Reviews

Catalytic antibodies as a new generation of biocatalysts*

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The present state and perspectives of the creation of a new generation of biocatalysts, catalytic antibodies (CA), and their application to organic synthesis are discussed. The problems to be solved for the development of the practical application of CA are especially noted.

Key words: catalytic antibodies, biosynthesis and application for organic synthesis; antigens (haptens).

The fact that organic synthesis is one of the most important directions in science and engineering is common knowledge. Along with purely chemical approaches, biosynthetic methods, which use natural enzymes at specific steps of complex chemical processes, have quickly come into practice during the last decade. The advantage of enzymes in directed chemical transformations is their very high selectivity toward the parts of molecules or the separate functional groups and chemical bonds that take part in a reaction (regiospecificity) and toward the steric orientation of a process (stereospecificity). The use of enzymes decreases side reactions to a minimum or excludes them, and allows one to carry out processes rapidly and efficiently and to achieve high yields of target products. Therefore, enzymatic and chemical-enzymatic synthesis is becoming a successfull laboratory and industrial practice.

Wide application of enzymatic methods is impeded by the fact that every reaction requires a separate enzyme with the necessary regio- and stereospecificity, and these enzymes must be found and obtained in sufficient amounts from natural sources. Laborious investigations are normally required for this purpose and a very common complication is the difficulty of obtaining an enzyme source.

In this context, a long-standing dream of investigators is the preparation in the laboratory of artificial enzymes with the properties they desire, primarily, the specificity of action that is the main requirement for a biocatalyst used in organic synthesis.

The direct solution of the problem might be chemical synthesis of the corresponding enzyme, which is a protein with a definite sequence of a large number of amino acids. Modern methods of chemical synthesis in principle allow one to carry out such a synthesis but this approach is not effective for practical purposes since it is extremely laborious and requires preliminary knowledge of the exact enzyme structure. About 10 years ago, more practical ways to obtain protein molecules possessing catalytic activity with a predetermined specificity were

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found.[1,2] These methods involve biosynthesis of protein molecules by the powerful instrument of the animal immune system intended to produce the protective proteins, antibodies. The so-called catalytic antibodies (CA) obtained by this procedure make possible a new generation of biocatalysts. Nowadays this area is one of the "hot spots" at the junction of chemistry and biology. It is already possible to obtain biocatalysts that accelerate a series of known reactions whose efficiency in some cases is close to that of natural enzymes.[3] More important is the fact that CA are able to catalyze chemical reactions for which natural enzymes are unknown, and, moreover, CA able to carry out chemical transformations that are impossible using known reactions without participation of CA, have been found. Although this area is just begun to develop and only the main fundamental problems have become clear, the possibilities of the practical application of CA can already be seen. It is obvious that the use of CA can significantly change the methodology of organic synthesis.

The chemical aspects of the problem and the possibility of using CA for fine organic synthesis are only briefly considered in this review. The complex problems of immunochemistry and enzyme kinetics, which are also of great interest, are not discussed either.

A natural enzyme is a high-molecular weight protein that contains an active center made up of a definite combination of amino acids that form a spatial cavity. The transformation of a substrate molecule occurs in this cavity. The catalytic effect of an enzyme, or, more exactly, its active center, is due to the fact that the transformation of a substrate molecule into a transition state is made easier in the active center. This is due to the steric and electronic correspondence (complementarity) of the active center cavity of the enzyme and the transition state configuration, which leads to stabilization of the latter and a decrease in its free energy. As a result, the energy barrier at the reaction coordinate is decreased and the rate of the transformation is increased.

In addition, the restriction of the mobility of the substrate molecule that occurs during the formation of its complex in the enzyme active center causes an advantage in entropy, favoring the reaction, while the presence of charged groups in the active center promotes the transformation of the substrate into a final product due to general acid-base or nucleophilic catalysis.

Thus, for a protein molecule to gain catalytic properties, a fragment able to perform all the functions of the catalytic center mentioned above, and able to stabilize the transition state must be formed inside the molecule. The generation of such a protein molecule by biosynthesis in an immune system was the main idea for the development of the method of the production of catalytic antibodies.

