Fundamentals and Applications of Supramolecular Chemistry
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W9L44_Design of Enzyme Mimics

So, hello, everybody. So, now let us continue the discussion. Today we are going to talk about the relevance of intermolecular interactions and non-covalent interactions in supramolecular processes related to biological systems.

And in this regard, we are going to look at a very important classification of compounds which is called enzymes. And we know that enzymes are very important biological molecules that function as very active catalysts. That bring about suitable reactions under the right conditions at extremely fast and accelerated rates.

So, enzymes are essentially the workhorses of molecular systems where they catalyze different chemical processes with unprecedented precision at very fast rates. And the ability to carry out this process is millions of times greater, without any loss in activity, and these enzymes function under physiological pH and room temperature conditions.

So, this makes naturally occurring enzymes extremely efficient. And so, what the chemists were thinking is whether we can actually make some systems, some specific systems where the chemical reactions performed in such systems model the enzymatic processes, and, whether we are able to replicate the same level of efficiency that occurs in a naturally occurring enzyme.

So, with this background in mind, a chemist got into the process of making specific molecules. For example, we have a biological receptor, and then we have a substrate molecule that is going to bind to the biological receptor, and then there is going to be a transition state, like a situation created where the reacting molecules come close to each other, recognize each other, the chemical reaction takes place, the products are formed, and then the products are again released back into the solution.

This process, where product formation takes place at unprecedented rates, is what makes these biological workhouses extremely efficient. So, chemists came up with the idea of trying to design enzyme mimics where we have, say, an enzyme that has a particular shape, and then we have, say, two reactant molecules, okay.

For example, we have two reactant molecules, A and B, and the idea is that they interact. These are called the substrates, and this is the enzyme, which is called a receptor, and now the enzyme is going to interact with the substrate via non-covalent interactions.

There is a slight conformational change to accommodate A and B. Then there is a chemical reaction between A and B to form D and C, and finally, decomplexation takes place. This is the complexation process; this is the reaction site, and at the reaction site, we can see that the A and B molecules come very close to each other and create a transition state-like situation.

So, essentially, they mimic the transition state such that the reacting partners are extremely close to each other, the chemical reaction takes place, and then the products C and D are released into the bulk. And this is what is going to be the topic of the current discussion: we are going to look at enzyme mimics.

And before we go on to that, let us look at the fact that we are trying to develop a structural model that mimics enzymatic activity. The reason for the enhanced enzymatic activity is the lowering of the activation barrier, which is a free energy associated with the process to a pretty reduced value, such that the reaction rates can now happen very fast and the reaction can take place in the shortest possible time. So, we need to mimic enzyme activity, and we need to develop structural models for that.

However, we need to keep in mind that naturally occurring enzymes, which function very efficiently, now have very precise structural binding and catalytic features. It is not easy, or in other words, very difficult to incorporate all the structural binding and catalytic features into a given structural model, and that is where chemists have been trying to design suitable structural models that can mimic enzyme activity, and some of the most important features of enzymes, as I told you, are fast reaction rates under mild conditions.

That is unlike traditional organic reactions where you can employ very harsh conditions, such as high temperatures and stoichiometric amounts of catalyst, and use reagents to bring about transformations, yet the rates may not be fast. The reaction can still be sluggish or proceed at moderate rates. Enzymes have fast reaction rates in mild conditions.

There is a very high degree of structural recognition of their substrates. So, you can see here that although this particular region did not really have the right match for this particular substrate, there is now a change in the conformation of the enzyme to accommodate this reacting molecule.

So that the structural recognition that occurs between the enzyme and the B reactant happens with the highest degree of molecular recognition. The third factor, as I told you,

is that there is a very high turnover number. So, these enzymes catalyze the reaction of a large number of substrate molecules with high precision without being destroyed; thus, a very high turnover number and a large number of substrate molecules are catalyzed at very high levels of precision without being destroyed.

There is also another important process that operates: competitive inhibition by compounds that bind to the enzyme but do not react themselves. So, this is also a parallel process which operates; that is, you can sometimes have a substrate that is able to bind to the active site of the enzyme but actually does not have the necessary predisposition of the functional groups to react with the other reacting partner.

So this particular competition, which can actually inhibit the enzymatic activity, also operates in parallel in the case of enzymatic processes. So, this factor also has to be kept in mind. Now, when we need to have the design of the structural model, what are the prerequisites for the structural model? And to start with the structural model, it is analogous to the transition state of a biological reaction when the substrate binding occurs with the system of interest.

An intermolecular system that carries out the reaction concerned without the binding characteristics of the enzyme is called the functional model. So, there is a functional aspect, and there is a structural aspect. These aspects are very important when it comes to the design of the requisite structural model.

