Name Key

Biochemistry 5700
Fall, 2004
Final Exam
December 13, 2004

INSTRUCTIONS:

Do not begin until 9:30 AM. The exam must be turned in by 11:20 AM. This exam contains two sections: the "new material" section and the "comprehensive" section. The NEW MATERIAL section consists of 6 questions. Answer ANY FIVE OF SIX questions in this section in the spaces provided for 15 points each. Cross out and mark "do not grade" on the one question from the new material section you choose not to answer.
Answer the "comprehensive" questions (25 questions) by marking on the scantron sheet using a #2 pencil. Turn in both the scantron and the entire exam when you are finished. Good Luck!!

Equations: $\Delta G = \Delta G^{\circ\prime} + RT\ln Q$ $\Delta E = \Delta E^{\circ\prime} - \frac{RT}{nF}\ln Q$ $\Delta G' = -nFE'$

$\mathrm{pH} = \mathrm{pK_a} + \log\frac{[\mathrm{A^-}]}{[\mathrm{HA}]}$ $v = \frac{V_{max}\cdot[S]}{K_m + [S]}$

Constants: F = 96.5 kJ/V•mol R = 8.3144 J/mol•K

table 5–1

**Properties and Conventions Associated with the Standard Amino Acids**

| Amino acid | Abbreviated names | | $M_r$ | $pK_a$ values: $pK_1$ (—COOH) | $pK_2$ ($—NH_3^+$) | $pK_R$ (R group) |
|---|---|---|---|---|---|---|
| **Nonpolar, aliphatic R groups** | | | | | | |
| Glycine | Gly | G | 75 | 2.34 | 9.60 | |
| Alanine | Ala | A | 89 | 2.34 | 9.69 | |
| Valine | Val | V | 117 | 2.32 | 9.62 | |
| Leucine | Leu | L | 131 | 2.36 | 9.60 | |
| Isoleucine | Ile | I | 131 | 2.36 | 9.68 | |
| Methionine | Met | M | 149 | 2.28 | 9.21 | |
| **Aromatic R groups** | | | | | | |
| Phenylalanine | Phe | F | 165 | 1.83 | 9.13 | |
| Tyrosine | Tyr | Y | 181 | 2.20 | 9.11 | 10.07 |
| Tryptophan | Trp | W | 204 | 2.38 | 9.39 | |
| **Polar, uncharged R groups** | | | | | | |
| Serine | Ser | S | 105 | 2.21 | 9.15 | |
| Proline | Pro | P | 115 | 1.99 | 10.96 | |
| Threonine | Thr | T | 119 | 2.11 | 9.62 | |
| Cysteine | Cys | C | 121 | 1.96 | 10.28 | 8.18 |
| Asparagine | Asn | N | 132 | 2.02 | 8.80 | |
| Glutamine | Gln | Q | 146 | 2.17 | 9.13 | |
| **Positively charged R groups** | | | | | | |
| Lysine | Lys | K | 146 | 2.18 | 8.95 | 10.53 |
| Histidine | His | H | 155 | 1.82 | 9.17 | 6.00 |
| Arginine | Arg | R | 174 | 2.17 | 9.04 | 12.48 |
| **Negatively charged R groups** | | | | | | |
| Aspartate | Asp | D | 133 | 1.88 | 9.60 | 3.65 |
| Glutamate | Glu | E | 147 | 2.19 | 9.67 | 4.25 |

**TABLE 13–7** Standard Reduction Potentials of Some Biologically Important Half-Reactions, at pH 7.0 and 25 °C (298 K)

| *Half-reaction* | $E'^{\circ}$ (V) |
|---|---|
| $\frac{1}{2}O_2 + 2H^+ + 2e^- \longrightarrow H_2O$ | 0.816 |
| $Fe^{3+} + e^- \longrightarrow Fe^{2+}$ | 0.771 |
| $NO_3^- + 2H^+ + 2e^- \longrightarrow NO_2^- + H_2O$ | 0.421 |
| Cytochrome *f* ($Fe^{3+}$) + $e^-$ ⟶ cytochrome *f* ($Fe^{2+}$) | 0.365 |
| $Fe(CN)_6^{3-}$ (ferricyanide) + $e^-$ ⟶ $Fe(CN)_6^{4-}$ | 0.36 |
| Cytochrome $a_3$ ($Fe^{3+}$) + $e^-$ ⟶ cytochrome $a_3$ ($Fe^{2+}$) | 0.35 |
| $O_2 + 2H^+ + 2e^- \longrightarrow H_2O_2$ | 0.295 |
| Cytochrome *a* ($Fe^{3+}$) + $e^-$ ⟶ cytochrome *a* ($Fe^{2+}$) | 0.29 |
| Cytochrome *c* ($Fe^{3+}$) + $e^-$ ⟶ cytochrome *c* ($Fe^{2+}$) | 0.254 |
| Cytochrome $c_1$ ($Fe^{3+}$) + $e^-$ ⟶ cytochrome $c_1$ ($Fe^{2+}$) | 0.22 |
| Cytochrome *b* ($Fe^{3+}$) + $e^-$ ⟶ cytochrome *b* ($Fe^{2+}$) | 0.077 |
| Ubiquinone + $2H^+$ + $2e^-$ ⟶ ubiquinol + $H_2$ | 0.045 |
| Fumarate$^{2-}$ + $2H^+$ + $2e^-$ ⟶ succinate$^{2-}$ | 0.031 |
| $2H^+ + 2e^- \longrightarrow H_2$ (at standard conditions, pH 0) | 0.000 |
| Crotonyl-CoA + $2H^+$ + $2e^-$ ⟶ butyryl-CoA | −0.015 |
| Oxaloacetate$^{2-}$ + $2H^+$ + $2e^-$ ⟶ malate$^{2-}$ | −0.166 |
| Pyruvate$^-$ + $2H^+$ + $2e^-$ ⟶ lactate$^-$ | −0.185 |
| Acetaldehyde + $2H^+$ + $2e^-$ ⟶ ethanol | −0.197 |
| FAD + $2H^+$ + $2e^-$ ⟶ $FADH_2$ | −0.219* |
| Glutathione + $2H^+$ + $2e^-$ ⟶ 2 reduced glutathione | −0.23 |
| $S + 2H^+ + 2e^- \longrightarrow H_2S$ | −0.243 |
| Lipoic acid + $2H^+$ + $2e^-$ ⟶ dihydrolipoic acid | −0.29 |
| $NAD^+ + H^+ + 2e^- \longrightarrow NADH$ | −0.320 |
| $NADP^+ + H^+ + 2e^- \longrightarrow NADPH$ | −0.324 |
| Acetoacetate + $2H^+$ + $2e^-$ ⟶ β-hydroxybutyrate | −0.346 |
| α-Ketoglutarate + $CO_2$ + $2H^+$ + $2e^-$ ⟶ isocitrate | −0.38 |
| $2H^+ + 2e^- \longrightarrow H_2$ (at pH 7) | −0.414 |
| Ferredoxin ($Fe^{3+}$) + $e^-$ ⟶ ferredoxin ($Fe^{2+}$) | −0.432 |

