Magneto-Mechanical Model of an Enzyme

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Abstract: It has always been difficult to clearly explain to biochemistry students how enzymes work. The idea of enzyme–substrate stereospecificity, active site, coupled reactions, inhibitor or activator effects, mono- and bisubstrate reactions or any other aspect related to the mechanism of action of enzymes are in general abstract and difficult concepts for students taking introductory courses in biochemistry. On the other hand, students are more familiar with mechanical or magnetic objects, and normally they have no problem understanding how they work. Accordingly, a magneto-mechanical model is proposed as a didactic resource to show in class how enzymes can catalyze biochemical reactions. This model is also helpful for introducing the concept of coupled reactions and many other structural and mechanistic aspects of enzyme reactions.

Introduction

In most biochemistry textbooks enzymes are presented after the introductory topic of structural biochemistry, in which the concept of primary, secondary, tertiary and quaternary structures are treated with different degrees of complexity [1–4]. It is assumed that thereafter the students should easily be able to visualize how a biological molecule in general, or proteins in particular, acquire catalytic properties. In practice, however, although students can interpret fairly well three-dimensional structures or spatial-structure domains, they often have problems using these concepts to interpret the mechanism of action of an enzyme. Unfortunately, textbooks do not help much in overcoming this problem. They usually treat the topic more specifically, giving structural and thermodynamic explanations and describing elegant experiments that are more appropriate for advanced students or professors. Consequently, important issues, such as enzymatic active site, for example, are well-understood from a structural standpoint, but the students only poorly comprehend how the active site works and how the whole structure of the enzyme contributes to the catalytic properties of the active site. The same applies to other enzymatic issues, such as coupled reactions, action of inhibitors, activators, or cooperative effects.

Students, on the other hand, can more easily visualize how mechanical devices work than how molecular devices work. Hence, this ability can be used to explain the mode of action of different type of enzymes (e.g., transferases, hydrolases, lyases, isomerases, ligases) by using a simple magneto-mechanical model that is presented in this article.

Model Description

The model consists of a rigid structure that resembles a lever, similar to those studied in elementary physics (also similar to scissors or a pair of pliers). Figure 1 shows schematic representations of the various mechanical devices that can be used to exemplify the resistant core of an enzyme molecule. The structures shown in Figure 1 must have at least two rigid bars (straight or not straight), pivoting (Figure1a and 1b) or swinging (Figure1c) on a fixed point located somewhere in between the bar extremes. By analyzing the structures shown in Figure1 it is easy to visualize that these mechanical devices are based on levers of different classes and that reaction that can occur at the point *r* would depend, therefore, on where and how the action is exerted in a particular structure. The actions expected from the mechanical devices shown in Figures1a and b will yield an opposite effect at the point *r* than the mechanical device depicted in Figure1c, provided that we apply on the three devices opposite forces at the point *p*. In Figure1a and b the action at point *p* provokes the approximation of the sites *r*, the same action in Figure1c separates them. Thus, condensing and synthetic reactions require mechanical devices such as those represented in Figures 1a and b, and hydrolytic reactions require the one depicted in Figure 1c. Although the mechanistic difference between the reactions that can take place with the devices depicted in Figures1a and b may seem subtle, it is recommended that they still be shown because they are useful in showing the structure–function diversity normally observed in biochemical reactions.

The spring force included in the mechanical model at the point *s* (Figure 1) accounts for the elastic energy contained within the enzyme and can be used to explain single-substrate reactions when the substrate reaches the active site or why the enzyme–product complex relaxes after the reaction is completed. It is important to realize that the spring force may have a positive or negative potential energy (with respect to the relaxed status) depending on the type of reaction or mechanical devise used and that energy is utilized to restore the original relaxed conformational state of the enzyme.

The model assumes that the molecules or particles can be represented by magnets that interact with themselves or other surfaces, not only by their magnetic poles, but also by having complementary shapes. The idea of using magnets to mimic molecules is not new and can be used to represent chemical bonds and molecule-binding properties.