To make it a little simpler, the essence of the process is as follows.[3] When a foreign substance (antigen) is introduced into an animal, the animal's immune system "neutralizes" the substance by synthesizing a group of proteins belonging to the class of immunoglobulins (antibodies). The active centers of these proteins are formed in such a way that their three-dimensional structure corresponds to that of the active group of the antigen (called the hapten).* Hence, if an antigen, in which the space and electron structure of the hapten group mimics the transition state of the transformation of the substrate, is introduced into an organism, the immune apparatus synthesizes a protein whose active center favors the formation of the transition state, *i.e.*, a protein with catalytic activity. It should be stressed that the most important condition for the success of this exercise is the right choice of the hapten structure, and it is necessary to know (or to make a well-reasoned assumption about) the structure of the transition state of the reaction under study.

The scheme of the preparation of CA may be presented as follows. On the basis of data about the transition state of the reaction under study, a hapten mimicing the transition state is designed and prepared using the methods of organic synthesis. The hapten molecule must contain groups that correspond to the groups of the substrate structure in order to provide the forming antibody with the ability to "recognize" the substrate. After that, the hapten is transformed by immobilization on some protein into a high-molecular weight antigen able to cause the formation of antibodies. For this purpose, the hapten must contain an aliphatic chain with an active group (for example, carboxylic, called a spacer), by means of which the hapten is connected with one of the amino groups of the protein molecule. The antigen is administered to an animal, usually mouse, and after the usual immunization procedure, a group of monoclonal antibodies is isolated using the well-known hybridome technology.**[4] Antibodies are generated not only against a hapten fragment but also against other parts of the antigen molecule. Therefore, it is necessary to select antibodies that are complementary to the hapten fragment and, as a consequence, can mimic the transition state of the reaction under study and exhibit catalytic activity. The ability of antibodies, in which the structure of the active center is complementary to the administered hapten, to form a sufficiently stable complex with the hapten is used for this purpose. This is the basis for the selection of catalytically active antibodies by immunoenzyme analysis. Immunization usually affords several CA that differ in activity; the most efficient of them is selected for detailed study and application.

This scheme can be illustrated by the generation of CA that catalyze the hydrolysis of esters similarly to natural enzymes, esterases. By the way, these CA were shown to be the first synthetic biocatalysts.

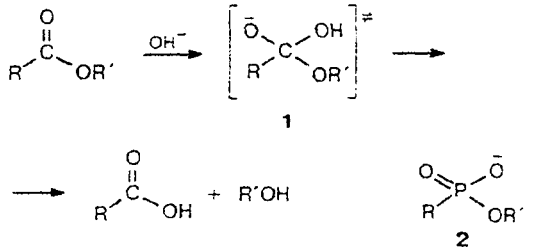
It is known that the transition state **1** (Scheme 1) that appears in the hydrolysis of an ester is a tetrahedral

* Antigens are high-molecular weight compounds; groups that determine the specificity of their structure are called haptens.

** Monoclonal antibodies are produced by one clone of cells that are antibody producers and differ from polyclonal antibodies in that they are significantly less heterogeneous.

