ASBMB ACCREDITATION EXAMINATION
SAMPLE QUESTIONS
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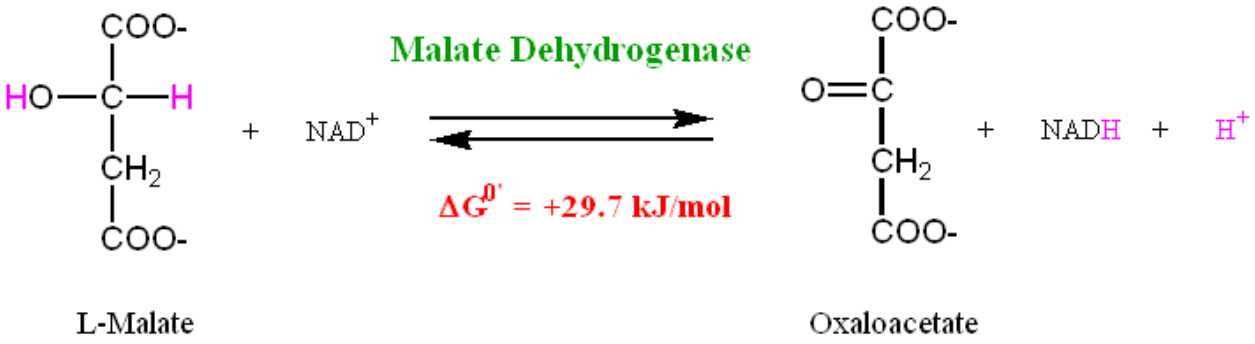
INTRODUCTION

The following sample questions have been prepared to assist students and their instructors in familiarizing themselves with the focus and format of the ASBMB Certification Exam for Baccalaureate Degrees in Biochemistry & Molecular Biology. Questions are grouped by core concept area and are accompanied by an answer key that describes the features of a Highly Proficient response. In the rationale section, we have tried to provide additional information illustrating the linkage between the question and fundamental concepts and skills in biochemistry and molecular biology.

CONCEPT AREA: Energy and metabolism.

1. The final reaction of the citric acid cycle (see reaction below) has a standard free energy change (ΔG°') of +29.7kJ/mol. Describe two specific ways cells drive this reaction forward.



KEY

When acetyl-CoA is available, the oxaloacetate produced by malate dehydrogenase is rapidly converted to citrate (a thermodynamically favorable reaction), which drives down the concentration of oxaloacetate and pulls the reaction to the right. In addition, this reaction is driven forward by the very low concentration of NADH and the higher concentration of NAD^+. [NADH] in the mitochondria is kept very low by continual reaction with the electron transport chain. Thus the reaction is driven forward by the electron transport chain functioning and keeping the [NADH] very low.

RATIONALE

This question is designed to probe the student's understanding of how the conditions existing inside a cell impact the thermodynamics of enzyme-catalyzed reactions. In this case, while ΔG°' is unfavorable, this value applies to a specific circumstance in which 1) all reactants are present in equal, 1 M, concentrations; 2) the reaction takes place in isolation; and 3) the pH is fixed at 7.0 (symbolized by the prime symbol ['']).

1) Inside the cell the concentrations of substrates and products generally fall at or below the millimolar range. Not only is this well below the 1M used to calculate the standard free energy, but for any given enzyme-catalyzed reaction the physiological levels of substrates and products are rarely equal to one another. For a typical enzyme-catalyzed reaction:

   $A + B \rightarrow P + Q$

   the actual free energy change, ΔG, under non-standard conditions can be calculated from the standard free energy as follows:

