University of Canterbury

Mid Year Examination and Test Period 2006

Prescription Number(s):	BCHM 301 BIOL 331
Paper Title:	Biochemistry 3

Time Allowed: TWO HOURS and 30 MINUTES

Number of pages: EIGHT

Answer **ANY THREE** questions

All questions are of equal value

For questions involving multiple discrete parts, an approximate marking scheme is given as a % value for each part of the question.

Some relevant biochemical structures for questions **THREE** and **FOUR** are included at the end of this paper.

TURN OVER

1. With regard to prions, give brief critical accounts of the following:

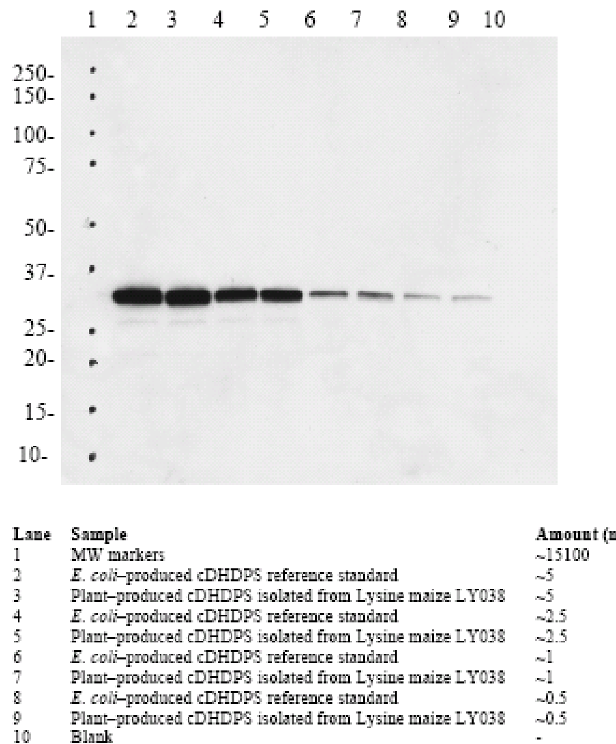
   (a) What makes them different from other protein aggregates? (25%)

   (b) What kind of experimental evidence has been used to distinguish between a protein aggregate and a protein aggregate that is a prion? (75%)

2. Corn line LY038 contains one novel gene, *cordapA*, from *Corynebacterium glutamicum*, which encodes the enzyme dihydrodipicolinate synthase (DHDPS). This enzyme is involved in lysine biosynthesis. It was extracted from the plant and compared to a version of the gene expressed in *E. coli* to determine if the protein was post-translationally modified in plants. Refer to the data below to answer the following **short answer** questions **(A – D)**. Please keep to the point.

5.0 Methods

5.1 *Protein purification.* The plant-produced cDHDPS protein was purified from Lysine Maize LY038 using a combination of cell extraction, anion exchange chromatography and hydrophobic interaction chromatography. The purification procedure preceded the characterization of the cDHDPS protein and assessment of the physicochemical and functional equivalence of the plant-produced and *E. coli*–produced cDHDPS proteins. Briefly, the grain powder was defatted and soluble proteins were extracted with a buffer containing 20 mM Tris-HCl, 100 mM KCl and 10 mM pyruvate, pH 8.0. The extracted proteins were concentrated by ammonium sulfate precipitation, desalted and applied to DEAE Sepharose (anion-exchange chromatography). Proteins were eluted with a potassium chloride (KCl) gradient from to 100 to 950 mM. The cDHDPS-containing fractions were pooled, brought to 0.5 M ammonium sulphate and applied to a hydrophobic interaction column of phenyl sepharose. Proteins bound to the column were eluted with a linear gradient from 0.5 M to 0 M ammonium sulfate. Fractions containing the cDHDPS protein were pooled, concentrated and desalted. The cDHDPS protein was further purified on a high-resolution anion exchange Mono Q column. The purified cDHDPS protein solution was concentrated, aliquoted and assigned the APS program lot number 60-100013.



