THE UNIVERSITY OF MANITOBA

April 15, 2006 FINAL EXAMINATION

PAPER NO: 345 LOCATION: 458 Parker Building PAGE NO: 1 of 2

DEPARTMENT & COURSE NO: CHEMISTRY 2.470 TIME: 3 HOURS

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

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You have 3 hours to complete the exam so you can spend about 3.5 minutes on a 2 mark question and 18 minutes on a 10 mark question. Wherever possible use diagrams to enhance your answers. Please answer Q. 5 & 6 in a separate booklet.

Marks

3 1a) What do "ppm", "Hz" and "MHz" stand for?

6 1b) In an 14.1 Tesla magnet ^{1}H atoms resonate at 600 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?

12 2) Describe a method for the purification of GFP-Calmodulin and explain the principles underlying each of the steps.

4 3a) In the Circular Dichroism of Nucleic Acids Lab 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?

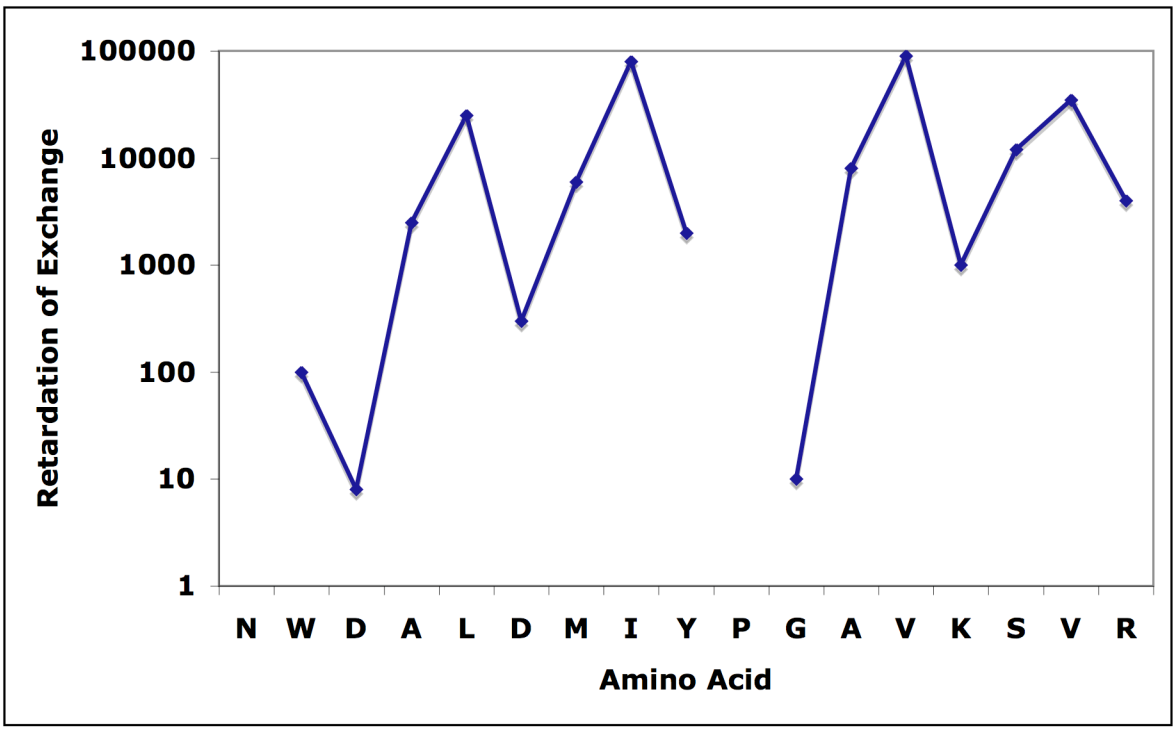
4 3b) You also measured the CD spectrum of ApA dissolved in a 40/60 (v/v) water/ethylene glycol mixture at 25^{o}C. What conclusions were you able to draw about the effects of solvent on the structure of ApA from CD spectropolarimetry?

5 3c) You measured the CD spectra of ApA dissolved in water at 10^{o}C, 25^{o}C, and 60^{o}C. Explain the effects of elevated temperature on the CD spectra making sure to refer to the structures of the molecules.

Marks

15 4a) In April of 2006 scientists reported that Enceladus, a small moon of Saturn, appeared to be jetting water vapour into space some of which falls back to the planet as snow. They speculated that the possibility of warm water on the moon makes it a logical place to search for extraterrestrial life. In May of 2016, the U.S. robotic spaceship "President Schwarzenegger" recovered a sample of water from Enceladus containing a mixture of peptides. The peptides were separated by reverse phase HPLC and the major fraction was sequenced by CID Mass Spectrometry and yielded the following sequence:
Asn-Trp-Asp-Ala-Leu-Asp-Met-Ile-Tyr-Pro-Gly-Ala-Val-Lys-Ser-Val-Arg.

Enough material was isolated to analyse the peptide by circular dichroism spectropolarimetry and NMR spectroscopy. Backbone amide hydrogen exchange rates 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 peptide. Using diagrams where appropriate, propose a structure for the peptide that explains the hydrogen exchange data obtained. Explain the relationship between your proposed structure, the sequence of the peptide and the exchange data. What do you think the CD spectrum looked like and why?



5 4b) Explain how saturation of the water resonance affects the NH intensities in the hydrogen exchange experiment.

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

10 5) 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?

8 6a) 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.

4 6b) What difficulties might be encountered if one attempted to measure NADH binding by changes in fluorescence of tryptophan in the citrate synthase molecule? [Hint: recall that NADH absorbs light at 340 nm, the same wavelength at which tryptophan emits fluorescent radiation].

3 6c) More extensive measurements of NADH binding have shown that as the pH of the medium is decreased, NADH binding is tighter (K_d decreases). Stopped-flow kinetic measurements show that this tighter binding is associated with slower dissociation of NADH from the NADH-protein complex. The ionizable group controlling this pH dependence has an apparent pK_a of about 7.4. Suggest an explanation for these findings, taking into account the known structures of proteins and NADH itself.

12 7a) Explain any 3 of the following 4 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) Resolution

5 7b) Briefly outline the procedures and principles used in the crystallization of lysozyme laboratory.

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