   $\Delta G = \Delta G^{\circ\prime} + RT \ln [P][Q]/[A][B]$

or, in this case

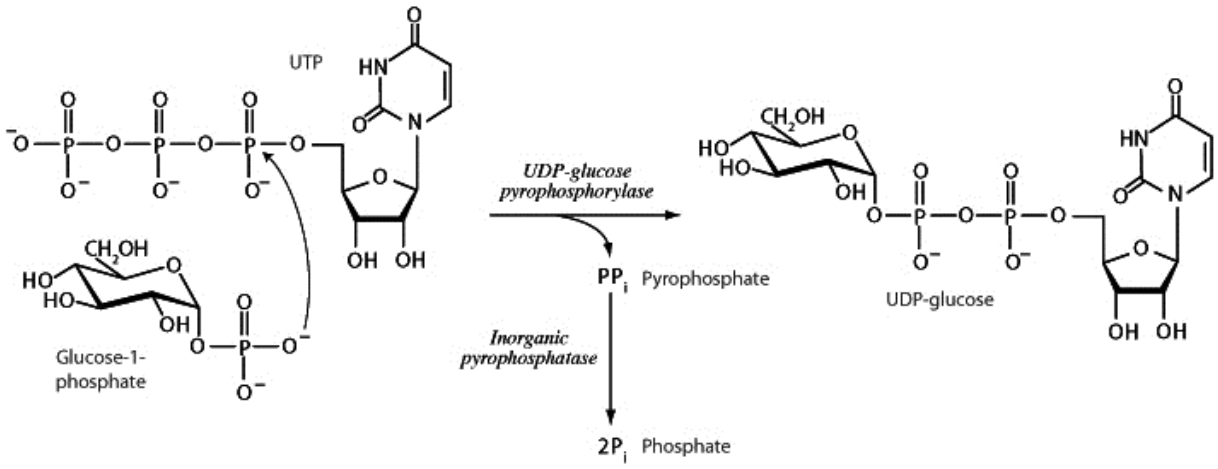
$$\Delta G = \Delta G^{\circ\prime} + RT \ln[\text{Oxaloacetate}][\text{NADH}]/ [\text{L-Malate}][\text{NAD}^+]$$

[Note that $\Delta G^{\circ\prime}$ is determined at a pH of 7.0; therefore, the concentration of protons has already been accounted for.] The ratio of [NADH] to [NAD^+] thus can significantly influence ΔG.

2) Inside the cell, metabolic reactions do not take place in isolation. In most cases the "product(s)" of a given enzyme-catalyzed reaction are oftentimes the "substrate(s)" for another enzyme in a biochemical pathway or cycle. Thus, as soon as these products appear, they are siphoned away through their chemical transformation via a subsequent enzyme-catalyzed reaction. Under such circumstances, the resulting reduction in the concentration of the reaction product(s) will pull the equilibrium to the right, as described by the equations above, enabling many seemingly thermodynamically unfavorable enzyme-catalyzed reactions to proceed at a useful rate *in vivo.*

CONCEPT AREA: Energy and metabolism

2. The equilibrium constant for the reaction catalyzed by UDP-glucose pyrophosphorylase is approximately one (1.0):



How does the presence of the enzyme **inorganic pyrophosphatase** in our cells increase the yield of UDP-glucose beyond that obtainable with UDP-glucose pyrophosphorylase alone?

KEY

By hydrolyzing one of the products of the reaction, inorganic pyrophosphate, and driving its concentration downwards, the net effect is to shift or pull the equilibrium to the right, in favor of the synthesis of more UDP-glucose.

RATIONALE

One of the major challenges faced by all living organisms is to devise ways to efficiently synthesize molecules whose formation is, under normal circumstances, thermodynamically disfavored. Understanding the mechanisms employed by living organisms to overcome or circumvent these barriers is thus fundamental to understanding the form and logic behind enzyme reaction mechanisms and the structure of many biochemical pathways. This problem probes student understanding of one of the most widely used of these mechanisms, the destruction/transformation or removal of one reaction product to promote the high-yield synthesis of a desired, but disfavored, product(s); the question determines whether students can **recognize** an example of this mechanism in action and **describe** it in a cogent manner.

CONCEPT AREA: Macromolecular structure-function-regulation.

3. Maximization of the entropy, and the consequent minimization of the free energy, of water is the predominant thermodynamic driving force behind which of the following molecular phenomena? Check all that apply:

a) Assembly of phospholipid bilayers _____

b) Assembly of glycogen particles _____

c) Protein folding _____

d) Formation of DNA double helix _____

e) Assembly of DNA and histones to form nucleosomes _____

f) Folding of transfer RNA _____

KEY

The correct choices are a), c), d) and f). Glycogen lacks the kind of amphipathic repeating unit found in lipid bilayers, proteins, and polynucleotides. Nucleosome assembly is driven primarily by charge-charge interactions.

