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Design of Biomimetic Catalysts by Molecular Imprinting in Synthetic Polymers: The Role of Transition State Stabilization

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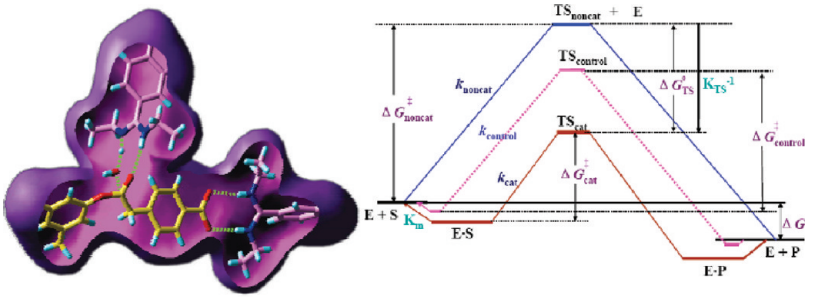

The impressive efficiency and selectivity of biological catalysts has engendered a long-standing effort to understand the details of enzyme action. It is widely accepted that enzymes accelerate reactions through their steric and electronic complementarity to the reactants in the rate-determining transition states. Thus, tight binding to the transition state of a reactant (rather than to the corresponding substrate) lowers the activation energy of the reaction, providing strong catalytic activity. Debates concerning the fundamentals of enzyme catalysis continue, however, and non-natural enzyme mimics offer important additional insight in this area. Molecular structures that mimic enzymes through the design of a predetermined binding site that stabilizes the transition state of a desired reaction are invaluable in this regard. Catalytic antibodies, which can be quite active when raised against stable transition state analogues of the corresponding reaction, represent particularly successful examples. Recently, synthetic chemistry has begun to match nature's ability to produce antibody-like binding sites with high affinities for the transition state. Thus, synthetic, molecularly imprinted polymers have been engineered to provide enzyme-like specificity and activity, and they now represent a powerful tool for creating highly efficient catalysts.

In this Account, we review recent efforts to develop enzyme models through the concept of transition state stabilization. In particular, models for carboxypeptidase A were prepared through the molecular imprinting of synthetic polymers. On the basis of successful experiments with phosphonic esters as templates to arrange amidinium groups in the active site, the method was further improved by combining the concept of transition state stabilization with the introduction of special catalytic moieties, such as metal ions in a defined orientation in the active site. In this way, the imprinted polymers were able to provide both an electrostatic stabilization for the transition state through the amidinium group as well as a synergism of transition state recognition and metal ion catalysis. The result was an excellent catalyst for carbonate hydrolysis. These enzyme mimics represent the most active catalysts ever prepared through the molecular imprinting strategy. Their catalytic activity, catalytic efficiency, and catalytic proficiency clearly surpass those of the corresponding catalytic antibodies.

The active structures in natural enzymes evolve within soluble proteins, typically by the refining of the folding of one polypeptide chain. To incorporate these characteristics into synthetic polymers, we used the concept of transition state stabilization to develop soluble, nanosized carboxypeptidase A models using a new polymerization method we term the "post-dilution polymerization method". With this methodology, we were able to prepare soluble, highly cross-linked, single-molecule nanoparticles. These particles have controlled molecular weights (39 kDa, for example) and, on average, one catalytically active site per particle. Our strategies have made it possible to obtain efficient new enzyme models and further advance the structural and functional analogy with natural enzymes. Moreover, this bioinspired design based on molecular imprinting in synthetic polymers offers further support for the concept of transition state stabilization in catalysis.

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Introduction

Enzymes, evolved in nature over vast periods of time, exhibit impressive catalytic capability by accelerating reactions with strikingly high efficiency and selectivity. For understanding and copying this admirable catalytic machinery, scientists have made great efforts to elucidate the nature of biological catalysis. Up to now, several different theories, with some variants, have been elaborated to understand enzymatic catalysis.[1,2] However, debates regarding the mechanism of action and the importance of different factors for enzymatic activity still exist.[3−5] It is widely accepted that enzymes accelerate reactions being sterically and electronically complementary to the reactants in their rate-determining transition states. The preferred binding of the transition state of a reaction in comparison to the substrate lowers the activation energy of the reaction and has thus a strong catalytic effect on the reaction rate. This transition state stabilization concept was proposed by Pauling[6] more than 60 years ago and later discussed more in detail by Jencks.[7] Studies of the structure of the enzyme's active sites as well as by the design of enzyme specific inhibitors having transition state analogous structures provide proofs for this concept,[2,8] To acquire new results elucidating the role of transition state stabilization in enzyme catalysis, two major strategies using artificial enzyme models were developed. They actually produced important progress in the present understanding of enzyme mechanisms. In this regard, catalytic antibodies generated against stable transition state analogues (TSAs) represented one successful example.[9,10] Monoclonal antibodies specific for defined TSAs were obtained by carefully optimizing the procedure for the immune response and the separation and enrichment of the most promising compounds. Antibodies of this type demonstrated high enzyme-like activities and specificities. They thus acted as hard-won evidence for the crucial role of transition state stabilization in enzymatic catalysis.

