THE UNIVERSITY OF MANITOBA

April 16, 2008 FINAL EXAMINATION

PAPER NO: 216 LOCATION: 539 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. 6, 7 & 8 in a separate booklet.

Marks

15 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.

12 2) In the CHEM 4700 laboratory you purified a fusion protein made of green fluorescent protein and calmodulin. Based on what you learned, describe a method for the purification of calmodulin and explain the principles underlying each of the steps. Would you expect GFP-calmodulin to behave differently from calmodulin in any of the purification steps you have just described?

7 3) Which X-ray crystal structure is of better quality?
1) R-factor: 33%, Resolution 1.7 Angstroms, or
2) R-factor: 19%, Resolution 1.7 Angstroms, or
3) R-factor: 33%, Resolution 3.2 Angstroms, or
4) R-factor: 19%, Resolution 3.2 Angstroms.
Support your answer.

8 4a) Draw or describe the circular dichroism spectra for polypeptides in pure α-helical, 3_{10}-helical, β-sheet, and irregular conformations. Predict what you would expect the circular dichroism spectrum to look like for a protein containing 75% of its polypeptide in the α-helical conformation and 25% of its polypeptide in the β-sheet conformation

3 4b) Name and draw the structure of a chromophore in proteins whose circular dichroism spectrum is sensitive to tertiary structure changes. Over what range of wavelengths does it absorb?

5 4c) You measured the circular dichroism 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. What chromophore in nucleic acids gives rise to the differential absorption of circularly polarized light and over what range of wavelengths is this observed?

15 5) Recently, some researchers in the Department of Chemistry at Washington State University in St. Louis measured H/D exchange of calmodulin using electrospray ionization to introduce the protein into the vacuum of the mass spectrometer. In the absence of Ca^{2+} about 115 backbone amide protons exchanged in about 1 hour. The rest of the amides exchanged more slowly. When Ca^{2+} was added to the protein and the experiments repeated, only about 91 amide protons exchanged quickly.

Explain briefly how ESI-MS can be used to measure hydrogen-deuterium exchange. Discuss the meaning of the calmodulin hydrogen exchange results in terms of the structure and function of calmodulin. Explain how hydrogen-deuterium exchange takes place and the relevance of solution pH; include the structure of the amide unit. Explain what is meant by "*sequence-dependent inductive effects*".

Marks		
10	6)	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	7a)	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	7b)	Why might this linear equation not be obeyed when the fluorophore consists of several tryptophan residues within a single protein?
2	7c)	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.
8	8a)	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	8b)	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	8c)	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.
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