And also we need to keep in mind that, unlike biologically occurring natural enzymes, where the rate of the reactions is extremely fast and they occur in a highly efficient manner, the biological mimics or structural mimics that we are going to make to model these biological processes will definitely happen much more slowly in comparison to that of a natural enzyme with a substrate.

Furthermore, what we should have is the hydrophobic binding site for the substrate, hydrogen bonding, or electrostatic interactions; that is, the binding sites that are complementary to the substrate.

Catalytic groups must be attached to this model. The structure must be well-defined and rigid, and finally, the model must be water-soluble; this is extremely important and catalytically active under physiological conditions. So these are the most important properties that must be present in the structural model, which are mentioned here, and the last five are extremely important.

We must have suitable hydrophobic sites, electrostatic binding sites, catalytic groups that are pretty rigid and have a pre-defined structure, and they must be water-soluble and catalytically active under physiological conditions. So, with this background in mind, let

us try to look at some of the relevant examples that are present, and in general, we would like to emphasize the fact that now, when you have got the enzyme that recognizes the starting materials, the reactants.

So, the entire reaction process has to happen essentially within this unimolecular species, which now contains the reactant molecules as well as the enzyme itself. So, overall, there is one large molecule that positions the reacting species in close proximity so that the chemical reaction takes place, products form, and then the decomplexation happens, such that the final products are now released into the bulk.

So, in this regard, it is the intramolecular process that takes place with the enzyme which is known to proceed with greater selectivity compared to the intermolecular processes. So, let us now emphasize the fact that intramolecular reactions proceed faster than their biomolecular equivalents. For example, let us consider this reaction.

So, here we are going to have a nucleophilic attack at the carbonyl center, and then this takes place with the rate constant k1, which is going to form this product; plus, say we have an X here, and then the hydrolysis of this is going to give me the dicarboxylic acid.

On the contrary, if this is an intermolecular process and if you look at the intermolecular reaction, then we will have O C O C S 3 plus O minus X, and the hydrolysis of this is going to give me two equivalents of acetic acid.

So, compared to the intramolecular, the intermolecular reaction gives two equivalents, whereas the intramolecular reaction gives one equivalent of the dicarboxylic acid. So, if this is k2, then k1 is much greater than k2; that means the intramolecular reaction proceeds at a much faster rate than the intermolecular processes. And in this regard, the first class of compounds that have actually been used as enzyme mimics is cyclodextrins.

So we will first look at cyclodextrins as esterase mimics. And in this regard, the observation is as follows: The observation is that the rate of hydrolysis of para nitrophenyl esters is increased by 750,000 by beta cyclodextrin. So, now you can appreciate that when we discussed cyclodextrin, it was an important class of molecules, and now people are trying to use cyclodextrin as biological mimics or enzyme mimics. And the first observation was that the rate of hydrolysis of para nitrophenyl esters is increased by 750,000 in the presence of complexing it with beta cyclodextrin. So, let us look at this process more carefully.

So, what has been observed is that we have this O minus here, and we have NO2 and COMe. And this is the rate constant in the presence of cyclodextrin, and it gives me this product. So, this is what is formed. So, we have this reaction here. It goes here, goes here,

it comes back, and this goes out, plus the phenolate ion, and then hydrolysis of this gives me the MeCO2H, plus my cyclodextrin back.

And I compare this now with the rate constant for this particular hydrolysis to give me the OHNO2 plus MeCO2H. This is now in the presence of water, so this will give me the corresponding cyclodextrin, and now what is observed is that kCD by k is 100 when the cyclodextrin has been employed. So, there is a rate enhancement for this particular ester, where the ratio is approximately 100. How does this reaction actually take place?

What has been shown to you here is that first we have the nucleophilic attack of O minus on this ester moiety, and then it forms the OCO OCO R group, followed by hydrolysis to give you the final product. And during the course of this reaction, what happens is that this particular species, when it reacts, you have the O- here, and now you have got the -COOR.

So, the nitro group is here. You can see here that this is a constrained situation because the ester carbonyl group is in the plane, and you have the O- on this side, and now there has to be a nucleophilic attack from O- at the carbonyl center.

So, it has to approach from the back to the front, hit the carbonyl carbon, and then form a tetrahedral intermediate. This entire process is taking place within the cyclodextrin. So, it forms this kind of intermediate.

So, this tetrahedral intermediate is formed, and now what has to happen is that there has to be a transfer of the acyl group. So, this is going to form O-CO-R and release the O minus NO2. So, this particular process has to take place now.

So, we now have the acylation which is happening here and when the acylation happens then the para nitrophenolate group will be now released. So, we see these interesting processes taking place.

