THE UNIVERSITY OF MANITOBA

April 15, 2004 FINAL EXAMINATION

PAPER NO: 261 LOCATION: 352 Parker Building PAGE NO: 1 of 4

DEPARTMENT & COURSE NO: CHEMISTRY 2.470 TIME: 3 HOURS

EXAMINATION: Advanced Biochemistry Laboratory EXAMINERS: H. Duckworth, J. O'Neil

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Section 1: *Put the answers to the questions in Section 1 in a separate exam booklet. You can spend about 45 min. on this part of the exam. Wherever possible use diagrams to enhance your answers.*

Marks

8 1a) What approach is used to measure NADH binding to *E. coli* citrate synthase by fluorescence? In your answer, describe the physical property measured; why it changes upon NADH binding; what experimental measurements are needed; and what calculations are needed to obtain the amount of binding and the dissociation constant for the complex.

3 1b) Could NADH binding be measured by this method if fluorescence of the nucleotide is lower when it is bound to the enzyme? If the fluorescence does not change upon binding? Explain your answers.

4 1c) The brightly-coloured dye Rose Bengal absorbs visible light strongly at 549 nm, and this absorbance peak shifts to 567 nm in the presence of excess citrate synthase. Outline an experimental approach that uses this property to measure the number of Rose Bengal binding sites on one subunit of citrate synthase.

10 2) Outline the steps by which, starting with the amino acid sequence of a protein from a fruitfly, you could use BLAST database searching techniques to identify the gene for a homologous protein in humans. In your answer, clearly indicate what a BLAST search does; for each step in the search, what kind of BLAST search is needed, and what kind of database you would search; and what you would expect the search output to look like. Do you expect to get complete and accurate information about the gene at the end of the search? If not, what factors may cause difficulties?

Section 2: *Put the answers to the questions in Section 2 in a separate exam booklet. You can spend about 135 min. on this part of the exam. Wherever possible use diagrams to enhance your answer.*

Marks

8 3a) Explain the step in the purification of Calmodulin that involved separation based on chromatography.

2 3b) What is the relationship between the method we used for the chromatographic purification of Calmodulin and the mechanism by which the protein functions?

3 4a) What do "ppm", "Hz" and "MHz" stand for?

3 4b) Write down the fundamental equation of NMR spectroscopy and explain the meanings of the three symbols.

6 4c) In an 11.74 Tesla magnet ^{1}H atoms resonate at 500 MHz. In the same field the resonances of an amide H doublet of an amino acid in an α-helix are located at 8.794 and 8.800 ppm. What is the ^{3}J-coupling constant, in Hz, between the amide H and the α-H? In the same magnetic field the resonances of a different amide H doublet of an amino acid residing in a β-strand are located at 8.613 and 8.632 ppm. What is the ^{3}J-coupling constant between the amide H and the α-H? What protein structural parameter do ^{3}J-coupling constants report?

4 5) In the Circular Dichroism of Nucleic Acids Laboratory you measured spectra of 5'AMP and ApA dissolved in water at 25^{o}C. What conclusions were you able to draw about the structures of the molecules from the differences in their CD spectra?

4 6a) Explain how saturation of the water resonance affected the NH intensities in the hydrogen exchange experiment.

