Catalytic Molecularly Imprinted Polymers Using Conventional Bulk Polymerization or Suspension Polymerization: Selective Hydrolysis of Diphenyl Carbonate and Diphenyl Carbamate

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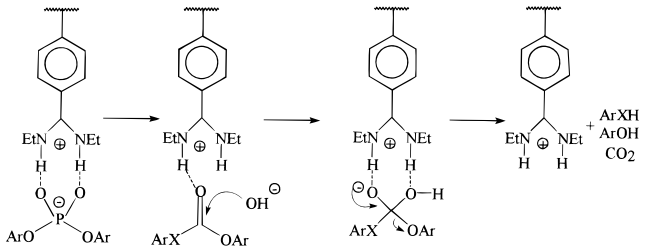
The molecularly imprinted polymer notion is conceptually attractive and has stimulated the design of many informative experiments and practical applications; indeed, this field is at an exciting stage of rapid development and progress.[1] In the area of catalysis, molecularly imprinted polymers would appear to be particularly attractive: cavities which bind to a transition state of a given reaction should be selective catalysts for that reaction.

However, in earlier experiments using stable mimics of the transition state (transition state analogue, TSA) as the imprint substance, in a fashion analogous to that used successfully for antibody-catalyzed reactions,[2] the resulting polymers displayed only limited catalysis.[3] In some cases, careful optimization of the conditions afforded improved catalysis, most notably in a dehydrofluorination reaction.[4] To fully complex the template, a large excess of binding/catalytic sites had to be employed in these experiments if weak noncovalent interactions with the TSA had been used. Improved catalytic performance results from imprinting procedures involving noncovalent bonds with sufficiently high association constants to afford complexes having a 1:1 ratio of template to binding site. We have termed this type of interaction "stoichiometric noncovalent interaction".[5]

A step in this direction involved the use of *N*,*N*′-diethyl(4-vinylphenyl)amidine (DEVPA)[6] as a functional monomer to bind phosphonate or phosphate TSA-imprint molecules. The strong ionic, double-bridged interaction between the amidine and the TSA can serve, if the TSA is replaced by the corresponding ester substrate, both for carbonyl group activation and also provide the "oxyanion hole" for TS stabilization in the subsequent hydrolysis reaction. The first DEVPA-based catalytic imprint polymers displayed significant rate enhancements of ester hydrolysis, Michaelis−Menten kinetics, selectivity, and TSA inhibition of activity, all important characteristics of enzyme-catalyzed reactions.[7] One disadvantage of this system was that it showed product inhibition similar to that observed with catalytic antibodies.[2]

Scheme 1. Depiction of the Amidine−Phosphate Complex (**1**) and the Catalysis in the Cavity of the Imprinted Polymer[a]



1 **2a** X=O **2b** X=NH **3** **4**

[a] Possible reaction mechanism, whereby the carbonate (**2a**) or carbamate (**2b**) is bound and activated via hydrogen-bonding to the carbonyl oxygen (**2**) followed by nucleophilic attack of hydroxyl and stabilization of the oxyanion (**3**) and subsequent breakdown to products with regeneration of the catalytic site (**4**). (We favor the $B_{Ac}2$ mechanism for carbamate hydrolysis on the basis of the analogous studies using a carbamate-hydrolyzing antibody elicited by a phosphonate).[9]

Table 1. Kinetic Parameters for Diphenyl Carbonate and Diphenyl Carbamate Hydrolysis Using Bulk-Type Imprinted Polymers[a]

substrate[b]	buffer HEPES: MeCN ratio	temp. (°C)	$k_{impr.}$ ($\times 10^{-4}$ min^{-1})	$k_{impr.}/k_{soln.}$	$k_{impr.}/k_{stat.}$
DPC	2:1	15	10.8 ± 0.5[c]	387 ± 33	8.1 ± 0.7
DPC	2:1	10	7.9 ± 0.1	588 ± 44	7.8 ± 0.4
DPC	1:1	15	2.8 ± 0.1	455 ± 19	10.7 ± 0.5
DPCM	2:1	15	93.9 ± 0.6	643 ± 48	5.8 ± 0.2
DPCM	2:1	5	38.4 ± 0.5	1435 ± 27	4.2 ± 0.1

[a] The composition of the monomer mixture for the preparation of the imprinted polymers consisted of 79.6 wt % of EDMA, 10.4 wt % MMA, and 9.0 wt % of DEVPA−DPP-complex, and 1 wt % of azobis(isobutyronitrile), diluted by the same weight of the porogen, acetonitrile. In the control polymer the DPP-template was omitted. For details see Supporting Information. [b] The substrates were either diphenyl carbonate (DPC) or diphenyl carbamate (DPCM). [c] Standard deviation values were calculated from at least five independent measurements.