It is a special challenge for chemists to try to mimic the catalytic machinery of nature by synthetic chemistry.[11−13] The design of tailor-made catalysts by rationally manipulating synthesized structures should facilitate the understanding of the nature of enzymatic catalysis. Initial efforts in this regard focused on the construction of small host molecules with defined cavities carrying catalytically active groups and cofactors. Notable achievements to date have been obtained with several host systems, such as cyclodextrins, dendrimers, cyclophanes, calixarenes, and other macrocyclic compounds.[11−16] However, the possibilities to generate high affinities and specificities by rational synthesis of suitable models are quite limited. Recently, promising success was achieved in the design of macromolecular receptors capable of selectively binding and catalyzing the reaction of defined substrates by using molecularly imprinted synthetic polymers. Thus, the other more recent remarkable development is the preparation of synthetic polymers imprinted by TSAs which display significant catalytic capability. Similarly, as with catalytic antibodies, the importance of TS stabilization in biological catalysis was thus clearly demonstrated in this system.

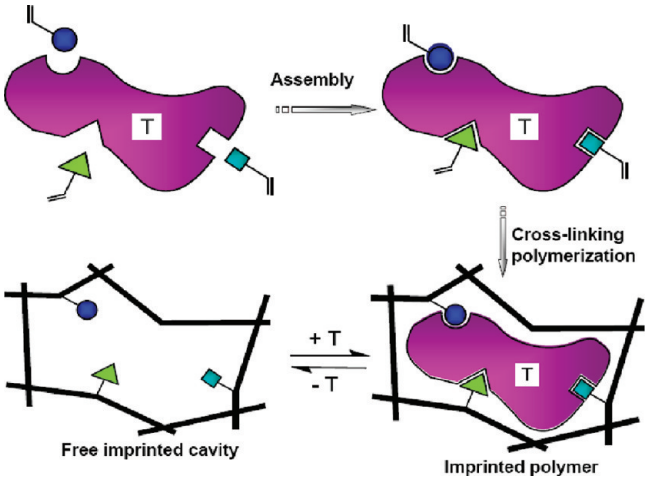
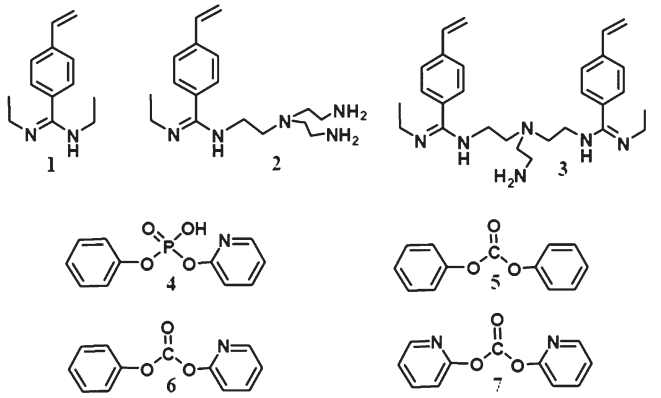


FIGURE 1. Schematic representation of the imprinting of specific cavities in molecularly imprinted polymers by a template (T).

Molecular Imprinting Strategy

Molecular imprinting is a promising strategy for generating active sites in highly cross-linked polymers with a specific shape having functional groups in a defined orientation[17] (see reviews, refs 18−20). Usually, polymerizable monomers containing functional groups are bound to a template molecule by covalent or noncovalent interaction (see Figure 1). These template monomers are copolymerized in presence of a high proportion of a cross-linker and other polymerizable monomers. Mostly the polymerization is performed by radical initiation in the presence of around the same volume of a solvent that can act as a porogen; thus, macroporous polymers with defined porosity are obtained. After polymerization, the template is removed and a free cavity is obtained with a shape and an arrangement of functional groups being complementary to the structure of the template. These functional groups can act as binding groups and as catalytically active groups. Polymers of this type have found broad application as selective adsorbents in solid phase extraction, as stationary phases for chromatographic separations (e.g., racemic resolution), as antibody

SCHEME 1. Structures of Functional Monomers (**1**–**3**), Template (**4**), and Substrates (**5**–**7**)[a]



[a]The function of these compounds and the position of their metal binding sites is seen in Figures 3 and 4.

mimics for immunoassays, as selective sensor layers in chemosensors, and as selective enzyme mimics and catalysts.[21,22]

