Carbon−Carbon Bond Formation Using Substrate Selective Catalytic Polymers Prepared by Molecular Imprinting: An Artificial Class II Aldolase

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A class II aldolase-mimicking synthetic polymer was prepared by the molecular imprinting of a complex between dibenzoylmethane (**1**) and cobalt(II) ion in a 4-vinylpyridine−styrene−divinylbenzene copolymer. This polymer was capable of selectively catalyzing the reaction of acetophenone (**2**) and benzaldehyde (**3**) to produce chalcone (**4**). The polymer, which demonstrated substrate selectivity and turnover, increased reaction rate eight-fold, relative to the solution reaction. The polymer was able to withstand vigourous reaction conditions, DMF and 100 °C for several weeks, while retaining most (80−95%) of its initial activity. This is the first reported example of catalytic carbon−carbon bond formation using the molecular imprinting technique.

Introduction

Molecular imprinting,[1−3] a technique for producing ligand selective recognition sites in synthetic polymers, has found use in the preparation of antibody combining site mimics with affinities and selectivities comparable to those of biologically derived antibodies[4−6] and in a range of other application areas.[7,8] The principles underlying the design[9] and preparation[8] of molecularly imprinted polymers (MIPs) are summarized in Figure 1. In parallel to the development of catalytic antibody technology,[10] attempts have been made to generate substrate selective catalytically active MIPs,[11−18] mainly focusing upon hydrolytic and dehydration-based processes, though to date success has been modest. The catalysis of carbon−carbon bond formation poses one of the most significant objectives for catalytic antibody technology and organic synthesis[19] and is addressed here.

We report the preparation and evaluation of a vinylpyridine−styrene−divinylbenzene copolymer imprinted with an aldol condensation reactive intermediate analogue, a complex between dibenzoylmethane (DBM), **1**, and Co^{2+}. The imprinted polymer demonstrated substrate selectivity, turnover, and rate enhancement when used to catalyze an entropically unfavorable C−C bond formation, namely, the aldol condensation of acetophenone (**2**) and benzaldehyde (**3**) to produce chalcone (**4**), Figure 2A. This class II aldolase-like activity, as found in primitive cells such as yeast and bacteria,[20] was competitively inhibited by the reactive intermediate analogue **1**. This weak enzymelike activity was achieved employing a combination of temperature (100 °C), reaction time (days), and solvent (dimethylformamide), not generally observed with biologically derived catalysts. This particular reaction system, which requires several weeks to reach completion, was selected to permit adequate kinetic analysis and to illustrate the robustness of these materials.

Results and Discussion

On the basis of a molecular model study, DBM (**1**) was perceived as a reactive intermediate analogue for the cobalt(II) ion-mediated aldol condensation of **2** and **3**.[21] The bidentate ligand **1** was expected to accommodate two of the coordination sites of the tetrahedrally configured Co^{2+}, Figure 1, with 2 equiv of vinyl pyridine (**5**) satisfy-

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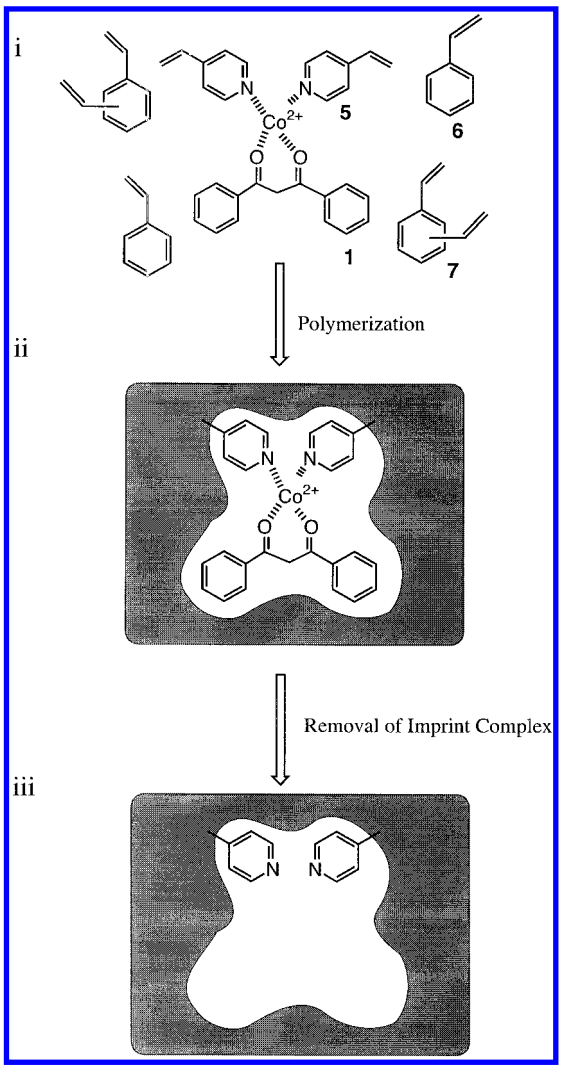

Figure 1. Schematic representation of molecularly imprinted polymer preparation: DBM-Co^{2+} MIP preparation. (i) The monomers, vinylpyridine (**5**), styrene (**6**), and divinylbenzene (**7**), are mixed with the imprint species, dibenzoylmethane (DBM) (**1**) and cobalt(II) acetate, and initiator in methanol, allowing formation of complementary sets of interactions between the monomers and the print species.[26] (ii) Polymerization produces a rigid bulk polymer. (iii) The imprint species is extracted to render sites topographically cimplementary to the print species in terms of shape and chemical functionality. (The schematic polymer structure dipicted represents an idealized case and does not take into account the accessibility of the substrate into the recognition site.)