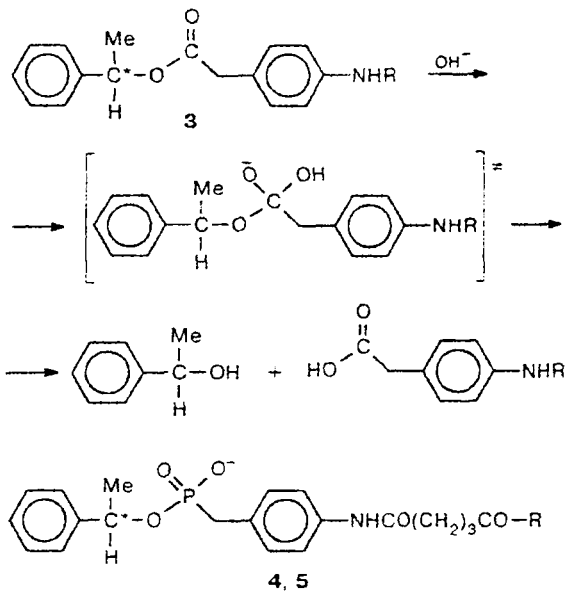
structure carrying a negative charge. The transition state cannot be obtained as a stable compound and therefore it is mimiced by the molecule having the most similar structure; this molecule serves further as a hapten to generate CA of given specificity. Derivatives of phosphoric (or phosphonic) acid **2** were chosen for this role since, like structure **1**, they contain a central tetrahedral atom and carry a negative charge.[1,2] For example, for the preparation of CA catalyzing the hydrolysis of *para*-substituted phenylacetates of α-methylbenzyl alcohols **3**,[5,6] haptene **4** (Scheme 2), whose structure is similar to that of the substrate but differs from the latter by the presence of the phosphoryl group in place of the carbonyl group, was used. Hapten **4** contains groups corresponding to those of substrate **3** and also a glutaric acid residue, whose carboxylic group links hapten **4** to the protein to form the high-molecular weight artificial antigen **5**.

Scheme 1



R = Ar, OR; R′ = Alk

Scheme 2



R = OH (**4**), protein residue (**5**)

Standard immunization of mice with antigen **5** followed by the application of hybridome technology resulted in the isolation of several CA. The most efficient of these possessed properties typical of the esterase enzyme, *i.e.*, it accelerated hydrolysis of compounds **3** by 10^5 times, the kinetics of the hydrolysis corresponded to the Michaelis—Menten dependence (indicating the formation of an enzyme-substrate complex as in the case with the natural enzyme), and the pH-dependence of its activity was typical of esterases. The activity of the isolated CA was reduced or completely lost after treatment with typical protein modifiers such as tetranitromethane, diethyl pyrocarbonate, *etc.*

The most important characteristic of CA is their specificity which is the main and the most valuable property of natural enzymes. It was found that CA are characterized by high regiospecificity: changing the structure of ester **3** in its alcoholic or acidic region resulted in a dramatic decrease or the total disappearance of the catalytic activity of the CA. The high stereospecificity of the obtained artificial enzyme is especially significant. Ester **3** contains a chiral center, the asymmetric carbon atom. If hapten **4**, which also contains a chiral center, is used as a racemate, immunization affords a mixture of antibodies containing CA that split either the (*R*)-isomer or the (*S*)-isomer of ester **3**. These CA with different selectivities can be isolated and used separately for hydrolysis of isomers with different configurations. Thus, each of the enantiomers of the hapten generates its own CA with stereospecificity corresponding to the steric structure of the enantiomer. When haptens are used as pure stereoisomers, CA with given stereospecificity can be directly prepared. Such CA have already been used for the kinetic separation of a mixture of stereoisomers, for example, of isomeric fluorine-containing alcohols.[7]

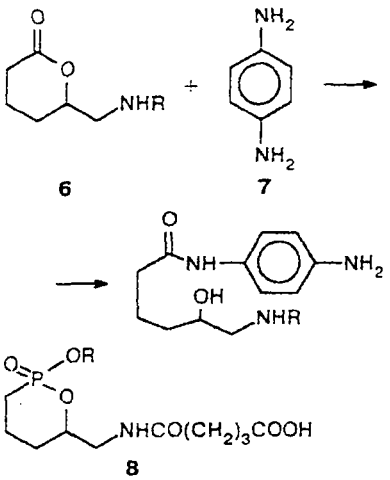
At present, more than ten CA that accelerate the hydrolysis of esters by several orders of magnitude with high stereospecificity and are in some cases comparable to natural enzymes in efficiency, are known. This opens the way to the solution of problems of fine organic synthesis. For example, one CA obtained is able to selectively remove identical ester groups located at different parts of a molecule (such as acetyl groups in monosaccharide polyacetate).[8] This process is very difficult to carry out using classic chemical methods.

