THE UNIVERSITY OF MANITOBA

April 14, 2005 FINAL EXAMINATION

PAPER NO: 27 LOCATION: 352 Parker Building PAGE NO: 1 of 2

DEPARTMENT & COURSE NO: CHEMISTRY 2.470 TIME: 3 HOURS

EXAMINATION: Advanced Biochemistry Laboratory EXAMINERS: J. O'Neil

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Section 1: *You urs to complete the exam so you can spend about 3.5 minutes on a 10 mark question and 18 minutes on a 10 mark question.*
Wherever possible use diagrams to enhance your answers.

Marks

15 1a) Outline how NMR spectroscopy can be used to determine the 3D structure of a protein in solution. Be sure to explain the roles of COSY and NOESY experiments, coupling constants, chemical shifts, and computation.

2 1b) Write down the fundamental equation of NMR spectroscopy.

2 2a) What is the effect of repeated freezing and thawing on *E. coli* cells during the purification of Calmodulin-Green Fluorescent Protein?

2 2b) What is the purpose of adding DNase and RNase to an *E. coli* cell extract?

1 2c) What does sonication do to *E. coli* cells?

2 2d) What is the function of mercaptoethanol in the purification of Calmodulin-Green Fluorescent Protein?

1 2e) During protein purification why are cell extracts kept at $4^{o}C$?

2 2f) What is the purpose of heating a cell extract to $65\,^{o}C$ during the purification of Calmodulin-GFP?

2 2g) Why is the phenyl-Sepharose column washed with 20% ethanol?

8 3a) When plane polarized light is passed through a medium containing chiral molecules, the light that is emitted is "elliptically polarized". Explain this phenomenon. Diagrams are essential for full marks.

3 3b) Name, and draw the structure of the chromophore in proteins whose CD spectrum is sensitive to secondary structure changes. At what wavelengths does it absorb?

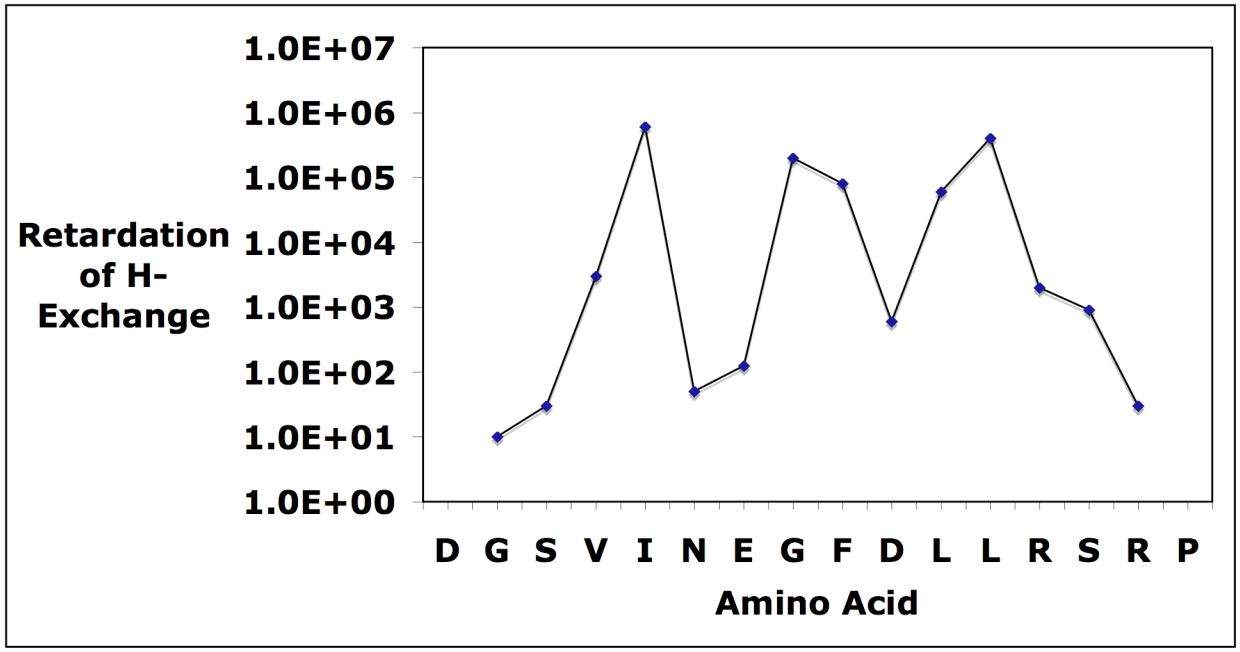
3 3c) Explain the meanings of the symbols in the following equation:

$$[\theta_{222}]_h = f_h\ (-31{,}500) + [f_\beta + f_i]\ (-2730)$$

4 3d) Explain how the equation above is used to extract the helix content of a protein.

15 4a) The following amino acid sequence was deduced from the genome of a newly isolated bacterium:
Asp-Gly-Ser-Val-Ile-Asn-Glu-Gly-Phe-Asp-Leu-Leu-Arg-Ser-Arg-Pro.
A peptide consisting of the above-mentioned amino acids was synthesized by solid-phase techniques. A circular dichroism spectrum of the deprotected and purified peptide dissolved in water showed two peaks of negative ellipticity at 208 and 222 nm and a peak of positive ellipticity at about 192 nm. Backbone amide hydrogen exchange rates were measured by ^{1}H-NMR spectroscopy and corrected for sequence-dependent inductive effects. The following graph was constructed

Marks 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 circular dichroism and hydrogen exchange data obtained. Explain the lack of data for the first and last residues.



7 4b) Explain what is meant by "sequence-dependent inductive effects" and how they can affect hydrogen exchange rates. What other techniques are available that could be used to measure the structure and / or dynamics of the peptide?

8 5a) 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. What calculations are needed to obtain the amount of binding and the dissociation constant for the complex?

3 5b) Could Rose Bengal binding to *E. coli* citrate synthase be measured if absorbance of the dye is lower when it is bound to the enzyme? If the absorbance does not change upon binding? Explain your answers.

4 5c) Measurements of NADH binding to *E. coli* citrate synthase 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.

16 6) Explain any 4 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

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