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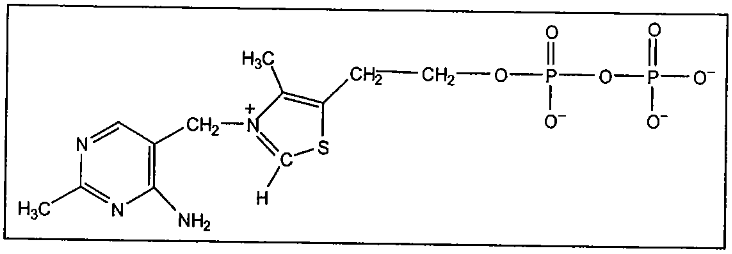

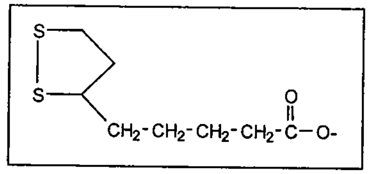

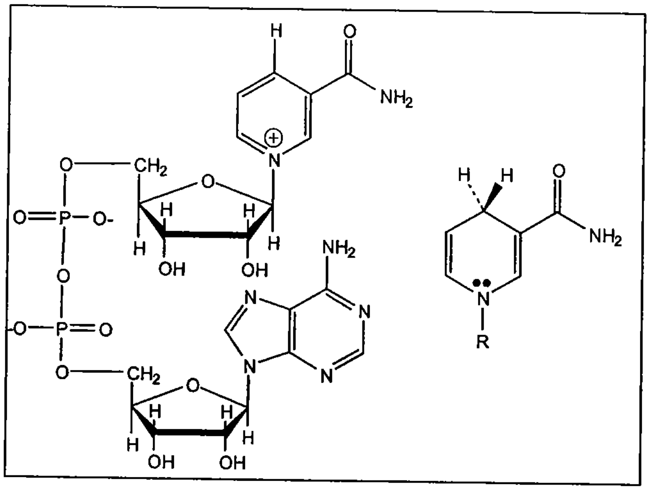

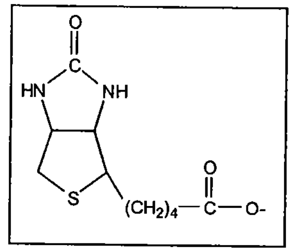

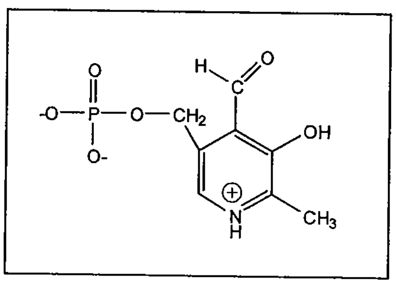

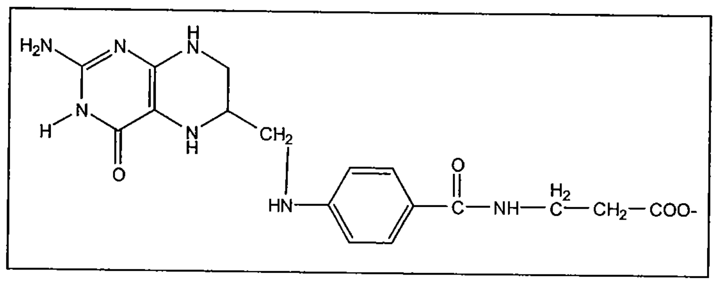

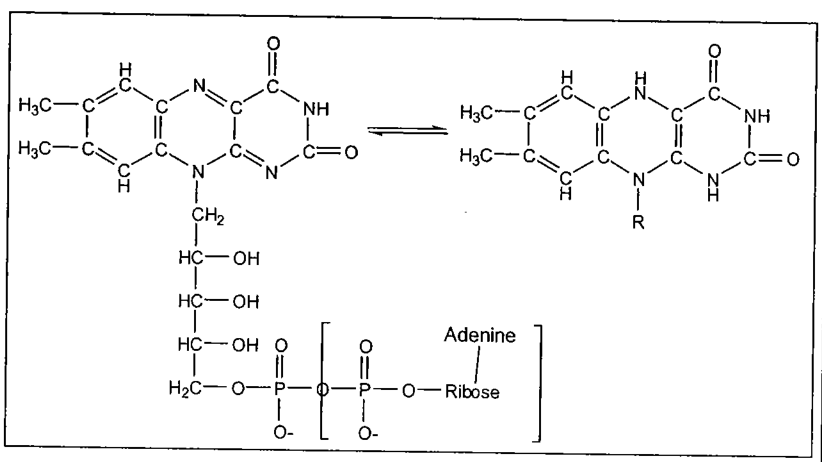


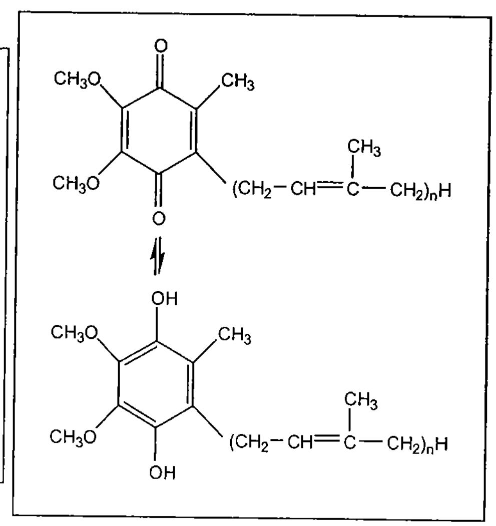

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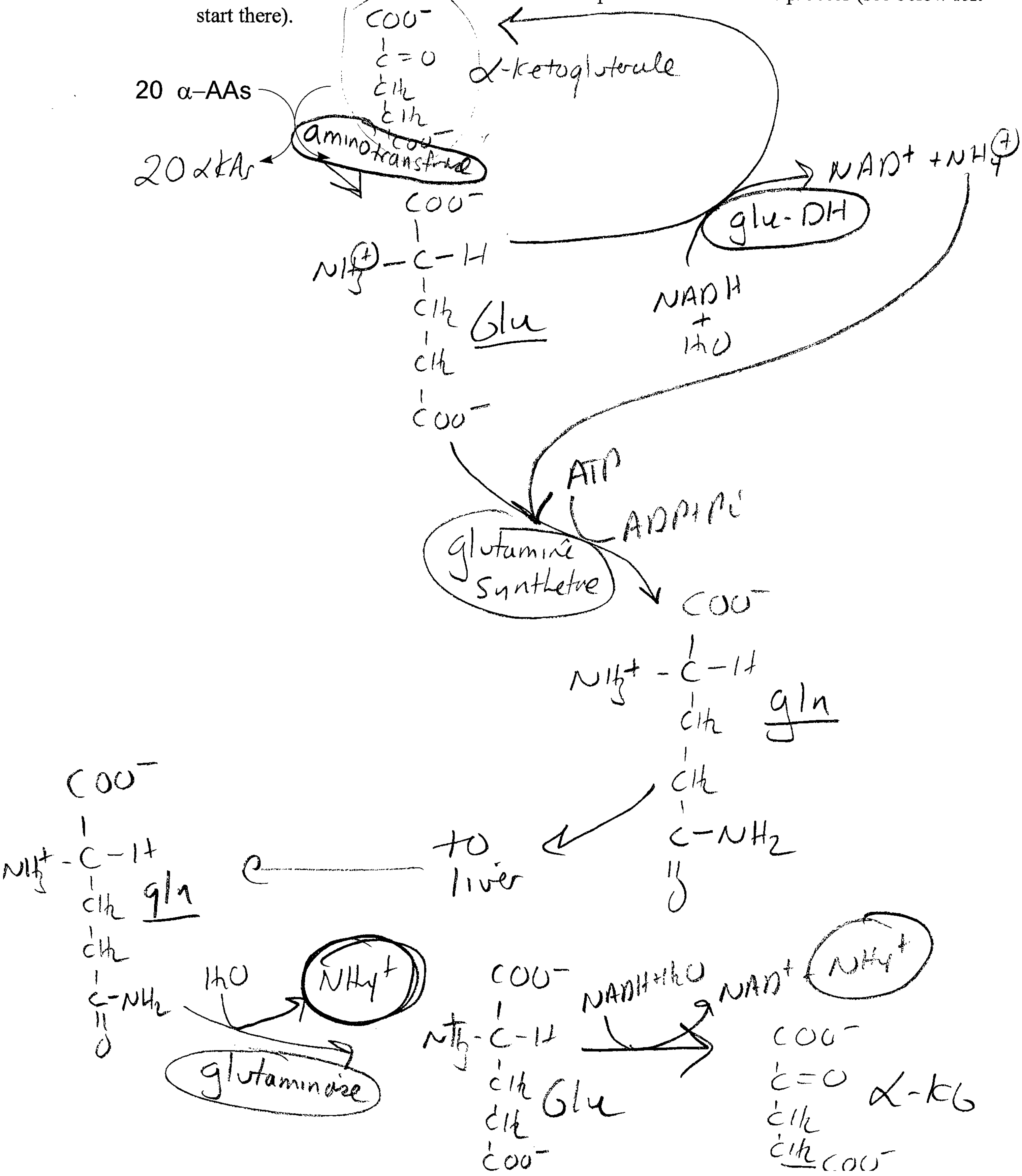
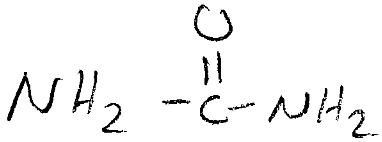
(1) (15 points) The amino groups from the **twenty dietary amino acids** are consolidated into the amino acid **glutamine** in tissue such as the muscle, then exported to the liver as glutamine, where the amino groups are released as **ammonia**. Provide a complete diagram for how this is done, showing necessary reactions, names and structures, and name the key enzymes involved. For simplicity, show a single reaction consolidating the amino groups from the 20 amino acids into the common acceptor at the start of the process (see below left- start there).

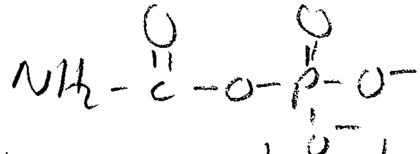


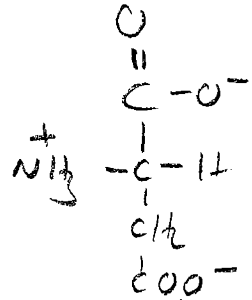
Name Key

(2)

(a) (5 points) Draw the structure of urea. What nitrogenous compounds <u>enter</u> the Urea Cycle to provide the nitrogen atoms present in urea? Draw their structures and name them.