Significant efforts have been undertaken to obtain CA catalyzing the hydrolysis of amide bonds, which is related to the solution of one of the most important problems of bioorganic chemistry, the selective cleavage of a protein chain. An organophosphorus analog, containing a group with a phosphamide bond instead of a carboxamide bond, has also been used as a hapten simulating the transition state of this reaction. The CA prepared by this method accelerated the hydrolysis of the simplest amides[9] but appeared to be insufficiently efficient for protein cleavage. To generate more active CA, haptens containing a metal ion were used.[10] The

CA generated against these haptens catalyze the cleavage of peptide bonds but at this time they are not used.

In addition to enzymes capable of cleaving ester or amide bonds, enzymes favoring the reverse reaction, *i.e.*, the formation of these bonds, are widespread in nature. Attempts to obtain CA catalyzing such processes have been made. In the course of these syntheses, two (or more) molecules are connected with each other in a strictly fixed way, *i.e.*, by a given type of bond involving atoms specified. The transition state of this reaction requires that the interacting molecules be mutually oriented in space in such a way that the bonds to be formed already "preexist" and the bonds to be cleaved are significantly weakened. In this case the hapten molecule must correspond as much as possible to the similar complex in its steric and electronic structure. For the CA catalyzing the formation of ester and amide bonds, all these conditions were reached using the same principle, the use of phosphorus analogs as haptens. For example, for the CA favoring the formation of the amide bond in the reaction of lactone **6** with *para*-phenylenediamine (**7**),[11] hapten **8** (Scheme 3), in which the phosphonic acid group took the place of the cleaving lactone and the forming amide bond, was used. The CA generated to this hapten exhibited properties typical of an enzyme with synthetase specificity and significantly increased the rate of the reaction over that of the noncatalyzed process. CA catalyzing the formation of an ester (or lactone) bond were obtained by a similar procedure.

Scheme 3

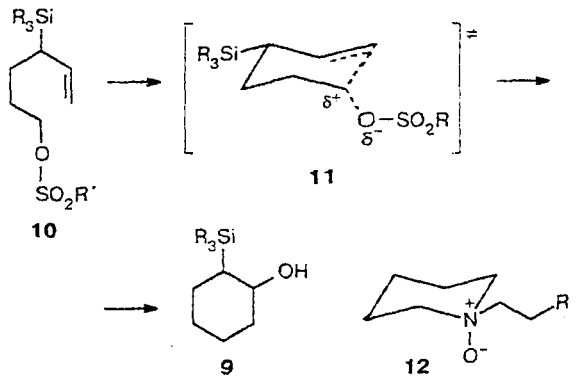


Preparation of CA that selectively catalyze the formation of definite bonds in the construction of a molecule of a target compound is of special value in organic synthesis, extending its range and affecting its methodology. Several tens of CA catalyzing a wide variety of processes, including oxidative-reductive processes, processes that transform or move some functional groups in a molecule, nucleophilic substitutions, synthesis of metal complexes, *etc.*, have thus far been studied.

Reactions involving the formation of a new carbon—carbon bond thus allowing one to create or directly change the carbon skeleton of a molecule are of special interest. A large number of such transformations can be carried out by chemical methods. Natural enzymes that increase their efficiency and specificity are known for some of them. For several reactions, artificial enzymes have also been obtained.

An example of catalysis of the formation of a carbon—carbon bond by CA is the synthesis of cyclohexane derivative **9** (Scheme 4) during the solvolysis of unsaturated sulfonate **10** (Johnson reaction).[12] The reaction proceeds as electrophilic attack on the unsaturated bond by the C atom of the CH_2 group connected with the sulfonate residue. The transition state is six-membered cyclic complex **11**, in which the new bonds are not completely formed and the old bonds are not completely broken. Hapten **12**, which structurally and electronically mimics the active center of the CA, is a the piperidine-*N*-oxide derivative whose N^+ and O^- atoms correspond electronically and sterically to the place at which bonds in the **10** → **9** transition are formed and cleaved, and which contains a six-membered cycle whose steric structure is close to that of the transition state. In the formation of the active center of the CA, the $^+N—O^-$ group initiates the appearance of negatively and positively charged groups in the center, which makes this transformation possible. Using compound **12** as a hapten it was possible to obtain CA that significantly accelerate the **10** → **9** reaction and, most importantly, make it strictly selective. For example, while the non-catalyzed reaction afforded a complex mixture containing only 30% target product **9**, the products in the presence of the CA contained 98% compound **9**.