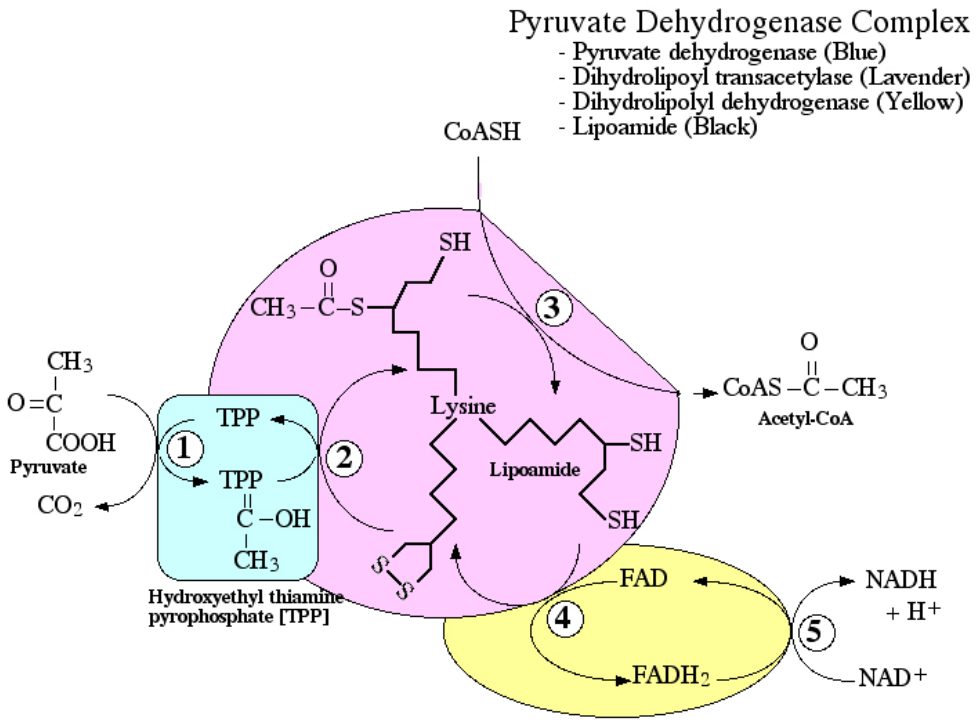
RATIONALE

Understanding how the interaction of biomolecules with water drives the folding of biopolymers into complex three-dimensional conformations, as well as the assembly of most macromolecular complexes, is fundamental to understanding numerous biochemical processes, including DNA replication, transcription, protein structure-function, membrane assembly, etc. The realization that this simple, universal phenomenon drives complexity — folding and assembly — across a wide range of organic biomolecules provides a key insight into evolution as a process driven by basic, comprehensible forces rather than a series of fantastically improbable events.

It is extremely important that students understand that the forces that drive macromolecular assembly and folding (hydrophobic effect, ionic bonds / salt bridges, hydrogen bonding) are universal and agnostic. This point is sometimes obscured by the employment of artificial constructs such as “protein folding”, which imply different “rules” apply to proteins versus polynucleotides, etc.

CONCEPT AREA: Macromolecular structure-function-regulation.

4. The image below offers a schematic representation of the catalytic mechanism of the pyruvate dehydrogenase complex. The prosthetic factor lipoamide (BLACK) is attached to the enzyme dihydrolipoyl transacetylase (LAVENDER) through a lysine residue. Three versions of the prosthetic factor as it participates in steps 2, 3, and 4 of the reaction pathway are shown. **What chemical entity or entities does lipoamide transfer/carry going from step 3 to step 4?**



KEY

H_2 **OR** 2e- and 2 H^+ **OR** H^- plus H^+

RATIONALE

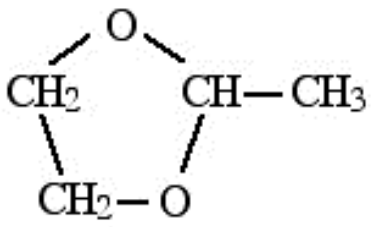
There are several ways one can reason out the answer to this question. The first is to compare the status of the lipoamide moiety before and after step 4. Two –SH groups are transformed to a disulfide S-S, losing two H atoms, the elements of hydrogen. Second is to look at the change in the status of the flavin prosthetic group, which changes from FAD to $FADH_2$, adding the elements of hydrogen. Third is to look at the ultimate product of step three, the reduction of NAD^+ to NADH + H^+. Lastly, the name of the enzyme catalyzing step 3, dihydrolipoamide dehydrogenase, provides a clear clue as to the type of reaction that is taking place.