Imprinted polymers show antibody-like recognition characteristics with high affinity and selectivity for the template molecules, and they bind ligands typically with association constants in the range of 10^3–10^7 M^{-1}.[23] More importantly, this strategy allows preparation of specific receptors for binding a wide range of different ligands from low molecular weight compounds to biomacromolecules of different classes.[21,22,24,25] Molecularly imprinted synthetic polymers provide an excellent possibility to generate predetermined specificities and prepare tailor-made catalysts by rationally manipulating their structures. In fact, not only suitable catalytically active groups and binding groups can be introduced into the active site in a predetermined orientation but, at the same time, the shape of the transition state can be mimicked by imprinting. The ability of synthetic chemistry to produce structurally and functionally complex molecules which might carry out the remarkable processes of life provides tremendous opportunities for artificial enzyme design. In the beginning, these enzyme models only slowly developed;[18,23,26] during recent years, these models could be improved considerably by the combination of the concept of *transition state stabilization* and a defined orientation of certain catalytic moieties in molecularly imprinted polymers.

Design of Catalysts by the Concept of Transition State Stabilization

In several examples, catalytic antibodies were described catalyzing the hydrolysis of esters and carbonates.[9,10] In these cases, mainly phosphonic esters or phosphates were used as stable transition state analogues. These stable transition state

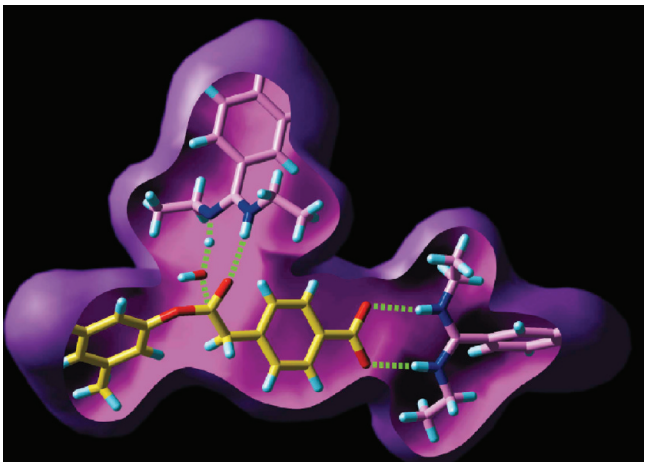


FIGURE 2. Computer graphic in the Molcad mode of the function of the amidines in the hydrolysis of an ester: One amidinium group binds the additional carboxyl group of the ester, and the other one stabilizes the tetrahedral transition state of the ester hydrolysis.[23]

analogues resemble the intermediate of the reaction in geometry and charge distribution and, according to the Hammond postulate, should be a good approximation also to the transition state of the reaction. Our plan was to approach in this way the mode of operation of the enzyme carboxypeptidase A (CPA, EC3.4.17.1), a zinc-containing metalloprotease removing the C-terminal amino-acid residue from a peptide chain and also splitting esters and carbonates. In the catalytic action of carboxypeptidase A, two guanidinium groups and a Zn^{2+} ion are predominantly incorporated.[27,28] One guanidinium moiety of Arg 127 binds the oxyanion generated in the rate-limiting step of the formation of the tetrahedral transition state. The zinc ion, coordinated tightly to the amino acid residues of His 69, Glu 72, and His 196, is decisive for the catalysis of the enzyme. Substrate specificity is brought about by a hydrophobic pocket and another guanidinium moiety of Arg 145.

In our earlier attempts, we wanted to mimic the function of the guanidinium moieties of arginine in the active site of CPA. If one would like to prepare efficient enzyme models by molecular imprinting, it is necessary to have the active functional groups exclusively inside the active sites. This should be possible by using a strong interaction of template to functional monomers. Due to weak binding, this is not possible with usual noncovalent interactions as earlier attempts by other groups have shown.[23] In such a case, usually an excess of functional monomers (4:1) has to be used with regard to the template. Therefore, the majority of functional groups after polymerization and removal of templates is randomly distributed in the polymer. Strong interactions are known to be present between guanidines or amidines with carboxylic acids, phosphonic acids, phosphates,

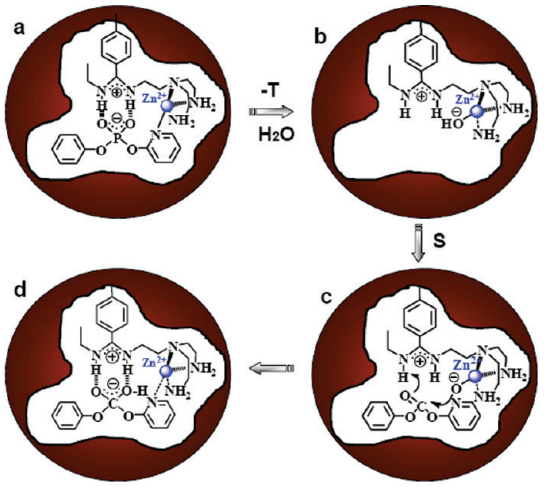


FIGURE 3. Schematic representation of the preparation and the function of the imprinted catalyst **PZn2,4**.[33] (a) Molecular imprinting with the template **4** and monomer **2** in the presence of Zn^{2+}. (b) Imprinted active site. (c) Substrate **6** bound in the active site. (d) The imprinted cavity stabilizes the tetrahedral transition state in the hydrolysis of **6**.

