Lincoln College Biochemistry Tutorials

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1st years, Hilary Term, Tutorial 4

Title: Biophysical Chemistry – Kinetics & macromolecular structure (Lincoln)

This week will provide an introduction to chemical kinetics and some of the ideas on macromolecular structure.

You should ensure you have notes on the following areas:

1. Basic concepts :-

Definition of rate of reaction and reaction co-ordinate (note - can have several different definitions for one reaction). Rate equations, rate constants (units vary with order), order (difference between order and molecularity, pseudo-order of reactions). 1st and 2nd order processes and half-lives. Applications of kinetics to systems approaching, and at, equilibrium. Kinetics of consecutive coupled reactions, rate-determining step, concentrations of intermediates with time, steady-state approximation (uses and conditions for validity). Relationship between rate equations and reaction mechanism. Variation of rate constant with temperature (Arrhenius equation -- also see below).

2. Experimental methods :-

Techniques for following reactions on different time scales (very briefly); continuous measurement (spectroscopic), analysis of aliquots, quenching, stop-flow techniques. Extraction of rate constants, orders and activation energies from kinetic data (very important).

3. Reaction Theories :-

Collision theory, conditions for a collision to produce a reaction, probability of collisions, activation energies. Transition state theory (thermodynamics applied to non-equilibrium systems), ΔH, ΔS, ΔG of activation. Arrhenius equation, significance of the constants in different theories. Relationship of activation energies to reaction energies.

4. Catalysis, enzyme catalysts and inhibitors.

5. Types of non-covalent interactions -- these almost all come down to charges of one form or another interacting (electrostatics). Types of charges :- mono-pole, dipole, induced-dipole. Types of molecules/functional groups that have different types of charges. Electrostatic interactions :- mono-pole--mono-pole, mono-pole--dipole, dipole--dipole, mono-pole--induced-dipole, etc. Distance, charge, angle dependence and relative magnitudes of energy terms. Size of these terms relative to thermal energy (kT). Effect of solvent (dielectric constant).

Specific types of interactions (mostly examples of above more general types) :- H-bonding, Van der Waals interactions (attraction and repulsion), salt-bridges. Interactions involving water :- structure of water, hydration of charges, hydrophobic/hydrophilic interactions. Molecular force fields (calculation of energy), energy minimisation and molecular dynamics.

6. Thermodynamics of folding --

ΔH and ΔS, cooperativity of interactions in macromolecules.

7. Protein structure --
Definition of primary, secondary, tertiary and quaternary structure. Primary structure, peptide sequence, backbone and side chain structures, peptide bond conformation, disulphide bonds. Types of secondary structure (a-helix, b-sheet, turns, loops) based on backbone conformation, interactions involved in stabilising secondary structure units, helix dipoles, Ramachandran plots. Tertiary structure based on side-chain packing, interactions involved in stabilising tertiary structure.
8. DNA and RNA structure --

References

Standard general texts and extra references in Price and Dwek (p.249).
A. Fersht, (1983) "Enzyme Structure and Mechanism", pub. Freeman.
L. Stryer, (1988) "Biochemistry", pub. Freeman
C. Branden and J. Tooze, (1991) “Introduction to protein structure”, pub. Garland
C.R. Cantor and P.R. Schimmel, (1980) "Biophysical Chemistry" (3 volumes), pub. Freeman.
T.E. Creighton, (1983) "Proteins: Structures and Molecular Principles", pub. Freeman -- *probably best book available*.
M.J. Pilling, (1975) "Reaction Kinetics", OCS, pub. Clarendon Press.
W. Saenger, (1984) "Principles of Nucleic Acid Structure", pub. Springer-Verlag.
G.E. Schulz and R.H. Schirmer, (1979) "Principles of Protein Structure", pub. Springer-Verlag -- *more advanced*.
L. Stryer, (1988) "Biochemistry", pub. Freeman.
M.C. Wahl and M. Sundaralingham, (1997) TIBS, **22**, p.97-102

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) The equation giving the total number of moles of ligand bound per mole of a protein (*r*) for a protein with *n* identical ligand binding sites is

$$r = n\frac{[L]}{K_d + [L]}$$

where [*L*] is the concentration of free ligand.

Derive this equation, stating any assumptions that you make, and explain the nature of the constant K_d. **[8]**

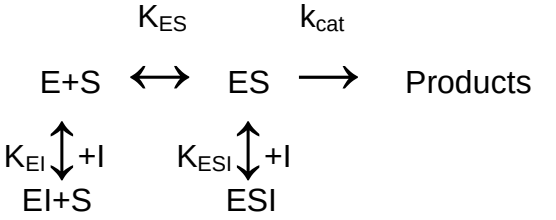
The following concentration data were obtained by equilibrium dialysis measurements for the binding of mannose to a 1 mmol dm^{-3} solution of a lectin.

Total mannose (mmol dm^{-3})	**Free mannose (mmol dm^{-3})**
0.5	0.24
1.0	0.51
1.5	0.78
2.0	1.10
2.5	1.43
3.0	1.75
4.0	2.53

What can you infer about the behaviour of the binding sites in this lectin from this data? Calculate the values of *n* and K_d for the lectin. **[9]**

In many cases, the binding of ligand to a multi-site protein does not obey the above equation. Explain why this may occur and discuss the implications. **[8]**

2) By reference to the following scheme, distinguish between the four types of reversible enzyme inhibition, *competitive, uncompetitive, mixed, and noncompetitive.* **[9]**



E - enzyme, S - substrate, I - inhibitor, K_{ES}, K_{EI} and K_{ESI} are the dissociation constants for ES, EI and ESI respectively.
Sketch plots of rate, v, versus substrate concentration, [S], for
(i) an uninhibited enzyme reaction,
(ii) the same reaction in the presence of a competitive inhibitor and
(iii) the same reaction in the presence of a noncompetitive inhibitor, all on the same graph. **[5]**

On the basis of the sketch, comment on the relative effectiveness of a competitive inhibitor and a non-competitive inhibitor with similar K_{EI} values to prevent flux through a metabolic pathway *in vivo* by inhibiting one of the intermediate steps. **[3]**

The enzyme xanthine oxidase is inhibited by an inhibitor I. The following kinetic data were obtained at three different inhibitor concentrations.

	Inhibitor concentration [I] (mol dm^{-3})		
	4×10^{-9}	8×10^{-9}	16×10^{-9}
	Initial rate ν (μmol min^{-1})		
Substrate concentration [S] (mol dm^{-3})			
50×10^{-6}	13.7	9.6	6.0
25×10^{-6}	11.0	7.4	4.4
16.7×10^{-6}	9.2	5.9	3.5
12.5×10^{-6}	7.8	5.0	2.9

By means of plots of $1/\nu$ against [I] and $[S]/\nu$ against [I], or by other methods, determine K_{EI} and K_{ESI} for this inhibitor and state what type of inhibition it demonstrates. **[8]**

3) (a) Give an account of attractive and repulsive non-covalent inter-atomic forces, including a discussion of their magnitudes and distance dependences. **[15]**

(b) Discuss:
(i) how such forces relate to the hydrophobic effect. **[5]**
(ii) the importance of these inter-atomic forces in stabilising a specific three-dimensional fold for a protein. **[5]**