It is important to note that this question can be answered without any prior knowledge of the catalytic mechanism of the pyruvate dehydrogenase complex.

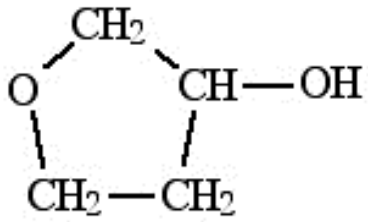
CONCEPT AREA: Macromolecular structure-function-regulation.

5. The two molecules shown below have the same chemical formula, $C_4O_2H_8$, and molecular mass; yet the boiling points (B.P.) of liquids comprised of solely and entirely (100%) of these compounds differ from one another by nearly 130°C.

Briefly **explain** why their boiling points are so different.



B.P. 82°C



B.P. 210°C

KEY

Molecules of the compound with the higher boiling point can hydrogen bond with one another, whereas molecules of the compound with the lower boiling point cannot. Both molecules possess oxygen atoms with lone pairs of electrons, but only the molecules on the right contain an unshielded hydrogen atom [hydrogen atom bound to an electronegative atom like O or N] available to engage these lone pairs to form a hydrogen bond.

RATIONALE

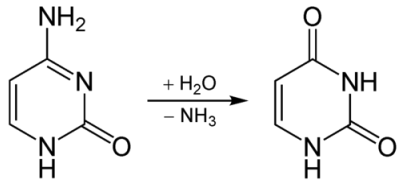
Hydrogen bonding is a fundamental factor in the ability of water to serve as the molecule of life, macromolecular folding and assembly, etc. This question probes a student's understanding of the structure and physicochemical impact of hydrogen bonding by presenting this concept in an unfamiliar context – one in which water molecules are neither shown nor mentioned.

NOTE

An error frequently encountered in questions of this type is the assumption that the question is asking about the properties of a solution of these molecules in water, rather than the pure liquids as intended.

CONCEPT AREA: Information storage and transfer.

6. The nucleotide base cytosine [C] can be deaminated *in vivo* to generate uracil [U]:



Cytosine Uridine

The table below gives the genetic code that determines the sequence of the polypeptide encoded by a given messenger RNA. Predict the impact on the polypeptide product of an mRNA molecule for which:

1. Cytosine deamination occurs in the codon for an asparagine residue:

KEY: No change. The amino acid sequence of the polypeptide product will be identical.

2. Cytosine deamination occurs in the codon for a glutamine residue:

KEY: Translation will stop, resulting in a shorter or truncated polypeptide.

UUU UUC phenyl alanine UUA UUG leucine	UCU UCC UCA UCG serine	UAU UAC tyrosine UAA UAG stop	UGU UGC cysteine UGA stop UGG tryptophan
CUU CUC CUA CUG leucine	CCU CCC CCA CCG proline	CAU CAC histidine CAA CAG glutamine	CGU CGC CGA CGG arginine
AUU AUC AUA isoleucine AUG methionine	ACU ACC ACA ACG threonine	AAU AAC asparagine AAA AAG lysine	AGU AGC serine AGA AGG arginine
GUU GUC GUA GUG valine	GCU GCC GCA GCG alanine	GAU GAC aspartic acid GAA GAG glutamic acid	GGU GGC GGA GGG glycine

RATIONALE

This question requires students to demonstrate the basic, yet fundamental ability to accurately translate the genetic code for the amino acids. In doing so, students will also be required to recognize and correctly apply the concept of redundancy in the nucleotide triplets that specify certain of the genetically-encoded amino acids.

CONCEPT AREA: Information storage and transfer.