and so forth.[29,30] Simple polymerizable amidines and guanidines did not work satisfactorily in molecular imprinting. We therefore synthesized a large variety of different amidines, and from these the diethyl amidine derivative **1** proved to be a good functional binding monomer (Scheme 1), being generally applicable in molecular imprinting.[31] Amidines are easier to synthesize compared to guanidines as in arginine, but still they have very similar properties and they show more favorable polymerization properties. If the association of acid and amidine is measured under imprinting conditions, that is, equimolar concentrations of 0.1 mol L^{-1}, in all cases values from 95 to 99% association of the template are obtained; thus, just mixing gives a nearly quantitative interaction (so-called stoichiometric noncovalent interaction).[29] The Molcad computer graphic[23] in Figure 2 gives an impression of the function of two amidinium groups in the hydrolysis of a homoterephthalic monophenolester: One amidinium group binds the free carboxyl group, and the other one activates the 3,5-dimethylphenolester and binds the generated oxyanion.[31] Compared to earlier experiments, this model gave significantly higher enhancement in rate (102 times that of the reaction in neat solution). It already showed a distinct substrate selectivity, and furthermore, it was shown that the template (the transition state analogue) is a very effective competitive inhibitor (K_m for the substrate = 0.60 mM; K_i = 0.025 mM for the template). This was the first example approaching analogues of CPA (in this case without a metal ion). Additional systematic studies were undertaken to investigate the catalytic hydrolysis of carbonates by molecularly imprinted polymers.[32]

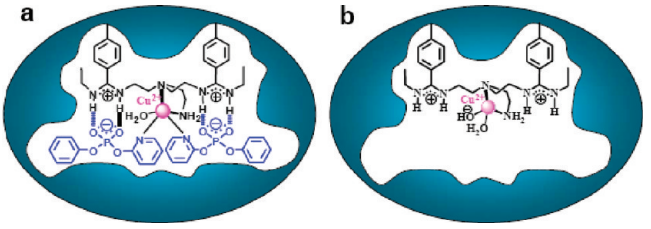


FIGURE 4. Molecularly imprinted polymer catalyst **PCu3,4**.[35] (a) Imprinting with functional monomer **3** and two molecules of the template **4** in presence of Cu^{2+}. (b) Catalytically active site of **PCu3,4** after removal of the template **4**.

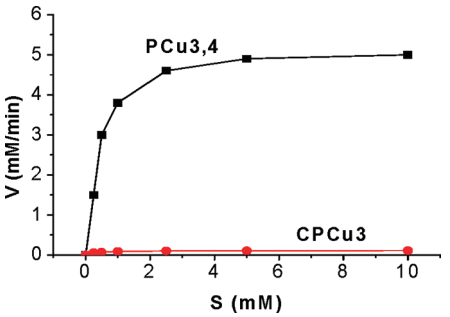


FIGURE 5. Michaelis–Menten plot of the hydrolysis of **7** by the imprinted polymer **PCu3,4** measured in HEPES/MeCN 1:1, pH = 7.3 at 20 °C (**CPCu3** control polymer).

Design of Catalysts by a Combination of Transition State Stabilization and a Defined Orientation of Special Catalytic Moieties

To approach the action of carboxypeptidase A further, imprinting with a stable transition-state analogue and introduction of an amidinium function in combination with a metal binding site was investigated. Thus, in a defined distance to the amidinium group, a triamine group was introduced resulting in the functional binding monomer **2** (Scheme 1). This group gives rise to a strong 3-fold coordination to zinc or copper ions, leaving one or two coordination sites free for other ligands (see Figure 3).[33–35] Of special interest is the binding in monomer **3**;[35] in this case, only one metal ion can be bound compared to the existence of two amidinium groups (see Figure 4). This structure changes the coordination sphere of the metal ion and its catalytic properties. As template phenyl-2-pyridyl-phosphate **4** is used, diphenyl-carbonate **5,** phenyl-2-pyridyl-carbonate **6** and di-2-pyridyl-carbonate **7** have been designed as the substrates (Scheme 1).