Similarly as with catalytic antibodies,[2] we therefore turned to systems in which less product inhibition is expected, and we investigated for the first time the hydrolysis of carbonates and carbamates (Scheme 1). These compounds have the very important feature that they do not form stable anionic products (as do esters) which can act as reaction inhibitors in the course of hydrolysis. Moreover, the advantages of the novel stoichiometric noncovalent interaction, based on DEVPA as functional monomer and diphenyl phosphate (DPP) as templated molecule, allowed the introduction of the suspension polymerization method for the preparation of imprinted polymer beads. Imprinted polymers have generally been prepared by bulk polymerization methods which produce a solid macroporous block which must then be crushed, ground, and sieved to obtain a desired particle size. This is a time-consuming and energy-wasteful process accompanied by large losses of material. In addition, the properties of the resulting irregular particles may not be ideal with regard to flow, reproducibility, and scale-up procedures, whereas suspension polymerization methods produce relatively uniform spherical beads which are far more suitable. For these reasons, suspension polymerization has also been considered by others;[1,8] however, because of the relatively weak interaction between the imprint substances and the functional monomers in these cases, simple suspension polymerization in

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Table 2. Kinetic Parameters for Diphenyl Carbonate Hydrolysis with Imprinted Polymer Beads[a]

sample	porogen	water phase composition[b]	particle size, μm (index of polydispersity[11])	$k^c_{impr.}$ ($\times 10^{-4}$ min^{-1})	$k_{impr.}/k_{soln.}$	$k_{impr.}/k_{stat.}$
SP1	toluene	PVA/PVP	141.0 (1.25)	8.2[7.9][d]	293[282]	9.7[2.0]
SP2	cyclohexanol−dodecanol	NaCl/starch	374.7 (1.16)	4.7	168	24.0
SP3	cyclohexanol−dodecanol	PVA/PVP	31.3 (1.23)	4.7[5.0]	168[179]	23.3[4.0]

[a] The monomer composition for the preparation of the polymer beads was the same as in Table 1; the porogen was changed (for details see Supporting Information). [b] Composition of suspending medium during polymerization, 2 wt % of poly(vinyl alcohol) and 1 wt % of poly(*N*-vinylpyrrolidone) solution in water (PVA/PVP) or 20 wt % of NaCl and 8 wt % of starch in water (NaCl/starch). [c] In brackets the corresponding values for bulk polymers with the same porogen are given. [d] Relative standard deviation values are in the range of 1−2% for $k_{impr.}$; 5−7% for $k_{impr.}/k_{soln.}$; and 2−5% for $k_{impr.}/k_{stat.}$

water could not be used. The new DEVPA-functional monomer allowed us to use the well-established suspension polymerization technique. In this case the interaction between template and binding site is very stable.

Imprinted polymers were prepared in bulk and in suspension from polymerization mixtures consisting of cross-linker, ethylenedimethacrylate (EDMA), methyl methacrylate (MMA), DEVPA as functional monomers, and DPP as template molecule in the presence of acetonitrile (MeCN), cyclohexanol-*n*-dodecanol, or toluene as porogen.[10] Classical aqueous suspension polymerization technique proceeded smoothly to give beads of 8−375 μm diameter, depending on the polymerization conditions used. The DEVPA−DPP complex does not appear in the aqueous phase in the course of polymerization, and the presence of this complex in the polymer matrix was confirmed by FTIR and nitrogen elemental analysis.[10] The free, imprinted active sites were obtained by removal of the template with a solution of 0.1 M NaOH and MeCN (1:1 by volume). Approximately 70−90% of DPP was removed, leaving cavities containing amidine groups whose quantity and pK_a values were determined by potentiomeric titration (pK_a 9.09−8.62).[10]

