Lincoln College Biochemistry Tutorials

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Michaelmas Term, Tutorial 3

Title: Biophysical Methods III – Absorption and emission spectroscopy (Lincoln)

This week's work will cover the basic concepts of spectroscopy and its application to the study of biological systems. There are many and varied techniques, most of them closely related in terms of the theory but with a wide differences in the information that can be extracted. As usual, the headers below are not exhaustive. Most time should be spent on section 3.

You should cover (make notes on) the following areas:
1. Nature of electromagnetic radiation; relationship between energy (E), wavelength (λ), frequency (υ), wavenumber; non-polarizsed, plane polarized and circularly polarized radiation. Electromagnetic spectrum; energies of radio-waves, IR, visible, UV, X-ray, γ-ray radiation.
2. Atomic/molecular energy levels (translation, rotation, vibration, electronic, nuclear).
Description of energy levels; Morse curve; Boltzmann distribution; transition energies for different types of molecular excitation. Absorption of electromagnetic radiation; transition probabilities, selection rules, Frank-Condon principle. Loss of absorbed energy; spontaneous (fluorescence and phosphorescence) and stimulated emission, non-radiative processes.
3. Types of spectroscopy -- classified according to type of radiation used (IR, UV, etc.), type of excitation (polarised, continuous or pulsed irradiation, etc.), process being monitored(absorption, emission, loss of polarisation, etc.).
In each case consider: the type of transition involved (the initial and final states), the timescale for the experiment, what type of chromophores can be used (both intrinsic and extrinsic) and, most importantly, what type of information can be obtained directly and inferred. IR spectroscopy -- types of bonds that give strong signals, bond force constants. UV/visible spectroscopy -- intensity and extinction coefficients (Beer-Lambert Law), solvent perturbation.
Fluorescence spectroscopy -- lifetime, polarisation anisotropy, intensity and quantum yield, quenching, energy transfer.
CD spectroscopy -- secondary structure analysis.
Surface plasmon resonance -- equilibrium and kinetic binding studies.

References

Standard general texts.
M.S. Braiman and K.J. Rothschild, (1988) Ann. Rev. Biophys. Biophys. Chem., **17**, p.541-570 - 'Fourier transform IR techniques for probing membrane protein structures' -- *interesting but more advanced*.

S.B. Brown, (1980) “An introduction to spectroscopy for biochemists”, pub. Academic Press.
T.E. Creighton (ed.), (1990) "Protein structure: a practical approach", pub. Oxford.
A.R. Holzwarth, (1989) Quart. Rev. Biophys., **22**, p.239-326 - 'Applications of ultra fast laser spectroscopy for the study of biological systems' -- *interesting but more advanced*.
J.R. Lakowicz,(1983) “Principles of fluorescence spectroscopy”, pub. Plenum.
B. Liedberg, C. Nylander and I. Lundstrom, (1995), Biosensors & Bioelctronics, **10(8)**, p.i-ix - 'Biosensing with surface plasmon resonance - how it all started' – *gentle introduction*.
Z. Salamon, M.F. Brown and G. Tollin, (1999) TIBS, **24(6)**, p.213-219 - ‘Plasmon resonance spectroscopy: probing molecular interactions within membranes’.
P. Schuck, (1997) Ann. Rev. Biophys. Biomol. Struc., **26**, p.541-566 - 'Use of surface plasmon resonance to probe equilibrium and dynamic aspects of interactions between biological macromolecules' -- *more advanced and theoretical*.
L. Stryer, (1968) Science, **162**, p.526-533 - 'Fluorescence spectroscopy of proteins' -- *very good*.
A. Szabo, L. Stolz and R. Granzow, (1995) Curr. Opin. Struc. Biol., **5**, p.699-705 - 'Surface plasmon resonance and its use in biomolecular interaction analysis (BIA)' -- *simple and lots of examples*.

Problems

All submitted material to be attached as one bundle from each separate student, to be clearly marked with the title of the tutorial, the date, the name of the student, and to clearly display “FAO Dr. Mark Leake, Clarendon Lab” on the first page. To be handed in to either the receptionist, or placed in the “L” pigeon-hole, of the Clarendon Laboratory, Dept of Physics by *12 noon* the day before the tutorial.

1). Write an essay on how the study of electronic transitions can give information about dynamic processes occurring in biological systems.

2). Describe the principles of solvent perturbation spectroscopy and outline the essential experimental details. To a good first approximation, N-bromosuccinimide-oxidised tryptophan has, unlike tryptophan, no solvent perturbation spectrum at 292nm. Lysozyme has six tryptophan residues. Assume that solvent perturbation studies indicate that three are exposed. (a) If the oxidation of three tryptophan residues completely eliminates the solvent perturbation, what can you conclude ? (b) If the solvent perturbation disappears only after six residues are oxidised, how is your conclusion altered?

3). Explain the following observations - Melittin is a 26-residue amphipathic peptide, a component of bee venom, containing a single tryptophan. An aqueous solution of melittin has a single fluorescence band with a maximum at 360 nm

and a fluorescence anisotropy of 0.06. ORD indicates the presence of » 12% helix. In 2M NaCl solution, the fluorescence band shifts to 348 nm, the fluorescence anisotropy increases to 0.13 and ORD studies indicate the presence of » 65% helix. In the presence of phospholipids, the fluorescence anisotropy is 0.14, both in 0M and 2M NaCl. Comment about the possible modes of action of melittin given these observations.

4). The X-ray crystal structure of calmodulin, a calcium binding protein containing two active thiols, one in the N-terminal and one in the C-terminal domain, shows the protein to consist of two globular domains separated by a rigid, solvent exposed helix (dumbbell-shaped). The central helix has been proposed to be an artefact of crystallisation. By what techniques could you investigate the extent to which this helix is present in the solution state?