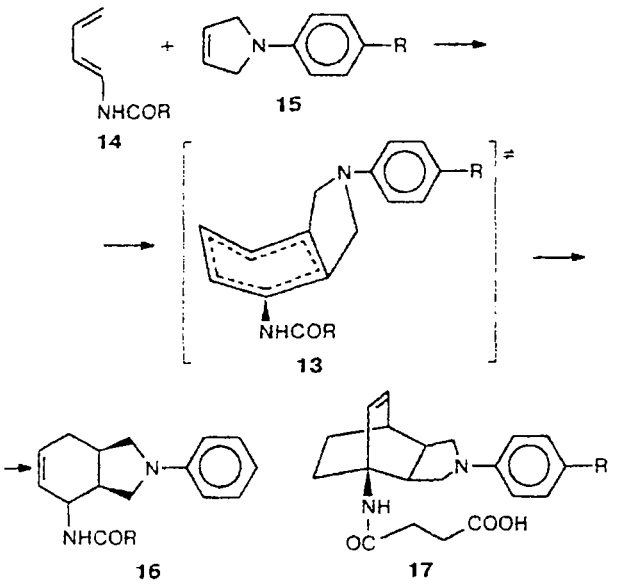
Scheme 4



R — spacer for linking to a protein

The most amazing example is the preparation of CA that accelerate the Diels—Alder reaction,[13] which is one of the classic reactions of organic chemistry, and for which no natural enzyme is known. This bimolecular [2+4]-cycloaddition proceeds as a pericyclic process. In transition state **13**, a diene (in this case, butadiene derivative **14**) is present in the cisoid form and together with the closest dienophile molecule (maleinimide derivative **15**) forms a "preexisting" cyclohexane structure with the boat conformation (Scheme 5). This transition state provides the best circumstances for the pericyclic process and the formation of the final product, cyclohexane derivative **16** with the corresponding stereochemistry.

Scheme 5



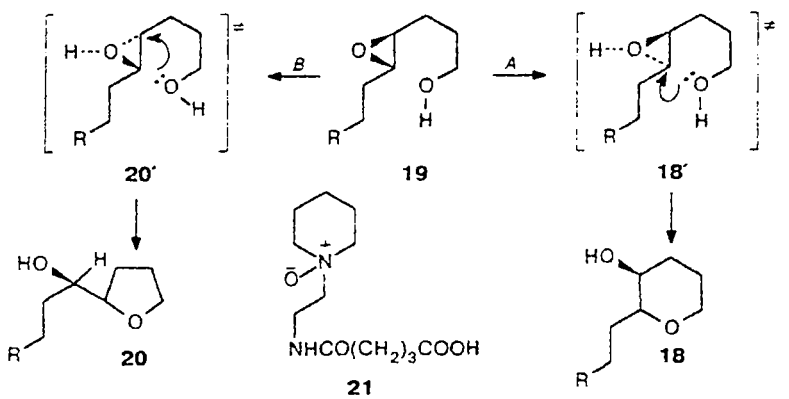
To form the sterically complementary active center in the CA, the model hapten must correspond to this transition state, and, most importantly, must imitate the boat conformation. This was achieved by using hapten **17** with the cyclohexene structure, whose boat-like structure is fixed by an additional ethene bridge. The CA obtained in this way actually exhibited catalytic action and accelerated many times the Diels—Alder reaction. A new type of enzyme, "dielsalderase," was thus synthesized for the first time!