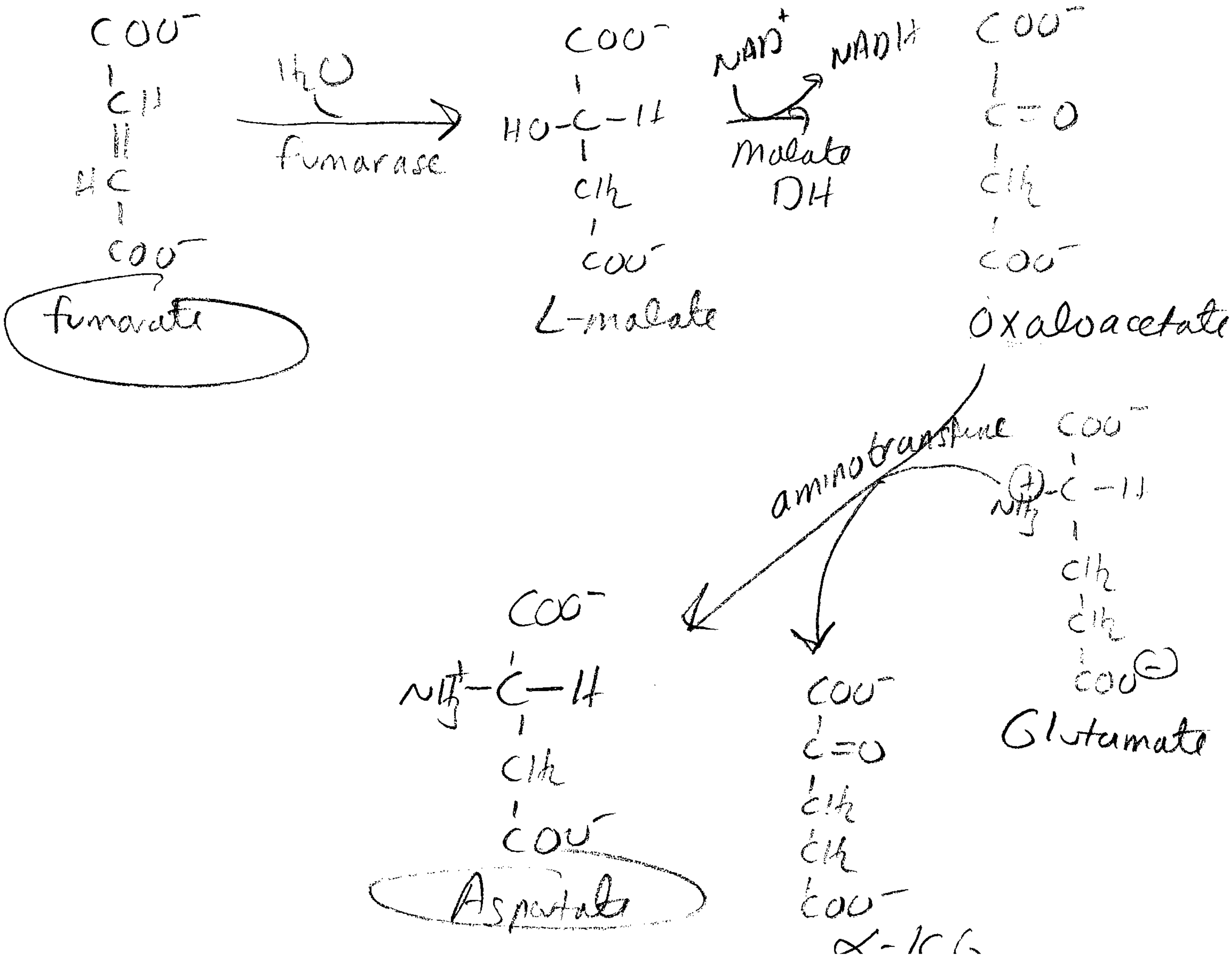




Carbamoyl phosphate

Aspartate

(b) (10 points) Fumarate is expelled from the Urea Cycle. Diagram the reactions necessary for converting fumarate back to the compound it's C skeleton originated from (i.e. the compound that went into the Urea Cycle), showing names and structures of intermediates, and name the enzymes that catalyze the necessary reactions.



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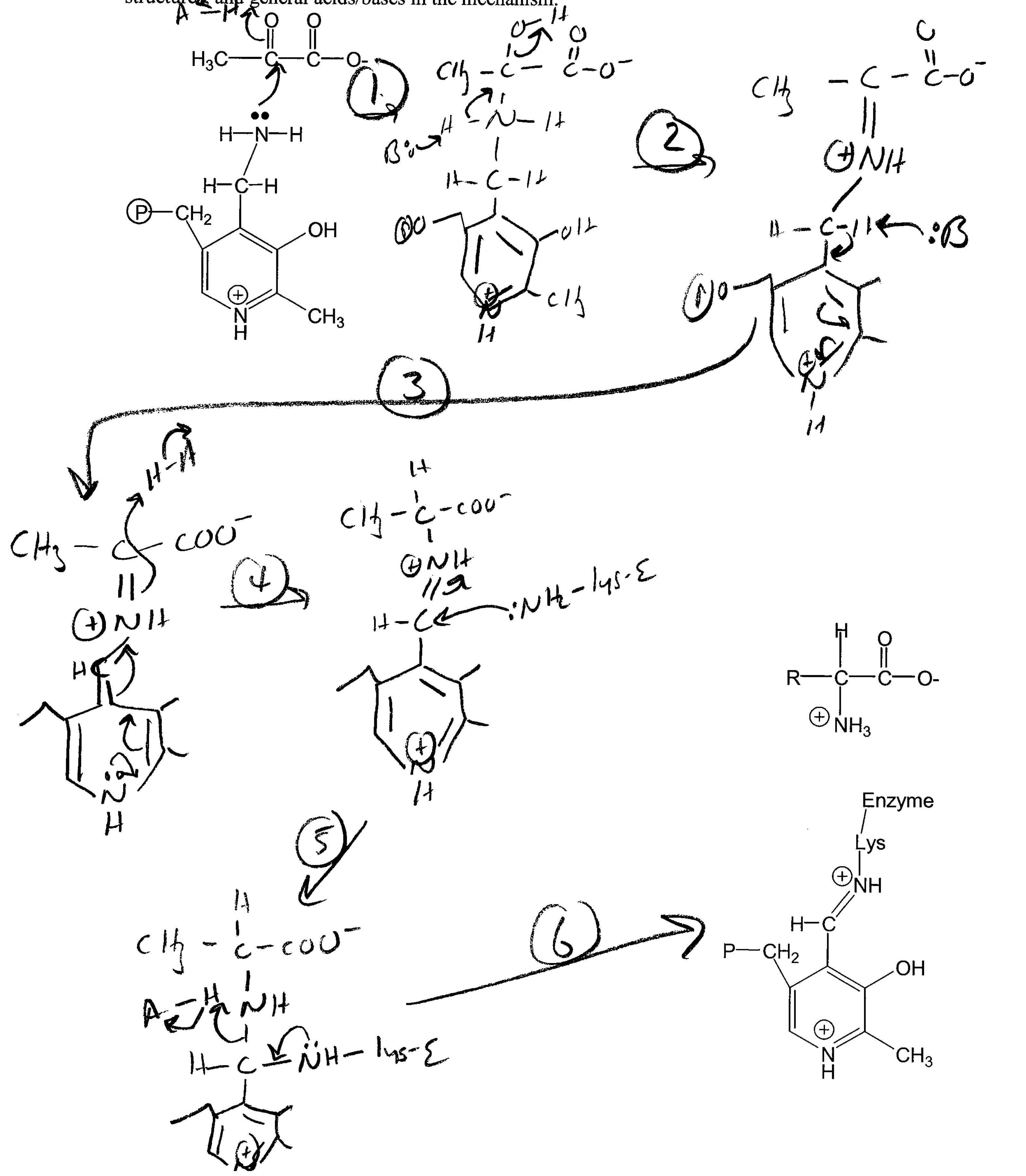
(3) (15 points) Starting with pyridoxamine and pyruvate (upper left), diagram the necessary mechanistic steps required to convert these compounds to alanine and the internal aldimine adduct of PLP and a lysine side chain (lower right). Show all intermediates, important resonance structures and general acids/bases in the mechanism.



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(4) (15 points) Electron transfer definitions

| | |
|---|---|
| Provide a proper enzyme name for the electron transfer complex I (i.e. ___ ___ oxidoreductase) | NADH: Ubiquinone oxidoreductase |
| Provide a proper enzyme name for the electron transfer complex II (i.e. ___ ___ oxidoreductase) | succinate: ubiquinone oxidoreductase |
| Provide a proper enzyme name for the electron transfer complex III (i.e. ___ ___ oxidoreductase) | Ubiquinone: Cyt C OR |
| Provide a proper enzyme name for the electron transfer complex IV (i.e. ___ ___ oxidoreductase) | CytC : $O_2$ OR |
| Name an electron transfer cofactor that is lipid soluble and not tightly bound to an enzyme | Ubiquinone (CoQ) |
| Name an electron transfer cofactor that is water soluble and not tightly bound to an enzyme | $NAD(P)^+$/NAD(P)H |
| Name a metal-free organic electron transfer cofactor that is always found associated with a protein | FAD, FMN |
| Name a metalloorganic electron transfer cofactor that is always found associated with a protein | Heme |
| Name an inorganic metal electron transfer cofactor that is always found associated with a protein | Iron-sulfur cluster, Cu |
| Name the transporter that brings ATP into the mitochondrion | adenine nucleotide translocase/ ADP/ATP translocase |
| In what portion of the mitochondrion does NADH oxidation occur? | matrix |
| In what portion of the mitochondrion does $O_2$ reduction occur? | matrix |
| Where in the mitochondrion is cytochrome C located? | inner membrane space |
| Where in the mitochondrion is ATP synthesized? | matrix |
| Into what portion of the mitochondrion are protons pumped? | inner membrane space |

