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A simplified electrostatic model for hydrolase catalysis

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ABSTRACT

Toward the development of an electrostatic model for enzyme catalysis, the active site of the enzyme is represented by a cavity whose surface (and beyond) is populated by electric charges as determined by pH and the enzyme's structure. The electric field in the cavity is obtained from electrostatics and a suitable computer program. The key chemical bond in the substrate, at its ends, has partial charges with opposite signs determined from published force–field parameters. The electric field attracts one end of the bond and repels the other, causing bond tension. If that tension exceeds the attractive force between the atoms, the bond breaks; the enzyme is then a successful catalyst. To illustrate this very simple model, based on numerous assumptions, some results are presented for three hydrolases: hen-egg white lysozyme, bovine trypsin and bovine ribonuclease. Attention is given to the effect of pH.

1. Introduction

Enzyme catalysis constitutes a well-established field of research in biochemistry. Pertinent literature reviews on this subject can be found in textbooks such as those by Frey and Hegeman [1], Bisswanger [2] and Purich [3], among others.

In this work, we consider a fundamental question: For a specific substrate reaction at a fixed temperature and pH, will a specific enzyme provide catalysis or not?

Toward an effort to answer this question, we initiate here a simple model based on electrostatics. To introduce the basic idea, consider two parallel metallic plates separated by a short distance. One plate bears a positive electric charge, while the other bears a negative electric charge, as indicated in Fig. 1. Into the gap between the plates, we put a dumbbell whose two ends are separated by a small distance compared with the height of the gap. The two ends of the dumbbell are charged; one end is positive and the other end is negative.

The two charged plates generate an electric field perpendicular to the surfaces of the plates. This field attracts one end of the dumbbell while repelling the other, generating a tensile force in the rod that connects the two ends. If the tensile force is sufficiently strong, the rod breaks.

We use this electromechanical picture as a basis for a simple model of enzyme catalysis. The two charged plates are replaced by the surface of a spherical cavity that represents the immediate region of an enzyme's active site. The charge on this surface is not uniform; it is determined by the positions of charges on the enzyme at a fixed pH.

The dumbbell is replaced by the pertinent chemical bond in the substrate, inside the cavity. The ends of the dumbbell bear partial charges of opposite sign. When the electric field in the cavity exceeds the chemical bond strength, the bond is broken, giving a catalyzed reaction.

Before presenting the essential details of our simple electrostatic model, we note that application of electrostatic physics to catalysis is not a new idea, as indicated by numerous previous publications, e.g. Warshel [4], Warshel et al. [5], Dao-Pin et al. [6]. The importance of the electric field in enzyme catalysis is evident in the THEMATICS method for identifying the active site [7–9]. Considering an enzyme of known structure but unknown active site, THEMATICS searches for unusual theoretically calculated titration curves, and associates the distortion in titration curves to catalytic activity. Electrostatic interactions also enhance the diffusion of substrate molecules to the enzyme's active site [10,11] and the steering of substrate molecules inside the active site [12]. Fried et al. [13] showed recently that large electric fields found in the active pocket of ketosteorid isomerase are responsible for the unusually high rate constants of the reaction catalyzed by this enzyme. However, to our best knowledge, no previous publication has tried to answer the question of concern here: for a given situation (enzyme, substrate, temperature, pH), can a relationship between the electric

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Nomenclature

Latin letters

$\underline{\mathbf{E}}_k$	electric field at position k
E_x	x-component of the electric field
$\underline{\mathbf{E}}_{jk}$	electric field generated by group j at position k
$\underline{\mathbf{F}}_m$	electrostatic force acting on atom m
$\underline{\mathbf{F}}_{mn}$	electrostatic force acting on atom n due to atom m
N	number of atoms in the enzyme molecule
q_i	charge on atom i
$\underline{\mathbf{r}}_{jk}$	vector from position (of atom/group) j to position (of atom/group) k
U_{mn}	electrostatic energy between atoms m and n

Greek letters

δ	cut-off distance in Eq. (7)
ε_0	vacuum permittivity
ε	dielectric constant
ϕ_k	electric potential at position k
λ	function defined by Eq. (7)

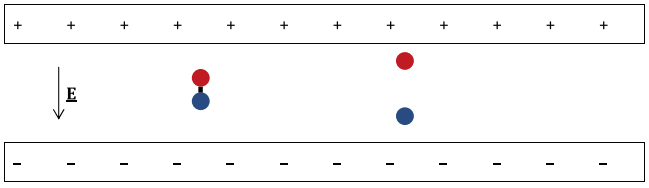

Fig. 1. Illustrative two-dimensional representation of bond cleavage in the active site. The two charged plates generate electric field $\underline{\mathbf{E}}$. (a) dumbbell representing the substrate's pertinent bond in the active site. The negatively charged atom is red, while the positively charged atom is blue. The electric field attracts the blue atom and repels the red atom, causing a tensile force in the (dumbbell) bond. (b) Atoms after cleavage. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

field generated in the enzyme's active site and the electric dipole of the bond in the substrate molecule be established? Based on simplifying assumptions, our model aims to provide an answer to the question.