7. One day, a graduate student working with all-white mice finds a single mouse with black ears in the mouse colony. Standard practice in the colony is to clip the tails of all newborn mice to provide tissue for genotype analysis. Using DNA from these tails clippings, the graduate student compares the sequence of the *agouti* gene – known to influence coat color -- from the black-eared mouse with the corresponding sequence from one of the all-white siblings of the black-eared mouse. The researcher finds that the sequences for the *agouti* gene are identical.

Next, the graduate student analyzes DNA extracted from clippings taken from the ears of these mice. While the sequence of the *agouti* gene from the ears of the all-white mouse matched that from the tails of both the white- and black-eared mice, the gene from the black ear tissue exhibited a mutation.

Confused, the student takes a third tissue for genotyping (toes) and once again finds that the *agouti* gene sequences are identical to those from the tail tissue. The findings are summarized in the table below:

Tissue	Comparison of *agouti* gene sequences reveals that the two mice are genetically
Tail	Identical (wild-type)
Toe	Identical (wild-type)
Ear	Different

The black-eared mouse was bred several times with multiple strains of white-eared mice. All of its progeny displayed white ears that harbored a normal, wild-type *agouti* gene.

EXPLAIN in two to five sentences why the mutant *agouti* gene was not passed on to the progeny of the black-eared mouse.

KEY

The fact that the mutation was found in some tissues but not others suggests that it is somatic in nature. Somatic mutations occur locally (such as in the ear) in relatively mature, differentiated cells. Germline mutations that occur in very early embryonic development (gametes or blastomeres) are passed to tissues throughout the body. If a germline cell had been affected, one would expect to see that the entire mouse would be black, not just the ears. Since only the genes contained in sex / germline cells will be passed on to progeny, none of the offspring will possess black ears (unless another, independent somatic mutation occurred).

RATIONALE

This question probes students' understanding of how the developmental and cellular context in which a mutation takes place can influence the impact of the mutation. In order to be

successful, students need to recognize that, unlike a bacterium, organisms composed of multiple, differentiated cell types will not automatically pass on genetic mutations to their progeny and that the point in a developmental process at which a mutation takes place determines what proportion of the cells / tissues in an organism will be impacted.

CONCEPT AREA: Information storage and transfer.

8. "Gene expression" is a term commonly used to identify the process by which the information contained in a given gene is employed to synthesize the encoded protein product. In many ways, the term "gene expression" is a misleadingly simple name for a multistep, complex series of events. For each of the molecules or processes listed below, identify whether they participate in the transcriptional, post-transcriptional, translational, or post-translational stages of gene expression (Check one item for each):

	Transcriptional	Post-transcriptional	Translational	Post-translational
mRNA splicing				
Repressor				
Signal peptidase				
RNAi				
mRNA deadenylases				
Ribosome				

KEY

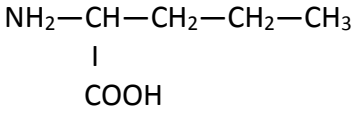
	Transcriptional	Post-transcriptional	Translational	Post-translational
mRNA splicing		+++		
Repressor	+++			
Signal peptidase				+++
RNAi		+++		
mRNA deadenylases		+++		
Ribosome			+++	

RATIONALE

Traditionally, gene expression has been taught as a two-stage process consisting of transcription and translation. It is important, however, that students understand that transcription and translation are not simple, monolithic processes of template-directed polymer synthesis but rather complex and highly nuanced mosaics in which the nascent polymer products must be processed in order to generate mature, functional mRNA and protein products. Moreover, regulatory mechanisms impinging on these many steps determine not just the timing and quantity of mRNA and protein synthesis, but also, through alternative splicing and other events, the physical and functional nature of these key products. The objective of this question is to probe the degree to which students appreciate the complexity of "gene expression".

CONCEPT AREA: Scientific method and quantitative reasoning.

9. A novel microorganism is discovered near the South Pole whose proteins contain a novel amino acid, Antarctine:



In order to map out the biosynthetic pathway responsible for the synthesis of Antarctine, Dr. Science exposes the microorganism to a chemical mutagen and selects for Antarctine auxotrophs (mutant microorganisms that can only grow if supplied with Antarctine in the culture media). DNA sequencing of auxotrophs identifies a mutation in the gene XYZ147.