We started with the preparation of Zn ion containing catalysts. The template is coordinated during imprinting by interaction with the amidinium group and in addition via a *N*-zinc complex. This gives a better imprinting result compared

TABLE 1. Michaelis–Menten Kinetics of Carbonate Hydrolysis with Imprinted Polymers Containing Zinc or Copper Ions[33–35]

polymer metal ion[a]	substrate carbonate	k_{cat} (min^{-1})[b]	K_m (mM)	k_{cat}/k_{noncat}[c]	k_{cat}/K_m ($min^{-1}M^{-1}$)	ratio efficiencies impr./control[d]
PZn2.4	**5**	0.035	2.01	6900	17.4	n.d.
PCu2.4	**6**	2.86	0.65	75 700	4400	537×
PCu2.4	**7**	28.0	0.58	110 000	48 200	790×
PCu3.4	**7**	105	0.36	413 000	292 000	1056×

[a]The imprinted polymer **PZn2.4** was prepared using monomer **2** and template **4** in the presence of Zn(II). The other polymers were prepared accordingly. [b]Hydrolysis of the carbonates in HEPES buffer (pH 7.3)/MeCN 1:1 at 20 °C. [c]k_{noncat} is the rate in the same solution without catalyst. [d]The efficiency is compared between the catalyzed reaction with the efficiency in the presence of the corresponding control. The control polymers were prepared in the same manner as **PCu2.4** and **PCu3.4**; only the template **4** was substituted by formic acid.

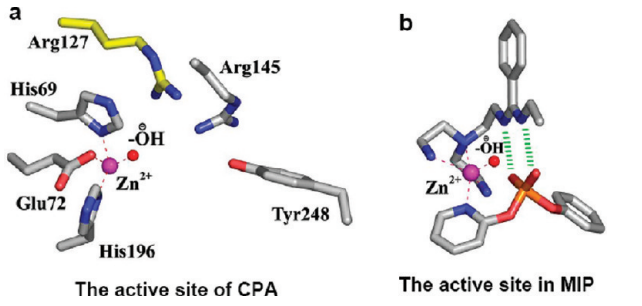


FIGURE 6. Schematic representation of the active site of the natural CPA (a) and the specific binding site in the imprinted polymer stabilizing the transition state (b).

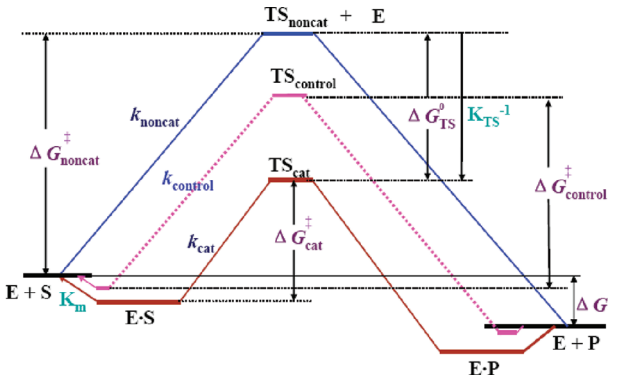


FIGURE 7. Energy levels (in free enthalpy ΔG) in catalysis (modified Polanyi's scheme). In addition to ΔG values, also equilibria constants (e.g., K_m) and rate constants (e.g., k_{cat}) are indicated. They can be calculated from the given ΔG values. In case of the equilibria constants, the direction of the arrow indicates either dissociation or association. TS_{noncat}, transition state in noncatalyzed reaction; $TS_{control}$, transition state in nonimprinted control catalyst; and TS_{cat}, transition state in imprinted catalyst. Proficiency = K_{TS}^{-1}. E = enzyme or catalyst, *S* = substrate, and *P* = product.

to just one binding interaction (see Figure 3 and ref 33). Similarly, but with a stronger 5-fold coordination, the corresponding copper complex can be used.[34] An interesting example with very high catalytic activity is shown in Figure 4. In this case, two molecules of template are used for molecular imprinting and the coordination of copper is different from those in the preceding examples.[35]

A good understanding of the catalysis can be obtained by measuring the Michaelis–Menten kinetics similarly as it is carried out with enzymes. An example is the most active catalyst **PCu3,4** and the corresponding nonimprinted control polymer **CPCu3** (Figure 5 and Table 1). In the case of **PCu3,4**, typical saturation kinetics are obtained at higher concentration with a constant catalyst amount but increasing substrate concentration. This indicates that all active sites are then occupied and the reaction becomes independent of substrate concentration (zero order). The typical form of the Michaelis–Menten plot as it is also found for natural enzymes clearly shows that although the models represent insoluble catalysts, they actually behave very similar to soluble enzymes. From these data, the Michaelis constant K_m and the turnover number k_{cat} can be calculated. Thus, data on the catalytic activity and the specific binding can be obtained independently. The control polymer is prepared in the same way but without using a template (nonimprinted control).