2. Calculation of the Electric Field and the Electric Potential

To calculate the electric field and electric potential inside the active pocket, the influence of all enzyme atoms is taken into account: each atom bears a full or partial charge that influences the electric field and the electric potential. The electric field $\underline{\mathbf{E}}_k$ is a vector. At position k of the active pocket, the electric field is calculated using the basic equation of electrostatics:

$$\underline{\mathbf{E}}_k = \frac{1}{4\pi\varepsilon\varepsilon_0}\sum_{j=1}^{N}\frac{q_j\underline{\mathbf{r}}_{jk}}{\left|\underline{\mathbf{r}}_{jk}\right|^3} \tag{1}$$

where ε is the relative permittivity of the medium, ε_0 is the permittivity of vacuum, q_j is the charge on atom j, and $\underline{\mathbf{r}}_{jk}$ is the vector from the position of atom j in the enzyme to position k anywhere in the active pocket. The summation is carried out over all N atoms in the enzyme.

In calculating $\underline{\mathbf{E}}_k$ we do not consider full or partial charges on the substrate molecule. These charges are present even when the substrate is outside the active cavity. The electric field calculated through Eq. (1) is in effect an "excess" field caused by the enzyme's active site. i.e. it is the net effect of the enzyme's active site on the atoms that constitute the bond. By disregarding the influence of the substrate molecule, we do not suggest that the substrate molecule does not interact with the active site. Instead, we imply that the effect of the charges of the substrate molecule on the electric field is the same, regardless of is environment. The effect of changes in the spatial arrangement in the substrate molecule is considered negligible for the calculation of the electric field. In all calculations, we set $\varepsilon = 1.0$ as discussed in Appendix.

The electric potential ϕ_k generated by the enzyme molecule at position k is obtained from:

$$\phi_k = \frac{1}{4\pi\varepsilon\varepsilon_0}\sum_{j=1}^{N}\frac{q_j}{|\underline{\mathbf{r}}_{jk}|} \tag{2}$$

where j is an atom in the enzyme. The summation is over all N atoms of the enzyme. While $\underline{\mathbf{E}}_k$ is a vector, ϕ_k is scalar.

We now consider the bond formed by atoms m and n in the substrate in the absence of an external electric field due to the enzyme. Atoms m and n bear partial charges q_m and q_n, respectively. The electrostatic force $\underline{\mathbf{F}}_{mn}$ acting on atom n due to atom m is a vector. This force is calculated from:

$$\underline{\mathbf{F}}_{mn} = \frac{1}{4\pi\varepsilon\varepsilon_0}\frac{q_m q_n \underline{\mathbf{r}}_{mn}}{|\underline{\mathbf{r}}_{mn}|^3} \tag{3}$$

We refer to the absolute value of force $\underline{\mathbf{F}}_{mn}$ as the bond force.

The energy U_{mn} of bond mn in the substrate is calculated as the minimum work necessary to separate the charges on m and n from the bond length to infinity. In the absence of an external electric field, the bond energy is calculated in a molar basis as:

$$U_{mn} = N_{\mathrm{AV}}\int_{|\underline{r}_{mn}|}^{\infty}\underline{\mathbf{F}}_{mn}\cdot \mathrm{d}\underline{\mathbf{r}}_{mn} = \frac{N_{\mathrm{AV}}}{4\pi\varepsilon\varepsilon_0}\frac{q_m q_n}{|\underline{\mathbf{r}}_{mn}|} \tag{4}$$

where N_{AV} is the Avogadro number.

We now consider that bond mn in the substrate is placed in the active cavity of the enzyme. In the presence of the electric field due to the enzyme, the resulting force on atom m of bond mn is:

$$\underline{\mathbf{F}}_m = q_m\underline{\mathbf{E}}_m + \underline{\mathbf{F}}_{mn} \tag{5}$$

A similar equation gives the force on atom n.

The bond energy for bond mn placed in the cavity is the minimum work to separate q_m and q_n from the mn bond length to infinity, but now we must include the influence of the electric potential generated by the enzyme. The bond energy for bond mn placed in the active cavity is:

$$U_{mn} = \frac{N_{\mathrm{AV}}}{4\pi\varepsilon\varepsilon_0}\frac{q_m q_n}{|\underline{\mathbf{r}}_{mn}|} + N_{\mathrm{AV}}(q_m\phi_m + q_n\phi_n) \tag{6}$$

where ϕ_m and ϕ_n are calculated at the positions of atoms m and n, respectively. The influence of the electric potential on the bond energy depends on the bond's orientation.

A specific enzyme may break a specific bond in the substrate, where the terminal atoms in the bond bear partial charges. Two methods are used here to estimate these partial charges. If partial charges for the ends of bond mn are given in one or more published force-field parameter sets, these partial charges are used, provided that the calculated bond energy (Eq. (4)) is close to the average bond energy of similar bonds. Otherwise, partial charges are estimated from Eq. (4) to be in general agreement with the average bond energy of similar bonds. In this case, we use partial charges of the same magnitude but opposite sign. Table 1 summarizes the partial charges used in our calculations.

3. Enzyme Structure and Ionization States

To illustrate the simple model proposed here, three bond-breaking enzymes (hydrolases) were considered: lysozyme, trypsin