(5) (15 points) What effects will the following compounds have on the rate of ATP synthesis (no effect, slow down, stop completely) in isolated mitochondria oxidizing succinate? Explain the chemical basis for their effects.
(a) a protonophore
(b) a potassium ionophore
(c) rotenone (an inhibitor of complex I)
(d) cyanide (inhibitor of complex IV)

(a) slow down, then stop completely. Protonophores allow free passage of $H^+$ across IM membrane, thus dissipating proton motive force required for ATP synthesis by ATP synthase

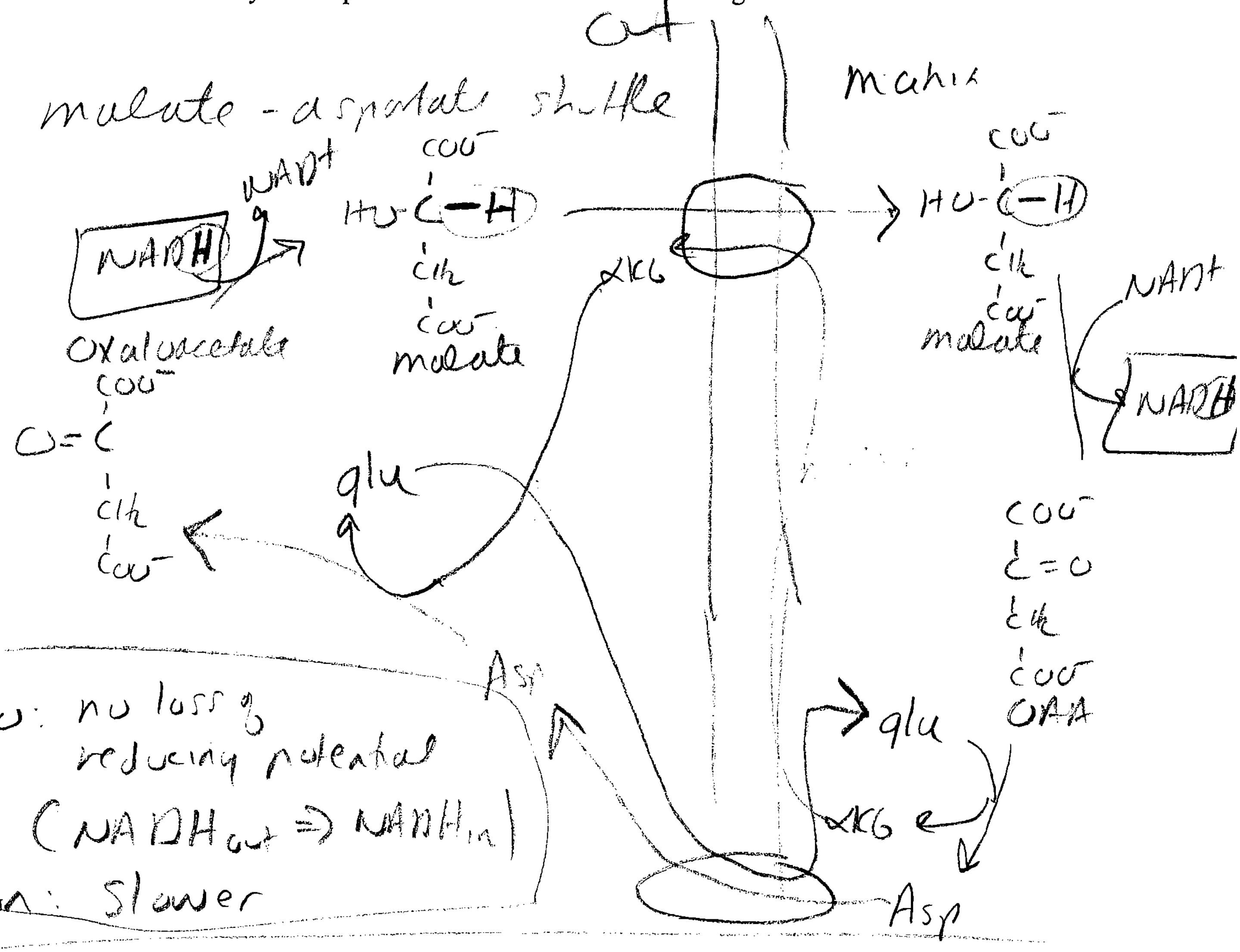
(b) Slow down, but not stop completely, A $K^+$ ionophore dissipates the electrical potential ($\Delta\psi$) component of the $H^+$ motive force but does not alter the chemical $H^+$ gradient, so some ATP synthesis continues

(c) no effect: succinate donates $e^-$ to complex II, not complex I

(d) Stop completely cyanide inactivates c IV, so no $e^-$ transfer can occur. Since $e^-$ transfer, $H^+$ pumping & ATP synthesis are coupled, all processes stop.

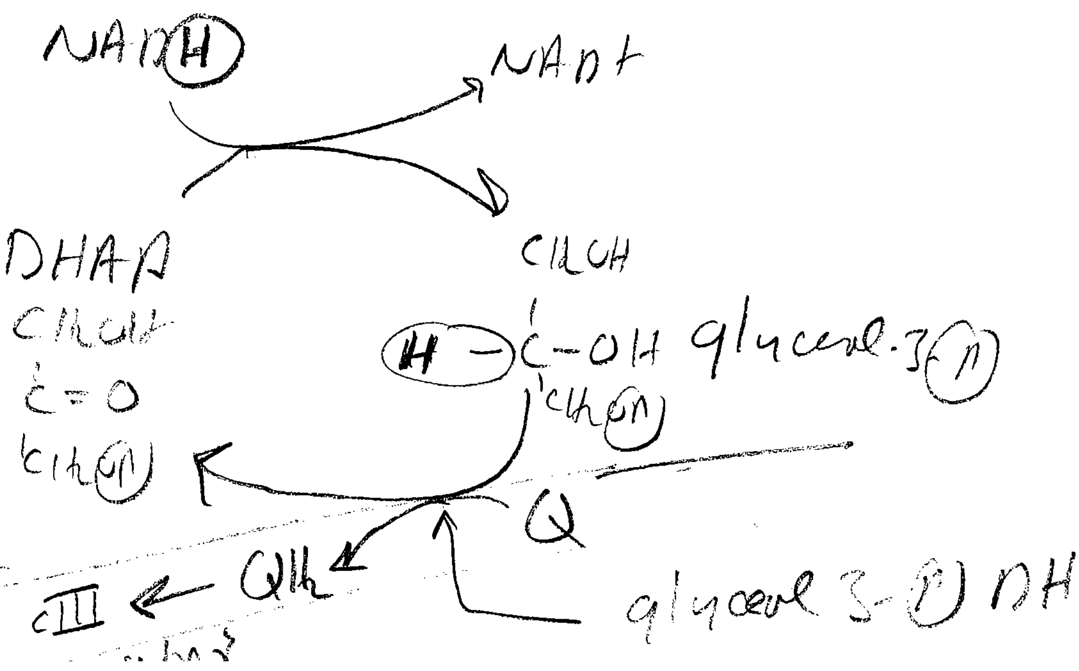
(6) (15 points) What two strategies (shuttles) exist for bringing the reducing equivalents from cytosolically-formed NADH into the mitochondrial electron transfer chain? Diagram the shuttles, then comment briefly on the pros/cons of the two different strategies.