In Table 1, the ratio k_{cat}/k_{noncat} ($k_{noncat} = k_{soln}$) is used to express the catalytic activity of our catalysts similarly as it is done with antibodies and natural enzymes. Our most active compound (**PCu3.4**) shows an increase of rate of up to 413 000-fold, a figure that is by far the highest obtained for molecularly imprinted catalysts. These values are even higher by more than 2 orders of magnitude compared to catalytic antibodies for which k_{cat}/k_{noncat} = 810 were reported for carbonate hydrolysis.[36] A high catalytic efficiency k_{cat}/K_m (min^{-1} M^{-1}) for the imprinted polymers in comparison to the nonimprinted controls is observed. Imprinted catalysts are more efficient by a factor of 790 and 1056 (imprinting factor). These differences are remarkable since the controls also contain the same catalytic functional groups; the excellent catalysis therefore relates to a very efficient imprinting procedure. Figure 6 shows a comparison of the active sites from the natural and the artificial carboxypeptidase A. The imprinted cavity shows a similar spatial structure with the capability for the stabilization of the TS

TABLE 2. Binding of Substrate, Template (TSA), and Transition State to Imprinted and Nonimprinted Polymer Catalysts[35]

polymer	K_m (M)	K_i (M)[a]	K_{TS} (M)[b]
PCu2.4	0.58×10^{-3}	2.5×10^{-5}	0.53×10^{-8}
PCu3.4	0.36×10^{-3}	n.d.	0.87×10^{-9}
Controls			
CPCu2	6.10×10^{-3}	n.d.	0.42×10^{-5}
CPCu3	4.16×10^{-3}	n.d.	0.92×10^{-6}

[a]The inhibition constant was determined from the competitive inhibition caused by the template during the catalysis of the hydrolysis of the carbonate. [b]K_{TS} is the reciprocal proficiency ($K_{TS} = k_{noncat}K_m/k_{cat}$).

and with a similar orientation of catalytic moieties being responsible for high catalytic activity.

Now it is interesting to compare the binding of our catalyst to the substrate, to the transition state analogue (TSA), and to the real transition state of the reaction. Binding to the substrate is obtained from the Michaelis constant K_m, and binding to the TSA can be evaluated from the competitive inhibition caused by added template during the catalysis of the substrate (K_i). In Figure 7, a modified Polanyi's scheme with the different energy levels during catalysis is shown. The noncatalyzed reaction shows a high free activation enthalpy ($\Delta G_{noncat}^{\ddagger}$) and therefore a slower reaction. The catalyzed reaction shows at first an equilibrium of catalyst with the substrate characterized by the substrate dissociation constant K_M, and overall a faster reaction due to a lower free activation enthalpy $\Delta G_{cat}^{\ddagger}$. For comparison, the energy levels of the control are given in $\Delta G_{control}^{\ddagger}$ having an intermediate value. Formally, the free transition state of the noncatalyzed reaction can be associated with the catalyst to form the bound TS_{cat}; this association constant is called the catalytic proficiency K^{-1}_{TS}.[37,38] The proficiency describes in the assessment of enzymes or other catalysts the difference in the difficulty of the task that they perform. For this, it is necessary to know the rate under noncatalyzed conditions and relate the efficiency k_{cat}/K_M to this reaction rate. Thus, the catalytic proficiency is calculated from $(k_{cat}/K_m)/k_{noncat}$. The catalytic proficiency is formally the equilibrium constant for the formation of the complex between the transition state and the enzyme or enzyme model. It therefore reflects the hypothetical binding affinity of an enzyme or an enzyme analogous catalyst for the transition state of the catalyzed reaction.

Our new imprinted catalysts show rather high K_{TS}^{-1} values (10^8–10^9). Thus we see in Table 2 that increased binding in the imprinted cavities is observed from the substrate of the reaction, to the template (a stable analogue of the transition state) to the real transition state of the reaction. This indicates that cavities with high affinity for the TS are responsible for the high rate enhancements. This is a further proof for the role of the transition state in this type of catalysis and for the efficiency of molecular imprinting.

The pH-rate profile for the carbonate hydrolysis in presence of **PCu2,4** is quite different from that of **PZn2,4**. The Zn-containing catalyst shows a strong increase in rate with the pH having an inversion point at pH 7.5; the copper containing one shows a bell-shaped profile with an optimum at pH 7.2. The maximum rate is obtained when the copper is predominantly in the catalytically active *aqua hydroxy* form. Similar copper- catalysts but without using TSAs and molecular imprinting were investigated by Suh.[39] In the Zn-containing catalysts, the amount of OH^- bound to the Zn is increasing with higher pH in parallel with the catalytic activity. For analogous low molecular weight zinc model substances, see the work of Kimura.[40]

In the foregoing examples, hydrolytic reactions were investigated. In the case of entropically less favorable reactions such as the Diels–Alder reaction, it is more difficult to obtain a high enhancement in rate using molecularly imprinted catalysts, similarly as it was observed with catalytic antibodies. Nicholls and his group prepared catalysts imprinted with a putative transition state analogue of a Diels–Alder reaction. An enhancement of rate of around 20-fold was obtained.[41]

Design of Soluble, Nanosized Enzyme Models with the Concept of Transition State Stabilization

The enzyme models described until now are molecularly imprinted insoluble synthetic polymers with a porous structure. Natural enzymes usually perform their functions with soluble proteins as scaffolds and possess a radius of gyration of 5–15 nm and a molecular weight of 30–500 kDa; they evolve their active structure typically by folding of one polypeptide chain (such as CPA in Figure 8a). It is a great challenge to create similar structures with the molecular imprinting strategy in synthetic polymers.