Lane	Sample	Amount (n
1	MW markers	~15100
2	*E. coli*–produced cDHDPS reference standard	~5
3	Plant–produced cDHDPS isolated from Lysine maize LY038	~5
4	*E. coli*–produced cDHDPS reference standard	~2.5
5	Plant–produced cDHDPS isolated from Lysine maize LY038	~2.5
6	*E. coli*–produced cDHDPS reference standard	~1
7	Plant–produced cDHDPS isolated from Lysine maize LY038	~1
8	*E. coli*–produced cDHDPS reference standard	~0.5
9	Plant–produced cDHDPS isolated from Lysine maize LY038	~0.5
10	Blank	-

"Figure 30. Western blot analysis of the plant-produced cDHDPS protein isolated from Lysine maize LY038. Samples of the plant–produced cDHDPS protein and *E. coli*-produced cDHDPS reference standard were separated by SDS-PAGE, electroblotted to a nitrocellulose membrane and detected using goat anti-cDHDPS polyclonal antisera followed by development [of the autoradiograph]. Amounts loaded correspond to total protein. Approximate molecular weights (kDa) are shown on the left and correspond to the markers loaded in lane 1 and lane 10 (not indicated)."

(a) What is post-translational modification? (25%)

(b) Describe how post-translational modification might have influenced the protein isolation procedure. (25%)

(c) Describe two ways in which post-translational modification might have influenced the protein detection procedure in the figure. (25%)

(d) What procedure could have been used to identify all isoforms of cDHDPS? Indicate how that procedure would be superior. (25%)

3. Nitrogen assimilation in a cyanobacterium, *Synechocystis*, had been studied in detail. Some data related to nitrogen assimilation in this photosynthetic prokaryote are given below (**a - f**). Using these data, **give a brief critical account of nitrogen assimilation in *Synechocystis***. In your account you should explain the biochemical significance of the data presented and compare nitrogen assimilation in *Synechocystis* with that in other organisms.

   (a) The rate of nitrogen assimilation by a *Synechocystis* mutant strain sp. PC6803 which lacks glutamate dehydrogenase (GDH) is the same as the wild-type strain.

   (b) The apparent K_M for ammonia of GDH from *Synechocystis* is 1-3 mM. The apparent K_M for ammonia of glutamine synthetase from *Synechocystis* is <0.2 mM.

   (c) Under low nitrogen conditions, *Synechocystis* produces high levels of nitrate reductase, nitrite reductase and glutamine synthetase. The presence of high concentrations of ammonia leads to lower levels of these proteins.

   (d) Glutamine synthetase (GS) from *Synechocystis* is not subject to feedback inhibition. In response to high ammonia levels, the activity of glutamine synthetase from *Synechocystis* is decreased by adenylylation. In addition, under these conditions, two inhibitory polypeptides, IF7 and IF17, are produced which bind to, and inhibit, GS.

   (e) The rate of nitrogen assimilation by wild-type *Synechocystis* is dramatically reduced by the presence of methionine sulfoximine.

   (f) An important enzyme for nitrogen assimilation in *Synechocystis* is a ferredoxin-dependent glutamate synthase (Fd-GOGAT). This enzyme catalyses the conversion:

$$\text{L-Glutamine} + \text{2-Oxoglutarate} + 2\,H^+ + 2e^- \rightarrow 2\ \text{L-Glutamate}$$

(question 3. (f) is continued on the next page)

Reduced ferredoxin is the electron donor. The crystal structure of Fd-GOGAT from *Synechocystis* shows that the enzyme has two active-sites, which are connected by an intramolecular tunnel. The activity at the two centres is carefully synchronized. In its unliganded state the enzyme cannot bind glutamine. When the enzyme binds ferredoxin and 2-oxoglutarate a conformational change is induced in the loop connecting the two catalytic centres. This rearrangement allows the enzyme to bind, and process, L-glutamine at the other active-site.

4. This question (given after the quotation) concerns the tailoring of nitrogen metabolism to specific biochemical situations.

The following is a quote from the paper, by Krebs and Henseleit, describing the discovery of the first biochemical cycle:

"We therefore draw the conclusion that arginine is the desired intermediate product of urea synthesis in the liver … In this way the abundance of arginase in the liver acquires its physiological meaning. The arginase reaction is a partial reaction in the synthesis of urea from ammonia. This is why one finds arginase in significant quantities only in those places where urea forms from ammonia and carbon dioxide: in the livers of mammals. It is clear why, in other organs, and in the livers of birds, which produce no urea, no arginase is found, or only 100 to 1000 times less than in mammalian livers."

(From H. Krebs & K. Henseleit, Kreislauf des Ornithins, *Z. Physiol. Chem.*, 1932, **210**, 33-66. Translated by F.L. Holmes.)

