31	nd	20	14	20	34	4	2
Aspartate	186	100	125	29	2	40	38	49	111	nd	26	38	60	15	25
Alanine	77	44	100	16	<1	23	21	38	40	29	9	10	33	4	3
Proline	23	14	18	100	1	5	3	4	5	5	1	2	11	2	<1
Threonine(1)	6	5	5	2	100	20	14	1	2	4	2	5	5	1	<1
Serine	9	9	12	2	14	100	50	3	2	2	2	6	9	1	1
Glycine	19	12	16	1	0	88	100	nd	nd	nd	3	nd	16	<1	2
Leucine	30	15	25	nd	<1	7	7	100	25	12	3	7	11	0	<1
Isoleucine	28	14	23	11	<1	9	8	34	100	15	8	12	10	4	3
Valine	34	19	29	7	0	12	10	46	41	100	5	6	14	1	2
Phenylalanine	24	12	18	2	<1	3	2	3	5	3	100	74	10	7	2
Tyrosine(2)	23	13	16	3	<1	4	4	4	5	4	44	100	9	1	1
Arginine(3)	34	23	20	18	2	6	1	nd	nd	nd	11	10	100	13	5
Lysine[3]	23	19	12	5	3	8	3	nd	nd	nd	6	nd	9	100	4
Histidine[3]	28	25	30	6	2	8	10	nd	nd	nd	9	nd	24	6	100

1. Slightly contaminated with ^{15}N serine. Data from one rat only.
2. Administered by a stomach tube. Animals killed after 12 hours.
3. ^{15}N excess significantly less than for other administered amino acids.