The problem of the preparation of soluble intramolecularly cross-linked polymers is that usually during their polymerization three-dimensional infinite networks of macrogels are obtained which are insoluble (Figure 8c). There are different methods to prepare soluble cross-linked microgels or nanogels (for definitions see ref 42); however, these methods are usually not very suitable for molecular imprinting. Graham and Hayes found that in special solvents (e.g., cyclopentanone) at low monomer concentration (e.g., 1%) it is possible to obtain soluble nanogels.[43] With this method, it was indeed possible to obtain molecularly

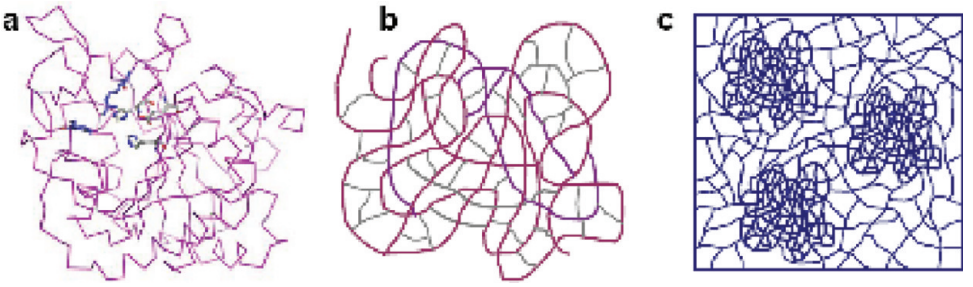


FIGURE 8. Comparison of different cross-linked chain structures: (a) natural enzyme, e.g., CPA, soluble, (radius of gyration 2−15 nm, $M_n = 30-500$ kDa); (b) intramolecularly cross-linked macromolecule, soluble (radius of gyration, e.g., ∼15 nm [see Figure 9], $M_n = 39$ kDa; (c) macrogels obtained by usual cross-linking polymerization, three-dimensional infinite network.

imprinted, soluble, highly cross-linked nanogels with defined structure[44] (Figure 8b).

For the preparation of catalytic nanoparticles (nanogels), we also used the concept of transition state stabilization and selected a rather simple catalytic system (**1** as functional monomer and diphenyl phosphate as a template) that was already employed with insoluble, molecularly imprinted polymers.[32] In a longer optimization procedure, we were able to obtain very active catalysts.[45] Since these nanogel particles are soluble, typical polymer analytical methods could be applied for their characterization, such as gel permeation chromatography (GPC). From the retention time and the form of the peak using a calibration with polystyrenes of defined molecular weight, different important data could be obtained by computer calculations. Most important in the improvement of the properties was a novel synthetic method that we have called the "post-dilution method". In a polymerization at high concentration of monomers, we stopped the polymerization just prior to macrogelation and then diluted extensively with cyclopentanone to keep the concentration below the c_m-value (0.1−1.5 wt.%). Above the critical concentration c_m, macrogelation takes place. The optimization of the preparation yielded nanoparticles with much better catalytic properties. It was possible to reach with these soluble particles the same catalytic activity compared to the standard, insoluble macroporous polymers investigated earlier.[32] The nanoparticles showed Michaelis−Menten kinetics with a k_{cat}/k_{noncat} ratio of 2990.

Another example for our optimization of the imprinted nanogels (INGs) is given. **ING2**−**ING4** were prepared under identical conditions, except the dilution of the monomers in the "post dilution method" was 1.0, 0.5, and 0.1%. The absolute molecular weight $M_{n,abs}$ measured by membrane osmometry dropped from 624 (1% monomer) to 261 (0.5% monomer) and to 39.0 kDa (0.1% monomer). At the same time, the polydispersity M_w/M_n dropped from 6.0 to 3.6 and to 1.54. The reason is a much lower aggregation of the primary particles. A polydispersity of 1.54 is a really good value for a radical polymerization of this type. The $M_{n,abs}$ values are considerably greater than the M_n values obtained from GPC measurements. This is not surprising, since highly cross-linked nanoparticles dissolved in good solvents possess a much more densely packed structure compared to linear polystyrenes used as standards in GPC. They have with the same molecular weight considerably higher hydrodynamic volumes. The factor $M_{n,abs}/M_n$ gives a good indication of the density of the nanoparticles. It increases from 16.7 (**ING2**) to 30.1 (**ING4**). Thus, the density of the optimized nanoparticles is 30 times higher compared to linear polystyrenes of the same molecular weight and nearly doubled during the optimization of the nanoparticles. This result gives a good explanation for the much better catalytic activity and the higher imprinting efficiency. It is interesting that the density of the enzymes is even higher since the peptide chains can intramolecularly interact. A calculation showed the existence of on average 1.03 active sites per particle for **ING4**.