(1) malate - aspartate shuttle




pro: no loss of reducing potential (NADH$_{out}$ ⇒ NADH$_{in}$)
con: slower

(2)

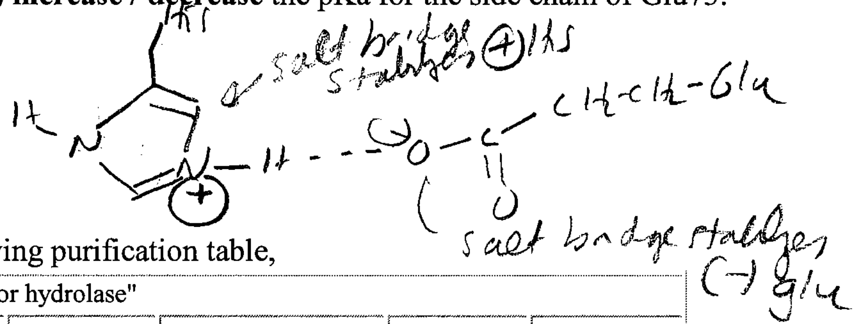



pro- faster
con- less reducing potential, as e⁻ are donated to Q, not CI
IM space

COMPREHENSIVE SECTION 3 points per problem. Mark answers on scantron.

(1) You have a protein "P" containing 100 amino acids that binds a ligand "L". Binding of ligand "L" induces a conformational change in the protein that brings the side chain of His24 in close proximity to the side chain of Glu73. This conformational change will (i) **increase / decrease** the pKa for the side chain of His24 and (ii) **increase / decrease** the pKa for the side chain of Glu73.

(a) (i) increase, (ii) increase
(b) (i) increase, (ii) decrease
(c) ~~(i) decrease, (ii) decrease~~
(d) (i) decrease, (ii) increase
(e) (i) no change, (ii) increase




(2) Given the information in the following purification table,

Purification table for the new enzyme "professor hydrolase"

| step of purification | volume (ml) | total protein (mg) | total activity (units) | specific activity (units/mg) | % recovery | fold purification |
|---|---|---|---|---|---|---|
| Lysate | 1000 | 20,000 | 90,000 | 4.5 | 100 | 1 |
| ion exchange | 200 | | 60,000 | 20 | | |
| gel filtration | 50 | 200 | 35,000 | 175 | 39 | |
| hydrophobic | 25 | 25 | | 800 | 22 | |

What is the "fold-purification" (relative to the lysate) for the enzyme at the gel filtration chromatography step?

(a) 2.6
(b) 4
(c) 39
(d) 100
(e) 180

$\frac{175}{4.5} = 39$

(3) Which of the following treatments will stabilize the R (oxy) form of hemoglobin?:

Stab.?

(i) lower 2,3-BPG R
(ii) higher $[CO_2]$ T
(iii) lower $[H^+]$ R
(iv) $O_2$ binding to the first of the four sites R

(a) (i), (ii), (iii) only
(b) (i), (iii), (iv) only
(c) (ii), (iii), (iv) only
(d) (i), (ii), (iv) only
(e) (i), (iv) only

(4) The rotation about which two atoms in a polypeptide chain defines the bond angle psi?

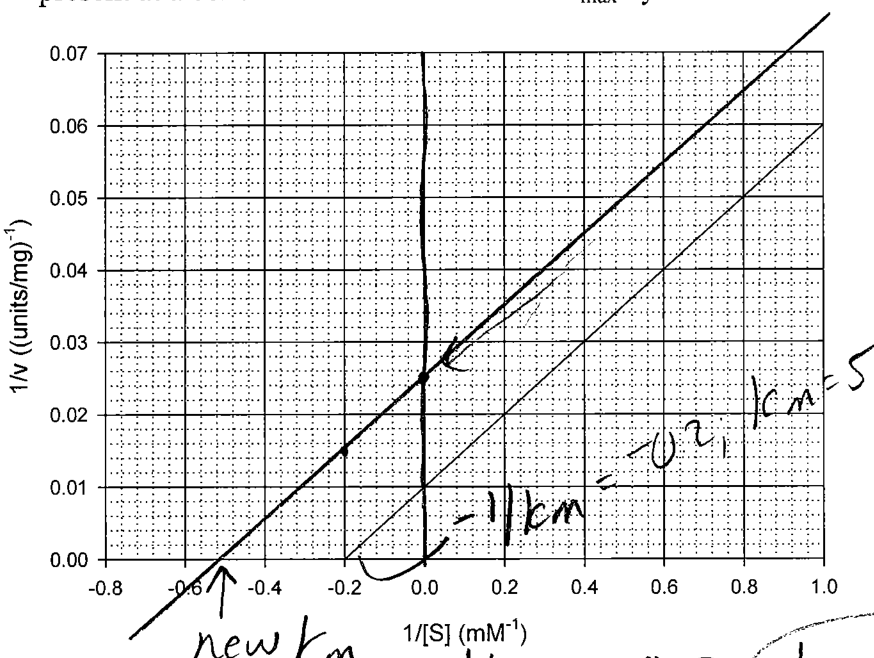
(a) N-$C_{alpha}$ (b) ~~N-$C_{carbonyl}$~~ (c) $C_{carbonyl}$-$C_{carbonyl}$
(d) $C_{alpha}$-R group (e) $C_{alpha}$-$C_{carbonyl}$

(5) You purify an enzyme from a crude bacterial lysate. The protein runs as a single band on nondenaturing polyacrylamide gel electrophoresis (PAGE) and as three distinct bands on SDS-PAGE. The sizes of the three protein bands on SDS-PAGE are 70,000, 50,000 and 10,000, and the staining intensities of the proteins indicate they are present in a stoichiometric ratio of 2:2:1, respectively. Your protein elutes from a size exclusion (gel permeation) column with an apparent molecular weight of 500,000. The quaternary structure of your protein is what (recall $\alpha$ is largest subunit, $\gamma$ the smallest):

(a) $\alpha_2\beta_2\gamma_1$
(b) $\alpha_3\beta_3\gamma_2$
(c) $\alpha_4\beta_4\gamma_1$
(d) $\alpha_4\beta_4\gamma_2$

$[(70)_2 (50)_2 (10)_1] \times 2 = 500$

(6) Using information from the following graph of 1/v vs. 1[S] for a particular enzyme, determine the apparent $K_m$ that will be observed for the enzyme in the presence of an uncompetitive inhibitor present at a concentration that decreases $V_{max}$ by 2.5-fold.