Give a critical, detailed, account of the way in which core nitrogen metabolic processes vary according to specific biochemical situations. You **MUST** illustrate your account with a wide range of **SPECIFIC EXAMPLES**, including

a discussion of the organism-specificity of the urea cycle (Krebs-Henseleit cycle) described above and some, or all, of the examples (**a - d**). In your answer include a discussion of how we know the “physiological meaning” of the biochemical processes you describe.

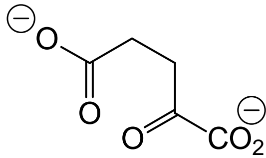
(a) *Bacillus subtilis* is a soil bacterium. It usually grows in an aerobic environment using oxygen as the terminal electron acceptor. In aerobic conditions, with nitrate as sole nitrogen source, it produces a nitrate reductase. *B. subtilis* can also grow under anaerobic conditions, but it requires nitrate to be present, irrespective of the levels of other nitrogenous compounds in the media. Under these conditions the bacteria express a different nitrate reductase isozyme.

(b) In *E. coli* the enzyme carbamoyl phosphate synthetase (CPS) is inhibited by both UMP and arginine and activated by ornithine. In humans there are two isozymes of CPS. One isozyme, CPS I, is found in mitochondria. It is activated by *N*-acetylglutamate. A second, cytosolic, isozyme, CPS II, is activated by PRPP; it is inhibited by UDP.

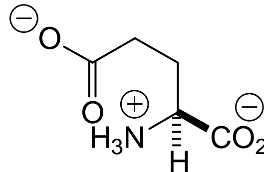
(c) *Cryptosporidium parvum* is a protozoan parasite which is a significant human pathogen. In evolving to a parasitic lifestyle it has lost all the genes for *de novo* nucleotide biosynthesis. In their place it has recruited a series of enzymes for the salvage of nucleotides. It is now completely reliant on salvage pathways for the production of both purine and pyrimidine nucleotides.

(d) Glyphosate (Round-up) is a potent herbicide because it inhibits 3-enolpyruvylshikimate-5-P synthase (EPSP synthase). Glyphosate is a useful agricultural product because it shows no toxicity towards humans.

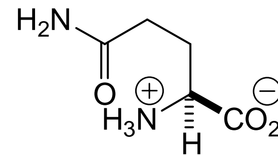
BIOCHEMICAL STRUCTURES RELEVANT TO QUESTIONS THREE AND FOUR



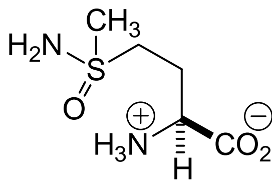
2-Oxoglutarate



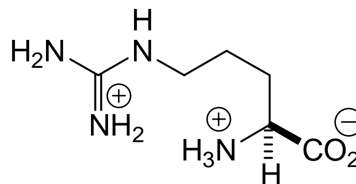
Glutamate



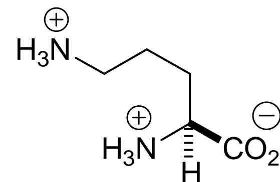
Glutamine



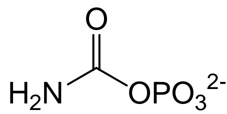
Methionine sulfoximine



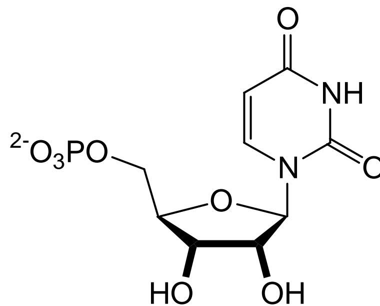
Arginine



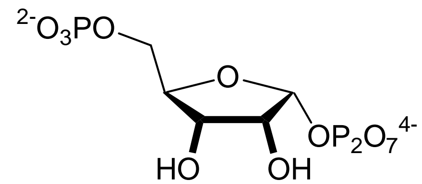
Ornithine



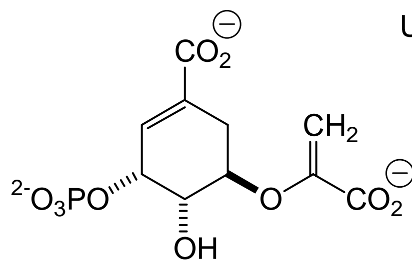
Carbamoyl phosphate



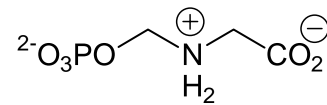
UMP



PRPP



3-Enolpyruvylshikimate-5-P



Glyphosate

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