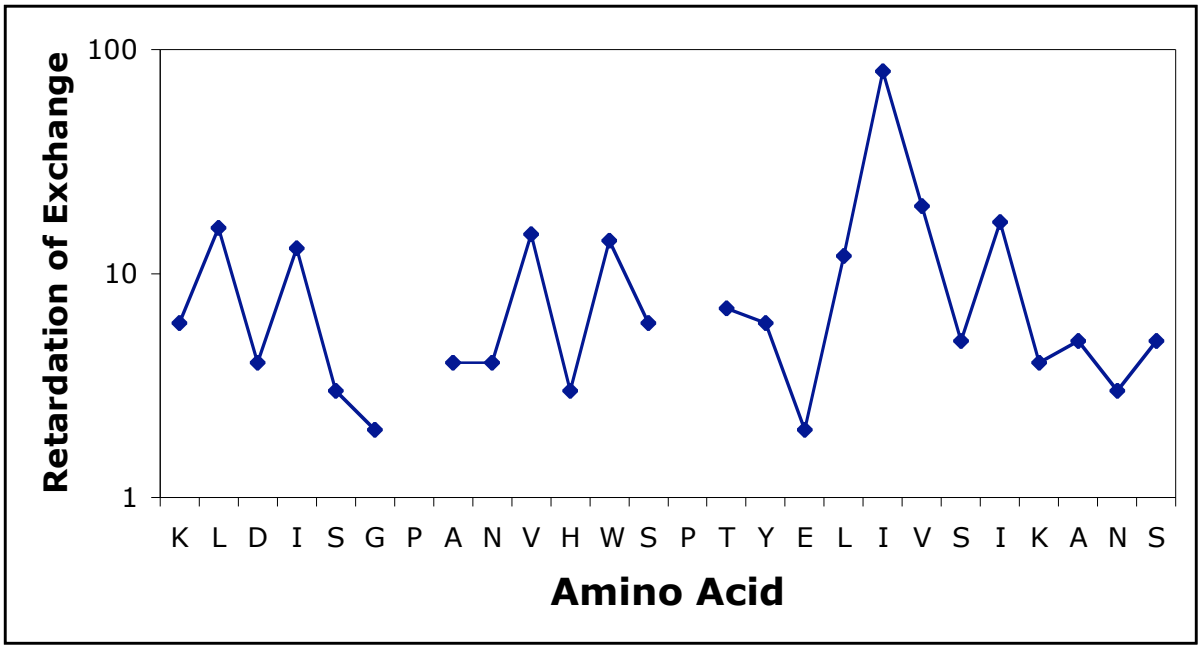
4 6b) Explain what is meant by a first order and a second order rate constant. What are the units for each?

Marks

15 7) Cyanobacteria or "blue-green algae," sometimes form large layered structures, called *stromatolites*. These structures form as a mat of cyanobacteria grows in an aquatic environment, trapping sediment and sometimes secreting calcium carbonate. When sectioned very thinly, fossil *stromatolites*, it is claimed, may be found to contain remarkably well-preserved fossil cyanobacteria and algae. Some microbiologists believe that the oldest cyanobacteria-like fossils are nearly 3.5 billion years old!

One scientist, Dr. Methuselah, claims to have isolated a small protein from a fossil cyanobacterium. Using Mass Spectrometry a tiny amount of the protein was sequenced and the following order was found: Lys-Leu-Asp-Ile-Ser-Gly-Pro-Ala-Asn-Val-His-Trp-Ser-Pro-Thr-Tyr-Glu-Leu-Ile-Val-Ser-Ile-Lys-Ala-Asn-Ser, which seems perfectly reasonable except that the amino acids are a random mixture of D- and L-isomers.

To explore the protein further a large amount was synthesized by solid-phase peptide synthesis and it was studied by NMR spectroscopy and circular dichroism spectropolarimetry. Backbone amide hydrogen exchange rates for the molecule were measured by ^{1}H-NMR spectroscopy and corrected for sequence-dependent inductive effects. The following graph was constructed which shows the degree to which hydrogen exchange rates are slowed down compared to the rates in an unstructured protein. What conclusion can you come to about the structure of the protein based on the hydrogen exchange data? What do the variations in exchange rate tell you about the structure in different parts of the protein? What do you think the CD spectrum of this protein looked like? Explain why attempts to crystallize the protein failed. Based on the evidence presented do you think that the protein originated from a living organism?



Marks

8 8) Explain how SDS-polyacrylamide gel electrophoresis can be used to determine the approximate molecular weight of a protein. How does Calmodulin behave in this system and why?

12 9) Explain any 3 of the following 5 terms relating to structure determination of proteins and nucleic acids by X-ray diffraction:

a) X-ray diffraction
b) Bragg equation
c) Unit Cell
d) Reciprocal lattice
e) Resolution

6 10) "The static 3-dimensional structures of proteins determined by X-ray diffraction contain all the information needed to deduce the biological activity of a protein". Discuss this statement critically.

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