The mechanical devices shown in Figure 1 have one or more cavities with special shapes (active or effector sites) where

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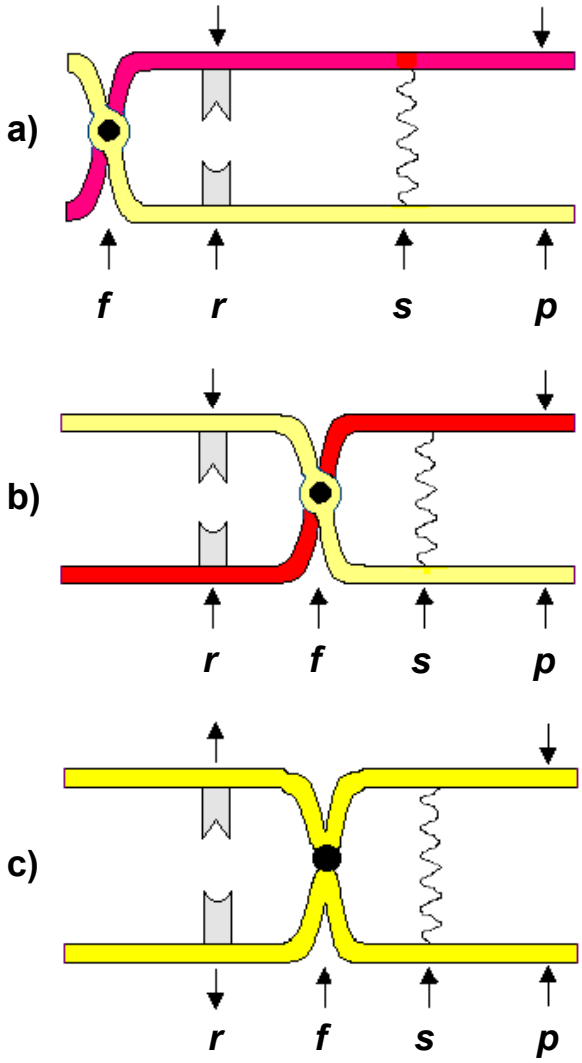

Figure 1: Mechanical devices used to exemplify the rigid structure of an enzyme-resistant core: a. Representative of a pair of pliers based on second-class levers; b. representative of a pair of pliers based on firs-class levers with the levers crossing over at the fulcrum, *f*,(scissors); c. representative of a pair of pliers based on first-class levers with levers swinging at the fulcrum *f*. Symbols denote the force (*p*), the resistance applied to the system (*r*), and the elastic force provided by the spring (*s*).

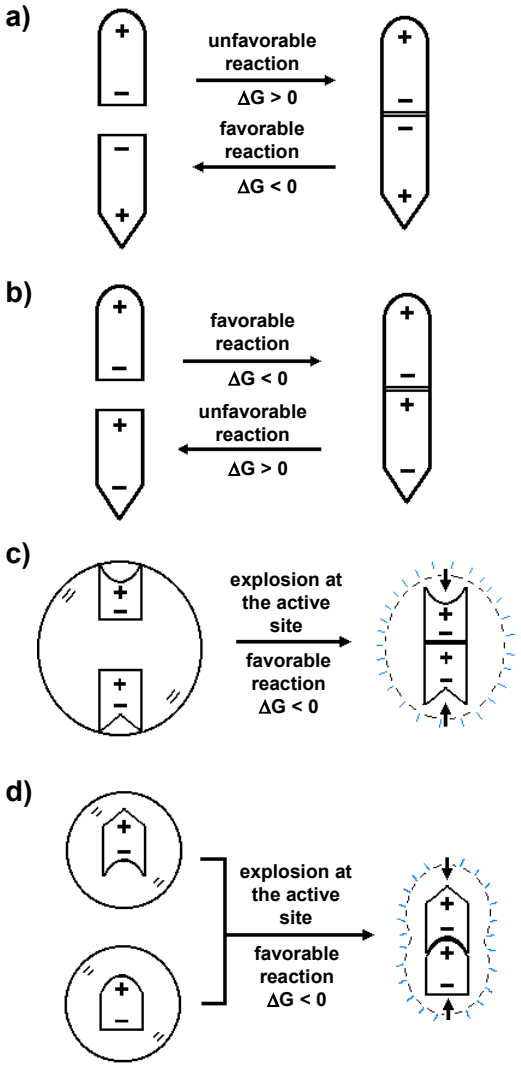

Figure 2. Different magnet arrangements used in the model: a. magnets bound in an unstable array; b) magnets bound in a stable array; c. array of magnets located on the surface of a single bubble; d. magnets included in individual bubbles. Reactions depicted in a and b can represent favorable ($\Delta G < 0$) or unfavorable ($\Delta G > 0$) reactions depending on the direction of the reactions. Reactions depicted in c and d represent favorable reactions ($\Delta G < 0$) that would only take place if the bubble explodes.