In order to verify that gene XYZ147 encodes an enzyme in the biosynthetic pathway for Antarctine, Dr. Science transforms the mutant microorganism with an intact, wild-type copy of gene XYZ147 carried on a plasmid. However, transformation with this plasmid fails to rescue the mutant phenotype. The plasmid-containing mutant still requires Antarctine for growth. Antibodies show that the copy of the gene XYZ147 that is carried on the plasmid produces levels of the protein product comparable to levels found in the wild-type microorganism.

Describe two plausible explanations that might account for the failure of the plasmid to rescue the mutant from its dependency on added Antarctine and that **do not** involve an error on the part of Dr. Science.

KEY

A highly proficient answer would contain any two of the following:

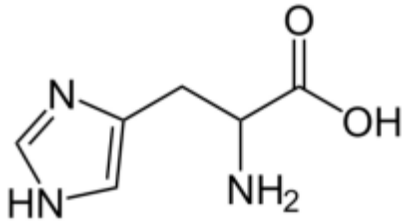
- A second, undetected mutation exists in the microorganism.
- The effect of the mutation was pleiotropic, affecting not just XYZ147 but other genes and their products as well.
- The protein expressed by the mutated form of gene XYZ147 exerts a dominant negative effect that blocks rescue.

RATIONALE

This question probes students' ability to formulate testable hypotheses from experimental data. In this example, the data set was kept as simple as possible — a plus or minus outcome — in order to focus student time and attention on formulating plausible explanations rather than crunching numbers. The question also was intentionally structured to suggest a variety of potential explanations rather than just one, all-or-nothing answer, to enable students to draw upon their individual perspective and strengths. The first explanation is technical in nature, the second draws upon a fundamental aspect of gene organization, and the third emphasizes the potential impact of gene products.

CONCEPT AREA: Scientific method and quantitative reasoning.

10. The structure of the amino acid histidine is shown below, along with the pK_a's of its acidic and basic functional groups.



Histidine

pKa of α–carboxyl group = 1.8
pKa of imidazole side chain = 6.0
pKa of α–amino group = 9.2

Estimate the **net charge** of the amino acid histidine to the nearest integer value (e.g. +5, not +4.76) when dissolved in an aqueous solution buffered to a pH of:

pH = 4 _____

pH = 7.5 _____

pH = 11 _____

KEY

pH = 4 +1
pH = 7.5 0
pH = 11 -1

RATIONALE

Understanding protonic equilibria and the ability to apply this concept to the behavior of biomolecules is fundamentally important. The question above is intended to probe whether the student has a good **operational understanding** of the relationship between pK_a, pH, and protonation/charge state. The choice of pK_a's and pH's is designed to let the student think through the problem without any need to resort to a calculator.

CONCEPT AREA: Scientific method and quantitative reasoning.

11. A scientist has cloned the genes encoding two potential transcription factors, factors T alpha and T-beta into plasmid vectors. The first plasmid encodes factor T-alpha, while the second encodes both T-alpha and T-beta. When bacteria were transformed with the first plasmid, no increase in the expression of a reporter gene was observed. However, when the second plasmid was used, expression of the reporter gene increased twenty-fold.

Analysis of the two bacterial strains indicated that both transcription factors were expressed at similar levels and that the protein products were intact and properly folded.

Construct statements describing two hypotheses that plausibly explain the results observed.

KEY

1. Expression of the reporter gene requires T-beta, but not T-alpha.
2. Expression of the reporter gene requires the presence of **both** T-alpha and T-beta.

Or words to that effect...

NOTE

Responses that take the form of a question, rather than stating a hypothesis, will be scored as Not Yet Proficient.

RATIONALE

Formulating hypotheses is one of the key tenets of the scientific method. This question attempts to present a realistic, straightforward situation that places the focus squarely upon the student's ability to formulate a hypothesis rather than to interpret a large and confusing mass of data. The use of the phrase "Construct statements…" is intended to cue students that while hypotheses are tools used to answer questions, hypotheses themselves are not questions but declarative statements.