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CYCLODEXTRIN DERIVATIVES AS MIMICS OF ENZYMES AND ANTIBODIES

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The cyclodextrin derivatives as enzyme mimics and imitators of antigens binding by antibodies are reviewed. The process of anisole bound into cyclodextrin in water and then catalytically chlorinated to produce only the p-chloroanisole product was the first catalytic enzyme mimic described as "artificial enzyme", where the simple cyclodextrin acted as an enzyme mimic and was more selective than the enzyme chlorinase. The cyclodextrins as artificial enzymes have been constructed for the transformation by which amino acids were biosynthesized, particularly phenylalanine and tryptophane, as well as for the cyclization and hydrolysis of RNA. The high hydrolysis rates and selectivity by the geometry of the enzyme-substrate complex can be achieved by using of cyclodextrins as artificial enzymes. The highly available cyclodextrins are an important start in the field of biomimetic chemistry.

Рассматриваются вопросы имитаторов ферментов, а также имитаторов связывающих антигены с антителами в качестве производных циклодекстринов. Первым имитатором каталитического фермента был процесс связывания анизола с циклодекстрином в воде и далее каталитического хлорирования для получения только подукта р-хлороанизола, описанный как "искуственный фермент", где простой циклодекстрин является имитатором фермента и действует более избирательно, чем фермент хлориназа. Сконструированы искуственные ферменты для трансформации с целью получения аминокислот, в частности фенилаланина и триптофана, а также для циклизации и гидролиза РНК. Высокие скорости гидролиза и селективность достигаются по геометрическим показателям фермент-субстратного комплекса с использованием циклодекстринов в качестве искуственных ферментов. Имеющиеся циклодекстрины позволяют сделать важный шаг в области биомиметической химии.

Քննարկվում են ցիկլոդեքստրինների ածանցյալները որպես ֆերմենտների նմանակիչների, ինչպես նաև որպես հակագենների հետ հակամարմինների կապման նմանակիչների վերաբերյալ խնդիրները: Ցիկլոդեքստրինի հետ անիզոլի կապակցման պրոցեսը ջրում և հետագայում կատալիտիկ քլորացումը միայն p-քլորոանիզոլի ստացմամբ, եղել է կատալիտիկ ֆերմենտի առաջին նմանակիչը (իմիտատորը), նկարագրված որպես «արհեստական ֆերմենտ», երբ սովորական ցիկլոդեքստրինը դառնում է ֆերմենտի նմանակիչ և ազդում է առավել ընտրողաբար, քան քրոլինազ ֆերմենտը: Ստեղծվել են արհեստական ֆերմենտներ տրանսֆորմացիայի միջոցով ամինաթթուների կենսասինթեզի, ինչպես նաև ՌՆԹ-ի ցիկլիզացիայի և հիդրոլիզի համար: Օգտագործելով ցիկլոդեքստրինները որպես արհեստական ֆերմենտներ կարելի է հասնել հիդրոլիզի բարձր արագությունների և ընտրողականության, շնորհիվ ֆերմենտ-սուբստրատային համալիրի երկրաչափական ցուցանիշների: Առկա ցիկլոդեքստրինները թույլ են տալիս կատարել կարևոր առաջընթաց բիոմիմետիկ քիմիայի բնագավառում:

The field of Biomimetic Chemistry has grown in interest ever since I first used this name in the literature [1]. Although mimics of enzymes include various partial mimics that use coenzyme derivatives and other catalytic groups, sometimes in intramolecular reactions, the most interesting enzyme mimics are those that bind a substrate, perform a catalytic process, and then release the products. There is also particular interest in enzyme mimics that function in water solution, among other reasons because water is an environmentally

benign solvent. Thus cyclodextrins are quite appealing as components of enzyme mimics, since they reversibly bind hydrophobic species in a reasonably well-defined geometry, and the binding is sufficiently reversible that the products can be released.

Cyclodextrin binding can also be considered as biomimetic in itself, imitating the binding of antigens by antibodies. However, simple cyclodextrin binding is often quite weak. For example, in water the hydrophobic sidechain of phenylalanine leads to a binding constant of only 20 M^{-1} or so, while antibodies bind complementary antigens with association constants ranging from 10^4 M^{-1} up to 10^{15} M^{-1}, and perhaps even higher. For this reason, we and others have examined the binding by dimers and even trimers and tetramers of cyclodextrins. In an appropriate case, where the cyclodextrin dimer is joined by two linking strands so that it is held in the correct geometry for substrate binding, we observed an association constant greater than 10^{11} M^{-1} for the correct substrate, while a close analog with not quite the right geometry lost a factor of at least 4000 in binding [2].

In this brief comment it will not be possible to describe all the work that has been done in this very large field. However, we have reviewed cyclodextrin derivatives as enzyme mimics very recently [3], so we will try just to indicate some of the high points in the development of this area.

The impetus for the field came from a publication by Cramer [4], describing his work and that of his predecessors on inclusion compounds in water between cyclodextrins and hydrophobic substrates. This stimulated our work, and at the same time Bender carried out some interesting studies showing that reactions of bound substrates with the cyclodextrins could be understood in geometric terms [5]. His reactions were not catalytic. We examined the first catalytic enzyme mimic, in which anisole bound into cycldextrin in water and then was catalytically chlorinated to produce only the p-chloroanisole product [6, 7]. The binding promoted a process in which the chlorine atom was passed from a hydroxyl group of the cyclodextrin to the accessible para position of the bound anisole, but not the otherwise reactive ortho position that was inaccessible in the complex. There was catalytic turnover as the product released from the catalyst and was replaced by more substrate.