only magnetic particles with the complementary shape and opposite magnetic polarity (specific molecules) can reach and bind. The reaction that can take place, therefore, would depend not only on the particular mechanical device selected to model an enzyme, but also on the type of particle involved. Figure 2 shows that these molecules/particles/magnets can be arranged in different ways, so that one can configure or choose the most appropriate set of magnetic particles to explain the particular reaction under analysis. Thereby, thermodynamically favorable ($\Delta G < 0$) and unfavorable ($\Delta G > 0$) reactions can be represented with interacting magnets as depicted in Figure 2. With a pair of magnets, we can show to students that although it is very easy to join them by their opposite magnetic poles (favorable reaction), it is extremely difficult to do so using the same magnetic pole (unfavorable reaction). On the contrary, it is easy to separate magnets if by any means they are connected at the same magnetic pole (favorable reaction) but much harder to separate them if they are connected at their opposite magnetic pole (unfavorable reaction). The model assumes that the magnets are anchored at their poles by a system that allows them to stay bound even when identical magnetic poles are put together; therefore, the mechanical device would be the instrument that provokes the junction or separation of the magnets, and that would mimic the process of the enzymatic formation or break- down of chemical bonds, respectively, and ultimately it would explain how enzymes can help a reaction to occur despite its thermodynamic status. A favorable (spontaneous) hydrolytic reaction can be represented by the process of separation of magnets that are initially bound from their identical poles and the corresponding unfavorable (nonspontaneous) reaction can be represented by the separation of magnets bound from their opposite poles (Figures 2a and b). Likewise, condensing or ligating reactions can be represented by the process of joining the magnets by bringing them

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together from their opposite poles (favorable reaction) or by their identical poles (unfavorable reaction). In this way, a specific enzyme or mechanical device accelerates a hydrolytic reaction, for example, if it can provide the energy (force *p* in the model) necessary to overcome the cohesive force of the anchoring system minus the magnetic repulsive force (in a favorable reaction) or plus the magnetic attractive force between the magnets (in an unfavorable reaction). It is clear, therefore, that for condensing (or ligating) reactions, mechanical devices, such as those shown in Figures1a b would be the correct choice, but for hydrolytic reactions the scheme shown in Figure 1c is correct.

The model also assumes that the magnets do not interact with each other in the medium. Sometimes it may be convenient to include the magnets in insulating bubbles (or balloons) that prevent the magnets from approaching each other and provide a relatively stable structure where the magnets can be fixed in different positions on the surface of the bubbles. The idea of using bubbles comes from the fact that they can easily collapse (or explode) under certain circumstances within the enzyme active site (see Figures 2c, 2d, and 3). The idea of insulating the magnets within a single or in individual bubbles has three advantages: (1) it allows the magnets to interact with each other, (2) it permits the allocation of smaller magnets in different positions (anchored separately) on the surface or within these bubbles, and (3) the bubbles provide a nice example of fragility to mechanical stress and of an irreversible process (Figure 2c and 2d). The model assumes that these bubbles do not explode spontaneously in the medium but only within the enzyme, due to the roughness found at the active site (Figure 3). An interesting property of the model is that it clearly shows how certain structures, such as these bubbles, are stable in the medium but collapse within the enzyme due to particular conditions found in the active sites; and that is precisely the concept that students normally have problems comprehending. The model shows that, because bubbles collapse, the enzymatic action is executed and the reaction takes place, provided that all permissible conditions are established (see Figure 3).

After analyzing many enzymatic samples with the model, students will easily conclude that if the energy needed to performed a particular task is higher than the energy provided by the enzyme, the reaction will not proceed unless another compound comes along to push the reaction in the expected direction. Thus, the concept of coupled reactions comes naturally out of this model.

