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Background

- *Enzymes* are large biological molecules responsible for thousands of metabolic processes that sustain life, including food digestion and DNA synthesis.
  - They are highly selective catalysts that greatly accelerate the *rate* and *specificity* of metabolic reactions.
  - Most enzymes are *proteins* and they adopt a specific three-dimensional structure.
  - Enzymes may employ organic or inorganic *cofactors* to assist in catalysis.
- *Cofactors* are non-protein compounds that are bound to a protein and are required for the protein's biological activity.
  - "Helper molecules" that assist in biochemical transformations.
  - Organic cofactors include biotin, flavin and heme. They can be further classified as:
    - *Coenzymes* → loosely bound cofactors that refer to the <u>functional</u> properties of a protein.
    - *Prosthetic groups* → tightly bound (covalent) cofactors that refer to a <u>structural</u> property.
  - Inorganic cofactors include:
    - *Metal ions* → metalloproteins
    - *Iron-sulfur clusters* → sulphide-linked di-, tri- and tetra-iron centres in variable oxidation states
- *Substrates* are molecules upon which an enzyme acts.
  - For a single substrate, an *enzyme-substrate complex* forms when the substrate bonds with the enzyme active site.
  - *Active sites* → small areas of an enzyme where substrates bind and undergo a chemical reaction.
  - *Binding* → usually enzymes have only one active site which fits with one specific type of substrate.
- Enzymes can be *denatured* at high temperatures or extreme pH values.
  - This causes the active site to change shape and the substrate molecules to no longer fit.
  - The *efficiency* of a reaction is believed to increase when there is a tighter fit between the active site and the substrate.
- *Solvation* is the interaction of molecules of a *solvent* with ions of a *solute* which leads to the stabilisation of the solute species in the solution.
  - Ions spread out and dissolve in a solvent and become surrounded by solvent molecules.
- *Dielectrics* are electrical insulators that can be polarized by an applied electric field.
  - Electrical charges do not flow through dielectric material when it is placed in an electric field.
  - Instead, there is a slight shift in average equilibrium positions causing dielectric <u>polarization</u>.

- *Solvation energy* resulting from polarization is quite considerable and must be accounted for when studying enzyme reactions. This allows:
  - Acidic groups to become ionized.
  - Charge distribution on substrate to be reasonable.
- *Bond cleavage* is the process of breaking down large molecules by splitting their internal bonds.
- *Charge distribution (density)* in quantum mechanics is summarised by the following equations.
  - $\rho_q(\mathbf{r}) = q|\psi(\mathbf{r})|^2$ → related to wavefunction
    - q = charge of the particle
    - $|\psi(\mathbf{r})|^2 = \psi^*(\mathbf{r})\psi(\mathbf{r})$ = probability density function → probability per unit volume of a particle located at $(\mathbf{r})$
  - $Q = \int_R q|\psi(\mathbf{r})|^2 \, \mathrm{d}^3\mathbf{r}$ → when wavefunction is normalized
    - Q = the average charge in the region $\mathbf{r} \in R$
    - $\mathrm{d}^3\mathbf{r}$ = the integration measure over 3-D space

Outline

- <u>*Aim:*</u> To develop a theoretical method for studying the reactions of enzymes, with particular emphasis on the factors that affect reaction pathways.
- <u>*Scope*</u>*:*
  - The system includes the entire enzyme-substrate (E-S) complex and its solvent (water).
  - The reaction examined is the cleavage of the glycosidic bond of *lysozyme*, focusing on the factors that affect the stability of the carbocation intermediate formed.
  - Classical approaches use *empirical energy functions* to study the <u>conformation</u> of E-S complexes → based on bond stretching, angle-bending, twisting and non-bonded interactions.
  - Quantum mechanical (QM) approaches can study the <u>mechanism</u> and <u>energetics</u> of enzyme reactions, although it has several limitations:
    - Model is oversimplified, including only a small fraction of atoms involved in the reaction.
    - Reactions are treated within an isolated system (vacuum) and are not able to account for "dielectric effects" (polarizability of atoms) which affect the energy contributions from electrostatic interactions.
- <u>*Method*</u>*:*
  - The complete enzyme-substrate-solvent system examined includes all the energy factors that might contribute to the reaction mechanism, including:
    - *QM energy factors:*
      - Bond cleavage (rearrangement of bonds)
      - Charge redistribution (density) of the substrate
    - *Classical energy factors:*
      - Steric interactions (substrate strain on binding to the enzyme)
      - Electrostatic interactions (between substrate and enzyme)
    - *Dielectric effects*:
      - Polarization of the enzyme atoms
      - Dipole orientation of water molecules

- *Model:*
  - The proposed *hybrid classical/QM* model evaluates:
    - *Energy* and *charge distribution* of the atoms that directly participate in the reaction (QM methods).
    - *Potential energy surface* of the rest of the system, which includes steric and electrostatic interactions (classical methods).
    - *Dielectric effects* from induced dipoles and polarization (QM/classical).
- *Analysis:*
  - *Potential energy partition*
    - The model partitions the potential energy surface into classical and quantum mechanical components, as well as a "coupling" term.
    - $V = V_{quantum} + V_{classical} + V_{quantum|classical}$
    - The combined coupling term accounts for:
      - Induced dipoles in the classical region
      - Electrostatic and van der Waals interactions between atoms in the quantum and classical regions.
    - $V_{classical} = \sum_i K_b(b_i - b_0)^2 + \sum_i K_\theta(\theta_i - \theta_0)^2 + \sum_i K_\theta \cos\{n(\phi_i - \phi_0)\}$
      $+ \sum_{i>j} \epsilon_{ij}\{(r_{ij}^0/r_{ij})^{12} - 2(r_{ij}^0/r_{ij})^6\} + \sum_i Q_i Q_j / r_{ij}$
    - $V_{quantum} =$ obtained by an extended form of *QCFF/PI* (quantum-mechanical consistent force field method for pi-electron systems) that includes <u>all</u> valence electrons → referred to as *QCFF/ALL*
    - $V_{quantum|classical} = \sum_{i,j} Q_i Q_j / r_{ij} + \sum_{i,j} \epsilon_{ij}\{(r_{ij}^0/r_{ij})^{12} - 2(r_{ij}^0/r_{ij})^6\}$
      $+ V_{\mathrm{ind}}^{\mathrm{E}} + + V_{\mathrm{ind}}^{\mathrm{W}}$
  - *Induced dipoles and the effective dielectric*
  - *Substrate conformational changes*
- *Results:*
  - *Steric effects in the ground state*
  - *Equilibrium conformation of the carbonium ion*
  - *Charge stabilization and dielectric effect*
- *Conclusions:*
  - By incorporating the polarizability of atoms into the hybrid model through dielectric effects, a more accurate description of electrostatic interactions and its energy contributions is achieved.
  - Electrostatic interaction is a major factor in the reaction rate leading to carbocation intermediate formation.
  - Steric strain was found to be of minor importance.