$V_{max} = \frac{1}{0.01} = 100$

2.5-fold decrease $= 40$

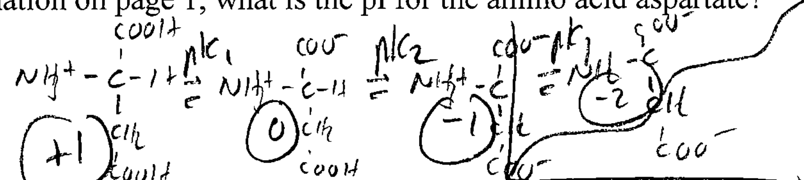
$1/V_{max\,new} = 0.025$

(a) 2 mM
~~(b) 5 mM~~
(c) 0.5 mM
(d) 12.5 mM
(e) 40 mM

$-1/Km_{new} = -0.5$; $Km_{new} = 2.0\ mM$

(7) Given the information on page 1, what is the pI for the amino acid aspartate?

(a) 2.76
~~(b) 6.62~~
(c) 5.74
(d) 5.04




(8) The products of the reactions of (i) $CO_2$ and (ii) acetone with the side chain of lysine are, respectively:

(a) (i) a Schiff base and (ii) a Schiff base
(b) (i) a Schiff base and (ii) an amide
(c) (i) a carbamate and (ii) an amide
(d) (i) a carbamate and (ii) a Schiff base

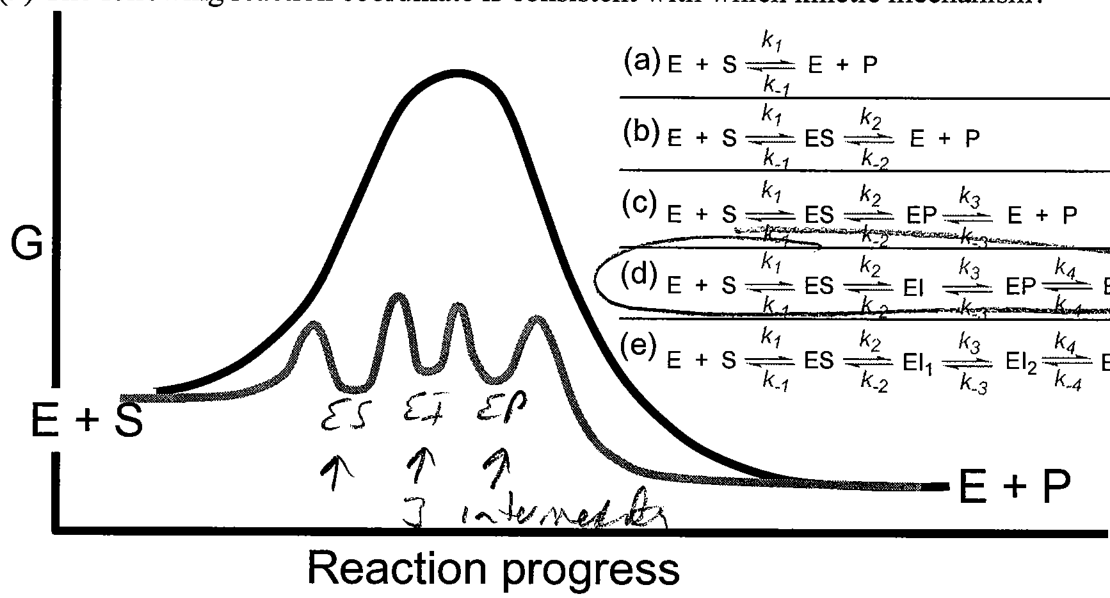
$pI = \frac{pK_1 + pK_2}{2}$

$pI = \frac{1.88 + 3.65}{2}$

$pI = 2.76$

 Name Key

(9) The following reaction coordinate is consistent with which kinetic mechanism?




(a) $E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E + P$

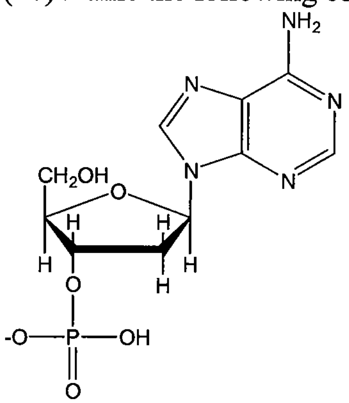
(b) $E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$

(c) $E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EP \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E + P$

(d) $E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EI \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} EP \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} E + P$

(e) $E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EI_1 \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} EI_2 \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} EP \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} E + P$

(10) Name the following compound properly:



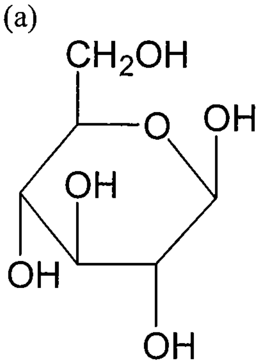
(a) deoxyguanosine-3'-monophosphate
(b) deoxyadenosine-3'-monophosphate
(c) deoxyadenine-3'-monophosphate
(d) adenosine-3'-monophosphate
(e) deoxyadenosine-5'-monophosphate

(11) What type(s) of carbohydrate linkage(s) are present in cellulose?

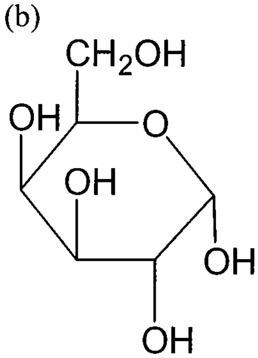
(a) α (1→4) only
(b) β (1→4) only
(c) α (1→4) and α (1→6)
(d) β (1→4) and β (1→6)

(12) D-Galactose is a C4 epimer of D-glucose. Which of the following is the correct structure for β-D-galactopyranose?

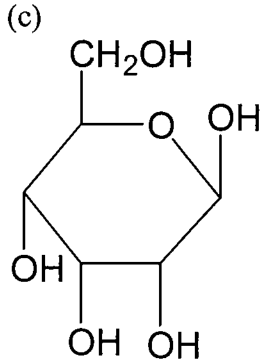
(a)



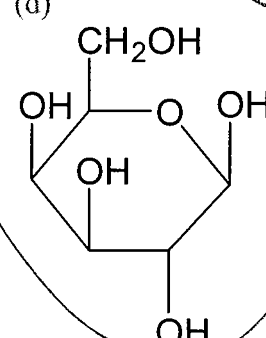
(b)



