THE UNIVERSITY OF MANITOBA

April 14, 2009 FINAL EXAMINATION

PAPER NO: 176 LOCATION: 356 Parker Building PAGE NO: 1 of 3

DEPARTMENT & COURSE NO: CHEM 4700 TIME: 3 HOURS

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

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.1-5 in one exam booklet, Q 6 in a second booklet, and Q. 7-9 in a third booklet.

Marks

Booklet 1

14 1) 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 2a) What is the purpose of repeated freezing and thawing of *E. coli* cells during the purification of a protein?

2 2b) When extracting protein from *E. coli*, what is the purpose of adding DNase and RNase to the cell extract?

2 2c) What is the purpose of adding mercaptoethanol to cell extraction and chromatography buffers during the purification of a protein?

1 2d) Why are cell extracts kept at 4^{o}C during protein purifications?

2 2e) What purpose would heating a cell extract to 80 ^{o}C serve during the purification of a protein?

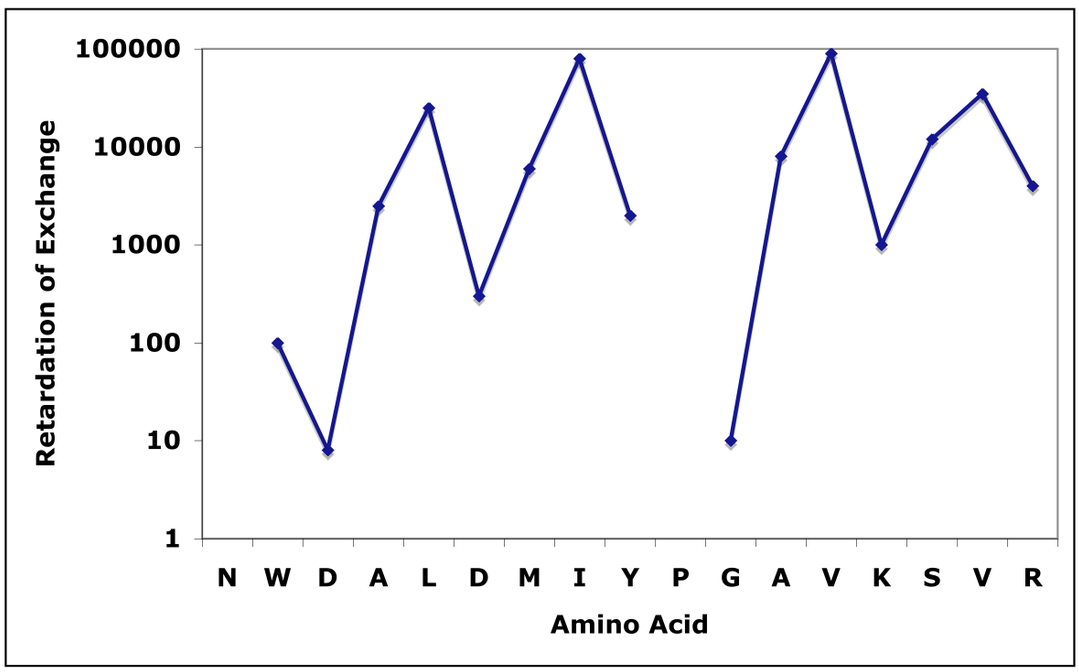
2 2f) Following hydrophobic interaction chromatography why is the chromatographic resin washed with 20% ethanol?

3 3) You 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?

15 4) 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 analyze 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?

Marks



12 5) Compare and contrast the ESI and MALDI methods of introducing proteins into the vacuum of a mass spectrometer. Explain briefly how the methods work, what information is obtained and what the spectra look like.

Booklet 2

5 6a) Describe the vapour diffusion method of protein crystallization.

5 6b) Give the equation for the crystallographic R-factor (residual-factor). Describe what it means and why it is used to judge the quality of a protein X-ray crystal structure.

Booklet 3

10 7) 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?

5 8a) What is the linear form of the Stern-Volmer equation for quenching of fluorescence by an added substance? Which parameter in this equation is indicative of how closely the quencher interacts with the fluorophore (fluorescent chromophore)?

3 8b) Why might this linear equation not be obeyed when the fluorophore consists of several tryptophan residues within a single protein?

2 8c) The experiments done in the CHEM 4700 laboratory all involved an ionic quencher, iodide. Suggest how the results might change, for a given protein, if a non-ionic quencher, such as acrylamide, were used instead.

Marks

8 9a) 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 9b) 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 9c) 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.

100