Now, what has been observed is that this step is closely related to the action of serine protease enzymes, in which an acyl group is transferred to serine OH in the first step. So, we can see this. I think there is an O here. Now, when it comes here, there is oxygen here. The step is closely related to the action of serine proteases, in which an acyl group is transferred to serine OH in the first step.

So, you can see this kind of rate enhancement when cyclodextrin is used. The second example I would like to focus on here is that example 2 is adenylate cyclase phosphodiesterase activity by cyclodextrin complexes. So, let me write down the chemical scheme for this process first. So, we have the O minus P = O, O minus.

So, we have this 5-membered ring. Here is the adenine. This is the adenine part, this is the sugar part, so we have the OH. And this will give first, in the presence of beta cyclodextrin and (promethium) Pr3+, which is a lanthanide. It will first attack here, so it will form this particular product O-P-O-H-O and a double bond O. So, it will form this 1, 2, 3, 4, 5, 6, 7-membered ring, 1, 2, 3, 4, 5, 6-membered ring, and it will form that.

And then here we have the OH; this is the adenine here, plus P2O7 4-. So, this species is referred to as cyclic adenosine monophosphate (cAMP), and this is the first stage of the process. Then we will have step 2, which is the hydrolysis that is done in the presence of gamma cyclodextrin and cerium (IV). So, this is step 1, this is step 2, and this gives the product. I am labeling it as A, and this gives the product.

I am labeling it as product B. So, what happens is that this AMP molecule, C-AMP molecule, is actually the cell-to-cell messenger, which is called the second messenger in biology. And there are two steps, as you can see here: the first step is catalyzed by adenylate cyclase, and the second step is catalyzed by phosphodiesterase.

So, when the cell receives a stimulus and that is the first messenger, whenever the first stimulus is received, compound A is formed. And this compound A is called CAMP; this compound is formed from ATP, which is adenosine triphosphate. So, what does CMP do now? It activates other intracellular enzymes to produce a cell response in step 2.

This response, which was triggered by CMP, is terminated by the hydrolysis of CMP by phosphodiesterase, which is a phosphate ester hydrolyzing enzyme. So, these are the two important steps which take place and these steps now are mimicked in a synthetic fashion as follows. So, step one is mimicked by a combination of beta-CD and Pr3+, and the second step is catalyzed by gamma-CD and Ce4+. And this is because the cyclodextrin, along with the lanthanide, is a hard oxophilic species that catalyzes the overall process.

That is what has been proposed. CD, along with this lanthanide, has these hard metal-oxygen interactions. And overall, the species catalyzes step 1 and step 2. The results are surprisingly interesting. At physiological pH and at 30 degrees centigrade, the half-life for the conversion of A to B is only 6 seconds, which, when compared to the uncatalyzed reaction, is 30,000 years. So now the time has dropped to only 6 seconds compared to the uncatalyzed half-life of 30,000 years.

This process of hydrolysis, which is the key step as well, takes place in a very short time. And this is what was achieved now, at physiological pH and at room temperature. If you go back to the previous step, the termination of this activation process is done by the hydrolysis of CMP by this phosphate ester hydrolyzing enzyme, which now takes place within only 6 seconds and therefore enhances the efficiency of the process. And we will

take another example to understand the relevance of biological mimics; here we will again consider cyclodextrin as glyoxalase mimics,which involves the isomerization of alpha-keto aldehyde to hydroxy acids through the involvement of the thiolate moiety.

So, let us see how this process is mimicked by a beta cyclodextrin having an amino ethanethiol group on its primary face.

So, let us see this process now. We have now got a cyclodextrin, okay. We have now got this process here. So, this is my alpha-keto aldehyde. We have this particular species here.

This is now my 2-naphthyl glyoxal. So, we now have the cyclodextrin. You can see here it is now positioned, and this is the primary face; now what we can have is the abstraction of the proton here. So, we have this reduction now, which has happened, and then in the next step, we will do a water wash, which will take out the corresponding acid, and this is the hydroxy acid.

So now you can see that this is the very important role that is played here by this particular cyclodextrin, catalyzing this particular reaction. So, again we can see that cyclodextrin can function as glyoxylase mimics, where it can actually lead to the isomerization of this keto aldehyde to the corresponding alpha hydroxy acid.

So, overall, we see in this particular lecture that we are able to design enzyme mimics that can bring about rates of chemical reactions by templating them at very specific sites on cyclodextrin, which mimics enzyme-like activity and achieves unprecedented rates, achieving the desired reaction in less time, and the product that is formed is also produced in high yields.

So, with this, we come to the end of this lecture, and in the next lecture, we will take up further studies on related systems.

Thank you.