How the Model Works

Because the model allows us to use various combinations of mechanical devices and magnets arrays, I have chosen a simple example to show how the model can be used in class. The example presented is the following generic coupled reaction that can be assigned to any real reaction carried out by ligase or synthetase:

$$A + B + ATP \rightarrow C + ADP + Pi$$

Some real examples are reactions catalyzed by pyruvate carboxylase, γ-glutamyl cysteine synthetase, glutathion synthetase, glycinamide ribonucleotide (GAR) synthetase, *N*-succinylo-5-aminoimidazole-4-carboxamide ribonucleotide (SAICAR) synthetase, or any other kinase [1, 2].

Figure 3 shows the model representation of the enzymatic steps involved in the reaction synthesis of compound C from the substrates A and B coupled to the hydrolysis of ATP. Single magnets represent substrates A and B, and ATP is represented by a pair of magnets anchored to the surface of a bubble with the polarities orientated as shown in Figure 3. In the proposed reaction, substrates A and B have to bind from their identical (negative) poles, a situation that does not give a spontaneous reaction. On the other hand, the structure assigned to ATP is such that in solution the magnets assigned to ATP will not stick to each other due to the separation imposed by the bubble, but if the bubble explodes, both magnets will stick spontaneously (see Figure 3). The enzyme is represented by a structure of the type presented in Figure 1b, although the structure shown Figure1a also fits well. The model locates the substrate sites for A and B inside the enzyme; they are represented by magnets located in cavities assumed to be the active site *r* (Figure 3). The topological configuration of the active site (shape and magnetic polarity of the magnets) prevents substrates from binding anywhere else within the enzyme structure except at their specific sites.

As shown in Figure 3 the reaction can be visualized in three stages. In the first stage, considered reversible, substrates (including ATP) can separately bind to their specific active sites, and as long as the three substrates do not simultaneously occupy their corresponding site, the reaction will not proceed. As soon as specific substrates and ATP occupy the three sites, however, the second irreversible stage of the enzymatic reaction takes place. Substrates A and B trigger the explosion of the bubble by changing the surface roughness of the site where ATP binds. The magnets of the ATP bubble stick to each other, dragging the whole structure of the "enzyme" to join their "arms," as occurs with a regular pair of pliers. This action provokes the critical approximation of the magnets A and B until they touch each other and get stuck by the anchoring system despite the repulsive force between the magnets. In this stage the irreversibility of ATP hydrolysis is represented by the bubble explosion and the collapse of the magnets contained within. The change of surface roughness at the ATP site can be explain as a distal structural distortion induced by the binding of both substrates (A, B) at their specific sites. This example satisfactorily helps students to understand the concept of substrate-mediated conformational changes and cooperative effects that are unavoidable issues in complex enzyme catalysis.

Once A and B are bound, the system relaxes and releases the reaction products C, ADP, and Pi. This constitutes the last reaction stage that can be considered reversible (Figure 3). At this point, it would be interesting to discuss the concept of the elastic energy, which was introduced in the preceding section as the spring force, *s*, (Figure 1), with the class. Although the spring was not shown in Figure 3 (for the sake of clarity) it is important to stress that the elastic energy is always present, and in this case it can be visualized as the energy contained in a compressed spring. Accordingly, once the reaction is completed and the products are released to the medium, the spring energy is liberated to restore the initial relaxed conformational state of the enzyme.