Efficient CA have also been obtained for two other important synthetic reactions that involve prosesses similar to pericyclic processes — stereospecific Claisen rearrangement[14] and oxy-Cope rearrangement.[15] For the latter reaction natural enzymes are also unknown. The same principle was applied to the preparation of other haptens that initiate the formation of CA with an active center complementary to the transition state. CA for Claisen rearrangement proceeding through a transition state sterically similar to the transition state in the Diels—Alder reaction were generated using a hapten in a the conformation that was also fixed as a rigid system. Oxy-Cope rearrangement also proceeds through a transition state with a group of six C atoms in the chair conformation. In this case the model hapten was a cyclohexane derivative in the chair conformation.

Thus, to obtain CA that catalyze the formation of a carbon skeleton, haptens based on a rigid structure that imitates the structure of the transition state are most often used.

CA that allow one to carry out reactions that cannot be realized by pure chemical means and for which natural enzymes are unknown are of even greater interest. Such transformations are impossible to carry out under normal conditions due to energy interdiction, *i.e.*, their high transition state energy creates such a high energy barrier that it cannot be overcome under relatively mild conditions (so-called "unfavorable" reactions). This difficulty can be overcome and the reaction can proceed in an "unfavorable" direction in the presence of CA. One examples is the preparation of the six-membered pyran derivative **18** from epoxide **19** (Scheme 6) through the opening of the α-oxide cycle by the hydroxy group.[16]

Scheme 6



This reaction can in principle proceed in two directions depending on the character of the opening of the α-oxide cycle to form either the six-membered pyran ring **18** (path *A*) or the five-membered furan derivative **20** (path *B*). The direction of the reaction is determined by the structure of the transition state (**18'** or **20'**). In the non-catalyzed reaction carried out in the absence of CA under the action of an acid, the more energy profitable transition state **20'** appears, the process proceeds by path *B* affording furan derivative **20**, and isomer **18** is not formed at all. To change the direction of the reaction to path *B* it is necessary to cause the formation of transition state **18** with the six-membered cycle, which is achieved by using CA generated by a hapten with a particular structure. The piperidine-*N*-oxide derivative **21**, which contains a six-membered cycle and a $^{+}N-O^{-}$ group located at the site corresponding to the site of the cleavage of the oxide ring bond in substrate **19** and the formation of the new bond in the reaction product **18**, was chosen as the hapten. This $^{+}N-O^{-}$ group causes the appearance of the corresponding negative and positive centers in the CA, which is the driving force of the nucleophilic opening of the α-oxide cycle. In fact, among the group of antibodies generated by immunization with hapten **21**, a CA was found that, in contrast to the non-catalyzed reaction, affords solely the pyran derivative **18**. Thus, in the presence of this CA, the reaction completely changes its direction and proceeds significantly faster. It should be added that the CA controls also the stereochemistry of the reaction, *i.e.*, in addition to regiospecificity it also possesses a high stereospecificity. This amazing result demonstrates vividly the emerging possibilities for the use of CA in organic synthesis.

Other examples of CA that allow transformations that cannot be carried out by purely chemical methods are also known. Among them, the preparation of molecules with complex steric structure that cannot be obtained by the common methods of stereochemically directed synthesis (for example, see Ref. 17) should be noted. This is especially valuable for the synthesis of complex natural compounds and biologically active preparations.

In this paper we have mentioned only some of the interesting results obtained with the help of CA. Although the efficiency of CA in most cases is below that of the natural enzymes known to catalyze similar transformations, the creation of biocatalysts with previously planned specificity that catalyze reactions for which natural enzymes are unknown, and, finally, allow transformations to occur that are inaccessible by purely chemical means, opens a new page in biocatalysis and promises interesting possibilities.

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Even at this first step in the development of the chemistry of catalytic antibodies it seems clear that in spite of the laboriousness of their preparation the use of CA in laboratory practice, especially after further development, will allow one to solve a number of complex problems.

Further improvement of haptene design by computer modeling of reaction mechanisms and also intensification of the immunization process, improvement of its directivity, and scaling are essential. In developing CA for use in industrial technology, which is quite real for the future, a series of engineering problems, such as scaling the preparation of CA, and giving synthesized CA technically convenient forms, for example, by immobilizing it on different carriers for use in columns, creating micellar forms of CA, *etc.*, must be solved.

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