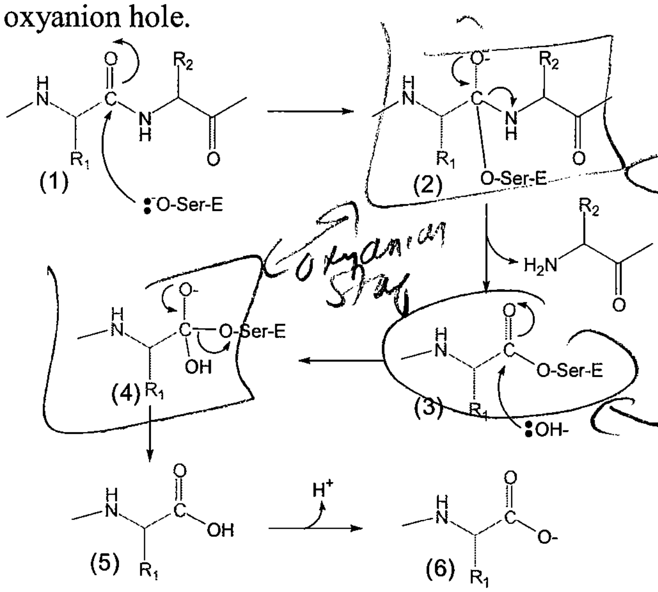
(c)



(d)

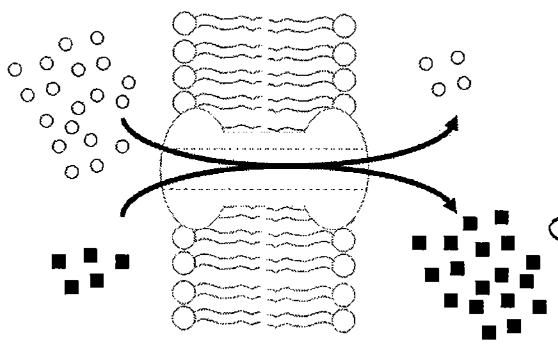


(13) In the reaction mechanism for chymotrypsin shown below, compounds __(i)____ represent acyl enzyme intermediates, and compounds __(ii)___ represent structures that are stabilized by the oxyanion hole.



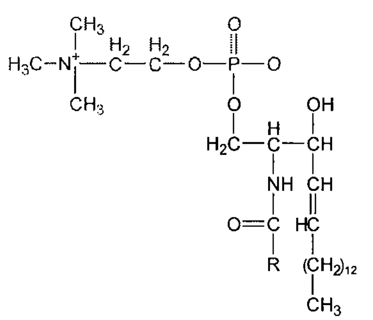

| | (i) | (ii) |
|---|---|---|
| (a) | 3 | 2,4 |
| ~~(b)~~ | ~~1,3,5,6~~ | ~~2,4~~ |
| (c) | 2,4 | 3 |
| (d) | 2,4 | 1,3,5,6 |
| (e) | 2,4 | 2,4 |

(14) Of the following choices, which matches the strategy of transport shown in this Figure?



(a) glucose permease
(b) bacteriorhodopsin
(c) lactose permease
(d) Chloride bicarbonate exchanger
(e) sodium potassium ATPase

(15) What type of lipid is diagrammed here?



(a) triacylglycerol
(b) ganglioside
~~(c) glycerophospholipid~~
(d) sphigomyelin
(e) ether lipid

(16) In B-DNA, the base orientation is ___anti___, the sugar pucker is _C3' endo_ and the two strands are ___antill___

(a) anti; C3' endo; antiparallel
~~(b) syn; C3' endo; antiparallel~~
(c) anti; C3' exo; antiparallel
(d) anti; C3' exo; parallel
(e) syn; C3' exo; antiparallel

(17) The compound resulting from the nucleophilic addition of an alcohol to an aldehyde is called what?

(a) hemiacetal
(b) acetal
(c) hemiketal
(d) ketal

(18) Of the 6 dehydrogenases listed below, which use(s) FAD rather than $NAD^+$ as initial electron acceptor.

(i) lactate dehydrogenase
(ii) malate dehydrogenase
(iii) succinate dehydrogenase FAD
(iv) glyceraldehyde-3-phosphate dehydrogenase
(v) isocitrate dehydrogenase
(vi) acyl-CoA dehydrogenase FAD

(a) (i), (ii), (iv), and (v) only
(b) (i), (iii), and (v) only
(c) (iii) only
(d) (iii) and (vi) only
(e) (ii) and (iv) only

(19) The CoA-dependent cleavage of acetoacetyl-CoA into two acetyl-CoA molecules is an example of:

(a) hydrolysis
(b) thiolysis
(c) phosphorolysis
(d) claisen condensation (in reverse reaction)
(d) oxidative decarboxylation

(20) Glycogen phosphorylase in the liver is a **(i)** glucose/AMP sensor, and binds this metabolite in the **(ii)** phosphorylated/unphosphorylated form. In contrast, glycogen phosphorylase in the muscle is a **(iii)** glucose/AMP sensor and binds this metabolite in the **(iv)** phosphorylated/unphosphorylated form.

| | (i) | (ii) | (iii) | (iv) |
|---|---|---|---|---|
| (a) | glucose | unphosphorylated | AMP | phosphorylated |
| (b) | AMP | unphosphorylated | glucose | phosphorylated |
| (c) | AMP | phosphorylated | glucose | unphosphorylated |
| (d) | Glucose | phosphorylated | AMP | unphosphorylated |
| (e) | glucose | phosphorylated | AMP | phosphorylated |

(21) The term "cis-enediolate" might correctly apply to an intermediate formed during catalysis for what class of enzymes in central metabolism?

(a) kinase
(b) isomerase
(c) aldolase
(d) mutase
(e) hydratase

(22) For the following reaction:

$$\text{lactate} + NAD^+ \rightleftharpoons \text{pyruvate} + NADH + H^+$$

What concentration of lactate would be in equilibrium with 1.0 mM pyruvate, 10 mM $NAD^+$, and 0.20 mM NADH? Use information and equations on the first page of the exam to work this problem.

(a) $7.4 \times 10^2$ mM
(b) $1.8 \times 10^6$ mM
(c) 50 mM
(d) 0.74 mM
(e) $1.4 \times 10^{-3}$ mM

$K_{eq} = 2.71 \times 10^{-5}$ (see key to Exam 3 p. 6)

$K_{eq} = \frac{[pyr][NADH]}{[lactate][NAD^+]}$ ; $[lactate] = \frac{[pyr][NADH]}{K_{eq}[NAD^+]}$

(23) Which cofactor facilitates C-C bond cleavage during decarboxylation of an alpha-keto acid?

(a) biotin
(b) thiamine pyrophosphate
(c) zinc metal ion
(d) lipoamide
(e) FAD

$[lactate] = \frac{(1 \times 10^{-3} M)(0.2 \times 10^{-3} M)}{(2.71 \times 10^{-5})(10 \times 10^{-3} M)}$

$[lactate] = 0.738 \underline{M}$

(24) For which of the following classes of enzymes might you expect to encounter a covalent phosphoenzyme intermediate during catalysis?

$= 738 mM$
$= 7.4 \times 10^2$ mM

(a) kinase
(b) dehydrogenase
(c) mutase
(d) phosphorylase
(e) thiolase

(25) Which of the following is a high energy acyl phosphate?

(a) phosphoenolpyruvate
(b) 2-phosphoglycerate
(c) 3-phosphoglycerate
(d) 1,3-diphosphoglycerate
(e) fructose-2,6-diphosphate