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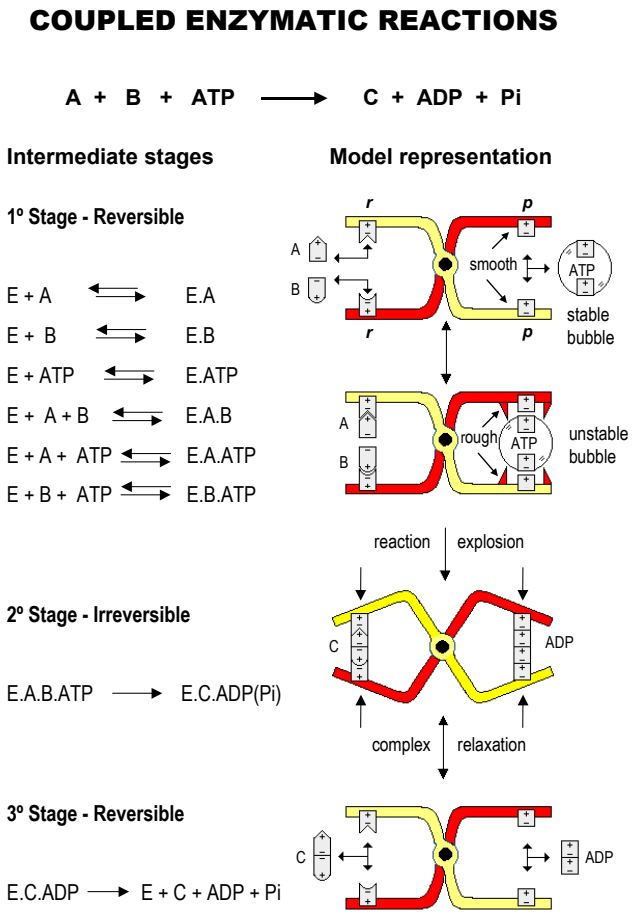

Figure 3: Representation of coupled reactions using the magneto-mechanical model. The reactions consist of the formation of the compound C by the condensation of A and B. The enzyme is represented by mechanical devices corresponding to a pair of pliers based on first-class levers (See Figure1b). Substrates are represented magnets A and B, which bind at the site *r* (active site), and ATP, which binds at site *p*. The first reversible stage (open complex) includes all possible complexes among substrates (A, B, and ATP) and the enzyme (E). The second irreversible stage takes place when the three substrates are located within the enzyme. Under this circumstance the inner surface of the enzyme changes (from smooth to rough) inducing the explosion of the ATP bubble and closing the complex. The relaxed open complex in equilibrium with the newly synthesized C and ADP is represented in the third reversible stage.

Model Limitations

It is important to realize that the model should not be considered as a perfect enzyme simulator. The model fails to simulate detailed realistic enzymatic reactions, does not account for the substrate concentration effect. Furthermore, many enzymatic reactions cannot be fully described using this model.

The model is a mechanical two-dimensional representation used to show how forces are transmitted and utilized to work on magnetic particles located at different positions of the mechanical device. In real enzymes, these forces are transmitted indeed, but through structural three-dimensional motions generated during the catalysis [1–4]. It is also often difficult to introduce the forces involved in the relaxation of the complex after the reaction using the model as presented. This is why it is sometimes convenient to introduce the spring elastic force. Students should be cautioned that the idea of using unstable bubbles to represent high-energy compounds such as ATP, although helpful for purposes of visualizing an enzymatic coupled reaction, by no means corresponds to reality.

Even with these drawbacks, however, the model does give an idea of what is happening within the microenvironment of the active site of an enzyme and how structure, substrate, and effectors contribute to making an enzymatic reaction possible.

Conclusions

The model presented in this paper illustrates, in a comprehensible and interesting way, how an enzyme works and how the intimate catalytic mechanisms occur. It also permits an interesting number of combinations among the mechanical devices and magnet arrays, so it is easy to find an optimal combination that can be used to exemplify an enzymatic reaction. By using the different mechanical devices and magnet sets to mimic different type of enzymatic reactions or reactions carried out by different types of enzymes, students can easily understand and retain basic concepts that are essential in this area of biochemistry. For example, the effect of inhibitors or activators can be introduced simply by incorporating in these mechanical devices other sites where different substrates can bind. The latter would approximate or separate the magnets in the sites *r*, consequently changing the likelihood of encounter or separation among molecules.

The model also permits a clear visualization of the concept of favorable (or spontaneous) and unfavorable (or nonspontaneous) reactions, and the role of the enzyme in facilitating the reaction. Although in the examples cited in this paper, and for the sake of clarity, the spring force has not been included in the analysis, the spring may add to a more complete understanding of the elastic forces involved in enzymatic reactions depending on the particular process modeled or enzyme used.

I have also used this model with high school students taking special courses in biochemistry and the results were excellent.

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