In principle, these nanogels can be separated like enzymes by affinity chromatography[45] as was shown recently for another type of nanoparticles by Piletsky et al.[46] Thus, a separation according to the affinity of the active sites would be possible if only one active site per particle is present. It offers an interesting new possibility for obtaining "monoclonal" molecularly imprinted polymers. For a recent review on nanoparticles and results from Resmini's group, see ref 47. In her group, instead of amidinium derivatives, guanidinium derivatives were used to prepare transition state analogous structures for the imprinting in nanoparticles.[48] They also followed the general scheme of Graham and Hayes[43] for the preparation of nanoparticles and obtained catalytically active nanoparticles with significantly enhanced rate of carbonate hydrolysis.

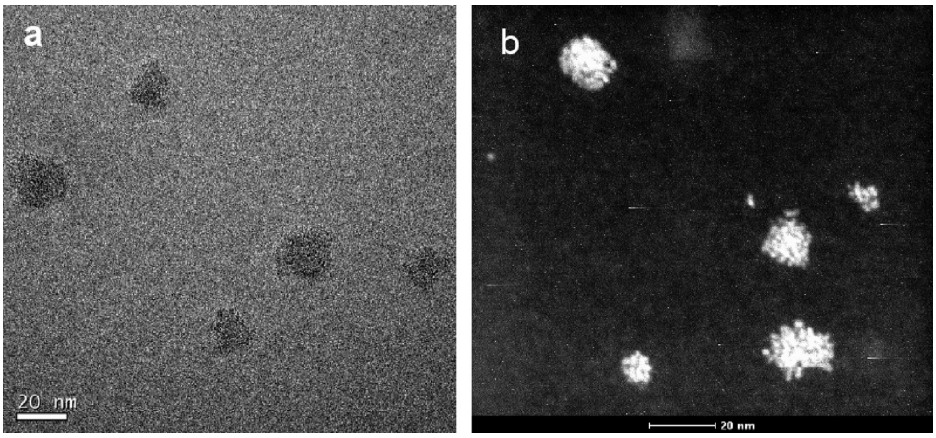


FIGURE 9. Transmission electron microscopy image by EDMA-based imprinted nanogel particles prepared with 0.1% monomer in cyclopentanone, stained with RuO4. (a) Measured in the imaging mode (HRTEM); (b) measured in the scanning mode (STEM). Part B reproduced with permission from ref 45. Copyright 2006 (Wiley-VCH Verlag GmbH & Co KgaA).

It was possible to visualize and characterize our new nanoparticles by scanning transmission electron microscopy (STEM) and in the imaging mode (HRTEM) (see Figure 9).[45] RuO_4 stained nanogel particles of **ING4** were used. Spherical particles with diameters between 10 and 20 nm can be clearly seen. These nanoparticles represent single molecule, intramolecularly cross-linked macromolecules which do not possess a marked fractal structure. The nanoparticles are rigid and do not collapse onto the support film. Since these soluble nanogels of 39 kDa can be prepared, on average, with one catalytic site per particle and show Michaelis–Menten kinetics, an increasing analogy to enzymes is reached.

Concluding Remarks

In this Account, we summarized recent efforts to prepare mimics of the enzyme carboxypeptidase A. It was not our concern to copy this enzyme as perfectly as possible, but instead we wanted to translate the principles of enzyme catalysis to the design of new catalytic materials. Thus, it was possible to prepare very efficient catalysts using a combination of transition state stabilization with a defined orientation of catalytic moieties in molecularly imprinted polymers. In qualitative respect, these catalysts show a good number of properties typical for enzymes; comparing them at the quantitative level, they are considerably less efficient relative to enzymes. Already in earlier investigations it was shown that catalysts prepared by molecular imprinting exhibit a pronounced substrate selectivity and enantioselectivity.[31,49,50] In our new models, typical enzyme properties, such as Michaelis–Menten kinetics, competitive inhibition by the TSA, and, more importantly, strong transition state stabilization, have been realized. Overall, an enhancement of rate of $k_{cat}/k_{uncat} = 4 \times 10^5$ for the hydrolysis of carbonates was obtained. The molecular imprinting contributes a factor of about 1000 in this system.

By using a special molecular imprinting strategy, soluble, single molecule catalytic nanoparticles with defined molecular weights and on average one active site per particle could be developed. In contrast to catalytic antibodies, it is possible with molecularly imprinted polymers to introduce desired functional groups in a predetermined orientation into a cavity of defined shape. With catalytic antibodies, the composition of the functionalities is the result of a more or less random selection from the functional groups of natural amino acids. Thus, the molecular imprinting strategy provides a basic tool and lays the foundation for a biomimetic design of artificial enzymes.

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FOOTNOTES

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