In this process simple cyclodextrin was acting as an enzyme mimic, and indeed it was more selective than is the enzyme chlorinase that also catalyzes the chlorination of anisole--in the enzyme case to a mixture of ortho and para chlorinated products. However, better enzyme mimics need not just binding near a cyclodextrin hydroxyl group, but binding near other catalytic groups. Thus we incorporated a metal binding ligand into the cyclodextrin, and saw that the resulting metal complex would catalyze the hydrolysis of substrate esters that bound to the cyclodextrin [8]. This was the first catalyst that was described in the chemical literature as an "artificial enzyme."

Many enzymes use coenzymes, not just simple catalytic groups. The first example in an artificial enzyme was our compound combining pyridoxamine with cyclodextrin [9]. We saw that this compound could selectively bind ketoacids with hydrophobic sidechains and convert them to amino acids, in particular to phenylalanine and tryptophan. This mimics the transamination by which amino acids are biosynthesized. Subsequently we and others have greatly expanded such examples, adding rigidity to the molecules and additional catalytic groups besides the pyridoxal [3].

Many enzymes use bifunctional catalysis, in which two catalytic groups cooperate in transforming a bound substrate. We constructed an artificial enzyme [10] that mimicked the enzyme ribonuclease A, which uses two histidine sidechains as important catalytic units in the cyclization and then hydrolysis of RNA. Our molecule had two attached imidazoles, the catalytic part of the histidines in ribonuclease, and we saw that their placement was critical in achieving catalysis [11]. We even saw that our catalyst exhibited isotope effects

very much like those of the enzyme [12], and suggested that the enzyme uses a chemical mechanism very much like the one we demonstrated for our artificial enzyme [13].

Artificial enzymes are interesting if they achieve very fast rates of reactions as a result of binding the substrate and catalyzing some process in it, and indeed we have examples in which cyclodextrin-based catalysts can achieve very high hydrolysis rates, for instance [14]. However, in many respects the selectivities of enzyme-catalyzed reactions are more striking, and more important to imitate.

Consider the contrast between selectivity in organic chemistry and selectivity in biochemistry. In organic chemistry, reactions occur at the most reactive parts of the substrate, directed by the intrinsic reactivity of the functional groups. For example, if we want to reduce a ketone there must not be an aldehyde group present, which is more reactive. Similarly, if we want to oxidize a carbon of a substrate it must be the most reactive carbon, for instance part of a double bond. By contrast, in enzymatic reactions the selectivity is directed by the geometry of the enzyme-substrate complex, which frequently overrides the intrinsic reactivity of the substrate.

As an example, in the biosynthesis of cholesterol enzymes oxidize methyl groups attached to saturated carbons of lanosterol, ignoring the much more reactive two double bonds and a secondary alcohol group. This is possible because the oxidizing group in the enzyme--an oxygen atom attached to the iron atom of heme--can reach the methyl groups but not the more reactive double bonds or alcohol carbon. Recently we have succeeded in imitating this geometric control, "liberating organic chemistry from the tyranny of functional groups." The catalysts we have prepared use cyclodextrins to bind the substrate in a well-defined geometry, and attack unactivated C-H bonds with an oxygen atom on a metalloporphyrin, just as in the enzyme group cytochrome P-450.

Our systems use a manganese derivative of a porphyrin, since this is more effective than the iron derivatives that are modeled on the enzymes themselves. In our earliest example [15] we used a tetraphenylporphyrin derivative carrying four cyclodextrin units, and achieved turnover catalysis of the hydroxylation of a steroid at a single unactivated methylene group. Furthermore, the product secondary alcohol was not oxidized to the ketone, for geometric reasons, even though such alcohols are more reactive than are unactivated methyene groups toward random oxidants. The geometry of the substrate-catalyst complex, and of the product alcohol-catalyst, do not permit attach on the C-H bond of the alcohol group, which is necessary for its oxidation to a ketone.

In later work we were able to improve the turnover capacity of our artificial cyctlochrome P-450 by incorporating fluorine atoms to make the catalyst more stable to oxidation [16]. In recent unpublished work we have raised the turnover numbers for a newer version of our catalyst to as much as 3000, again with complete positional and stereochemical product control, dictated by the geometry of the catalyst-substrate complex;.

In further work, we were able to shift the oxidation of the same substrate type to a new position by using three-point binding of the substrate to three cyclodextrin units in the catalyst [17]. Furthermore, in unpublished work we have seen that a carbon-carbon double bond in the substrate is not attacked when the binding geometry puts it out of reach of the manganese-oxygen group. Thus using cyclodextrins we have indeed achieved an important advance in biomimetic chemistry using artificial enzymes--overcoming the intrinsic reactivity of the substrate by imposing geometric control in a complex that mimics the enzyme-substrate complex in biochemistry.

In this most recent work the cyclodextrins were used as binding units to help achieve strong complexing with well-defined geometry. Of course other binding units can also be envisioned, which may ultimately have some advantages. However, the highly available

cyclodextrins have let us make an important start in the field of biomimetic chemistry, in which we hope to utilize the same principal by which enzymes achieve their selectivity. Cyclodextrins have also let us perform these selective transformations in water solution, and thus add an environmental advantage to their other features. We want to change the style of organic chemistry, and to achieve transformations that are otherwise impossible when using previous chemical styles. Cyclodextrins have helped open up this new field of chemistry.

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