ASBMB ACCREDITATION EXAMINATION
SAMPLE QUESTIONS
February 14, 2018

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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.

í. The final reaction of the citric acid cycle (see reaction below) has a standard free energy Z vP ~P'£[• }(= 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

dZ]• ‹µ •š]}v]• •]Pv š} ‰Œ} šZ •šµ vš[• µv Œ•š v]vP }(inside a cell impact the thermodynamics of enzyme-catalyzed reactions. In this case, šZ]o P'£[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 óXì ~•Çu }o]Ì Ç šZ ‰Œ]u •Çu }o €Z••X

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

or, in this case

P ' A P ' £ [= Z d o v € K Æ o }-Malate] [NAD⁺] • € Œ , • I € >

€ E } š š Z š P ' £ [ ] • š Œ u] v therefore, the concentration of protons has already been accounted for.] The ratio of [NADH] to [NAD⁺] thus can significantly] v (o µ v P ' X

2) Inside the cell, metabolic reactions do not take place in isolation. In most cases the ^ ‰ Œ } µ š ~ • • _ } (- P š À o Ç Ì v Ì Œ u š] } v Œ } (š v š] u • š Z ^ • µ • 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

CONCEPT AREA: Macromolecular structure-function-regulation.

ï. 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 assemblyv 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 }v•šŒµ š• •µ Z • ^‰Œ}š]v (}o]vP_U ÁZ] Z]u‰oÇ]((Œ vš ^ polynucleotides, etc.

CONCEPT AREA: Macromolecular structure-function-regulation.

ð. 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.

CONCEPT AREA: Macromolecular structure-function-regulation.

ñ. The two molecules shown below have the same chemical formula, $C_4H_8O_2$, 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.

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, š X d Z] • ‹ µ • š] } v ‰ Œ } • • š µ v š [• µ 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

CONCEPT AREA: Information storage and transfer.

ò. 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:

[redacted]mation storage and transfer.

[redacted]tudent working with all-white mice finds a single mouse with black
[redacted]. Standard practice in the colony is to clip the tails of all newborn mice
[redacted]type analysis. Using DNA from these tails clippings, the graduate
[redacted]uence of the agouti gene t known to influence coat color from the
[redacted]e corresponding sequence from one of the all-white siblings of the
[redacted]researcher finds that the sequences for the agouti gene are identical.

[redacted]t analyzes DNA extracted from clippings taken from the ears of these
[redacted]of the agouti gene from the ears of the all-white mouse matched
[redacted]the white- and black-eared mice, the gene from the black ear tissue
[redacted]

[redacted]s a third tissue for genotyping (toes) and once again finds that the
[redacted]re identical to those from the tail tissue. The findings are summarized

[redacted]	Tissue	Comparison of agouti gene sequences reveals that the two mice are genetically
[redacted]	Tail	Identical (wild-type)
[redacted]	Toe	Identical (wild-type)

[redacted]

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.

ô. ^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):

CONCEPT AREA: Scientific method and quantitative reasoning.

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

$$
\begin{array}{l}
NH_2 - CH - CH_2 - CH_2 - CH_3 \\
\qquad\;\; | \\
\qquad COOH
\end{array}
$$

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.

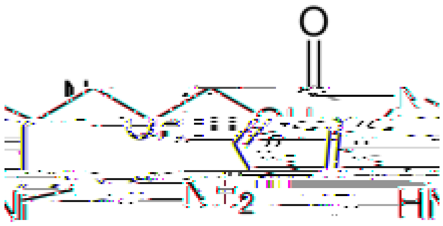
KEY
A highly proficient answer would contain any two of the following:

- 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.

1 ì. The structure of the amino acid histidine is shown below, along with the [pK$_a$ } ( ] š •]] and basic functional groups.



Histidine

pKa of D carboxyl group = 1.8
pKa of imidazole side chain = 6.0
pKa of D 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$_a$ [• v ‰ , [• designed to let the student think through the problem without any need to resort to a calculator.

CONCEPT AREA: Scientific method and quantitative reasoning.

1 í. 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 result 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[•]o]šÇ š} (}Œuµo š ZÇ‰}šZ •]• Œ šZ Œ šZ v š}]vš u •• }(š X dZ µ• }(šZ ‰ZŒ • ^ }v•šŒµ š •š š u všY_]• while hypotheses are tools used to answer questions, hypotheses themselves are not questions but declarative statements.