The hydrolysis of diphenyl carbonate and diphenyl carbamate was performed in a 2:1 and 1:1 solution of 2-[4-(2-hydroxyethyl)-1-piperazino]-ethanesulfonic acid (HEPES) buffer (pH = 7.3) and MeCN. The formation of phenol at 5, 10, or 15 °C was followed by HPLC analysis on an RP8 column. Substrate hydrolysis was treated as a pseudo first-order reaction with rate constants *k*. The rates of the catalyzed reactions ($k_{impr.}$) were compared with those of reactions carried out in a buffer solution of pH 7.3 ($k_{soln.}$) as well as to the rate of reaction in the presence of nonimprinted polymer produced under identical conditions but without the imprint template, DPP ($k_{stat.}$). The results are presented in Tables 1 and 2. It can be seen that rate constants can be enhanced by factors of 588 (in case of carbonate) and 1435 (in case of carbamate), compared to rates for reactions in buffer at the same pH. The enhancement with respect to nonimprinted polymers containing statistically distributed amidines is 10 (carbonate) and 5.8 (carbamate) for bulk polymers and up to 24 for imprinted polymer beads. Thus, the beads show a much higher selectivity, maybe due to better mass transfer. It is not surprising that carbamate hydrolysis is less specific with regard to the control polymer since the polymer was not imprinted with a TSA of the carbamate hydrolysis reaction. The large rate enhancement of the polymer-catalyzed carbamate hydrolysis relative to that in buffer solution is remarkable in comparison to those of earlier imprinted polymer hydrolyses.[3]

These values are among the best published until now for molecularly imprinted catalysts. However, these results show that nonspecific catalysis at the surface of the polymers *also* plays a role in the enhancement of reaction rates. The specific catalysis arises from the shape selective transition state stabilization in the cavities. In addition other nonspecific factors such as high local concentrations of amidine sites, differences in the pK_a values of amidines in solution and at the polymer, and specific adsorption of substrates to the polymer backbone play a role. Further studies are required to understand and control these effects. It is also important to note that the rate of diphenyl carbonate hydrolysis (k_{am}) in 4-ethyl-*N*,*N*′-diethylphenyl amidine−HEPES buffer solution containing the same amount of amidine groups as in the imprinted polymer is very similar to the rate constant of hydrolysis in buffer solution ($k_{am}/k_{soln} = 0.93$). Thus, the amidine is not an efficient catalytic group in solution at this pH.

The imprinted polymer beads possess the same catalytic activity (although higher selectivity) (Table 2) as bulk-type imprinted polymers prepared with the same porogens, cyclohexanol-*n*-dodecanol or toluene. Although the rate constants of diphenyl carbonate hydrolysis are apparently higher for polymers prepared on the basis of MeCN as porogen, it is impossible to use it in suspension polymerization due to the miscibility of MeCN with water.

Diphenyl carbonate and carbamate hydrolyses with imprinted polymer prepared in bulk and in suspension (sample SP3, see Table 2) reveals Michaelis−Menten kinetics and competitive inhibition typical for catalytic antibodies.[10] For diphenyl carbonate hydrolysis with imprinted polymer prepared in bulk, the kinetic constants are $K_m = 5.01$ mM, $V_m = 0.023$ mM min^{-1}, $k_{cat} = 0.012$ min^{-1}, $K_I = 0.094$ mM (K_I is the inhibition constant for the template molecule); for diphenyl carbamate hydrolysis with imprinted polymer prepared in bulk: $K_m = 3.33$ mM, $V_m = 0.044$ mM min^{-1}, $k_{cat} = 0.022$ min^{-1}, $K_I = 0.285$ mM; for diphenyl carbonate hydrolysis with SP3: $K_m = 13.4$ mM, $V_m = 0.008$ mM min^{-1}, $k_{cat} = 0.004$ min^{-1}, $K_I = 0.22$ mM. The slower hydrolysis with imprinted beads is due to the use of cyclohexanol-*n*-dodecanol as a porogen instead of the MeCN used in the bulk polymer preparation; bulk polymers prepared with the same porogen showed comparable kinetics (see Table 2).

The chemical potential of the immune system has been underscored by showing that antibodies raised to tetrahedral, negatively charged phosphate and phosphonate TSAs could selectively catalyze the hydrolysis of esters, carbonates, and carbamates.[2,9,11] This report is an initial attempt to approach the efficiency and selectivity of catalytic antibodies using synthetic polymers, which have distinct advantages (far less expensive, more convenient and chemically as well as mechanically more stable). In the case of carbamate hydrolysis, the rate enhancement with imprinted polymers relative to that with buffer solution already approaches the values recently obtained with catalytic antibodies for the same reaction.[9]

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Supporting Information Available: Experimental details of characterization and syntheses of monomers, substances and polymers (both in bulk and as beads) and Lineweaver−Burk plot (PDF). This material is available free of charge via the Internet at http:/pubs.acs.org.

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