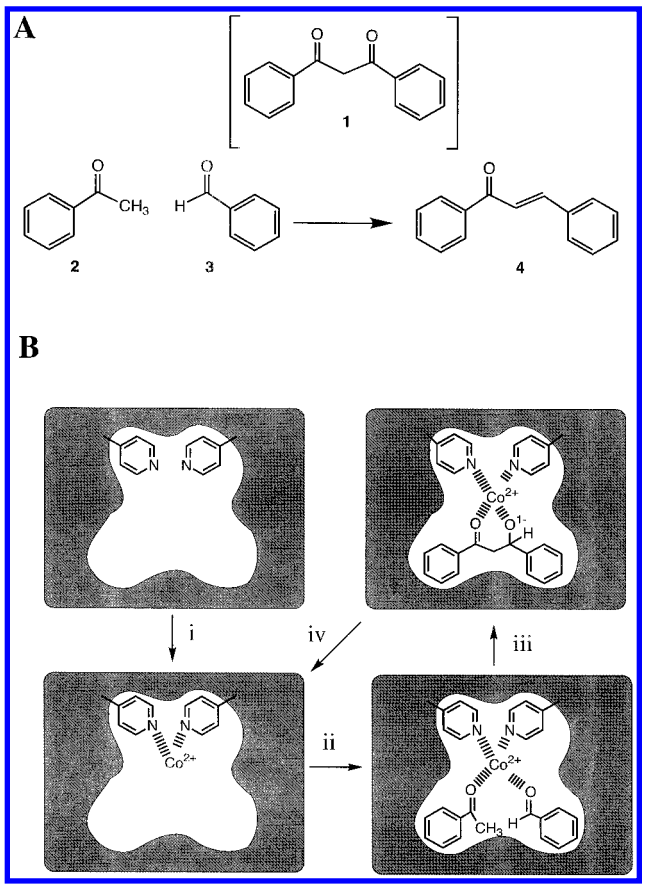

Figure 2. (A) The aldol condensation of acetophenone (**2**) and benzaldehyde (**3**) to yield chalcone (**4**) with the reactive intermediate analogue dibenzoylmethane (DBM) (**1**). (B) Schematic representation of MIP-mediated catalysis of the aldol condensation of acetophenone (**2**) with benzaldehyde (**3**) to yield chalcone (**4**). (i) Polymer incubated with cobalt(II) ion. (ii) Incubation of the polymer with reactants. (iii) Enolization and nucleophilic addition yields a polymer-stabilized reactive intermediate. (iv) Proton transfer to the stabilized reactive intermediate and subsequent dehydration affords chalcone (**4**), which has a lower affinity for the polymer than the stabilized reactive intermediate structure due to **4** having only a single point coordination to the metal center, motivating its diffusion from the recognition site. [Note: It is unclear whether the dehydration step takes place within the "active site" or after diffusion therefrom.]

Table 1. Chromatographic Recognition Study[a]

	HPLC column (imprint species)			
analyte	DBM-Co^{2+}	Co^{2+}	DBM	blank
dibenzoylmethane (DBM) (**1**)	9.3 (5.4)	7.0 (5.6)	2.5 (4.7)	3.6 (5.2)
acetophenone (**2**)	0.54 (0.55)	0.55 (0.56)	0.41 (0.47)	0.64 (0.61)
benzaldehyde (**3**)	0.47 (0.49)	0.44 (0.48)	0.39 (0.42)	0.61 (0.56)
chalcone (**4**)	3.50 (2.09)	2.01 (2.10)	1.59 (1.71)	2.34 (2.28)

[a] Data represent capacity factors (k') determined using the relationship: $k' = (t - t_0)/t_0$, where t is the retention time of a given species and t_0 that of the void (determined by injection of acetone). Polymers were packed in Teflon-lined stainless steel chromatography columns (100 × 5.4 mm). Flow rate 0.35 mL min^{-1} and detection at 260 nm. Figures in parentheses are k' values in the absence of cobalt(II) acetate (0.05 M) in the buffer.

ing the remaining two coordination sites of the Co^{2+} complex. In addition to metal ion coordination, the pyridinyl residues would provide the base necessary for generation of the enolate of acetophenone (**2**). Styrene (**6**)−divinylbenzene (**7**) was used as the cross-linking agent; these monomers were expected to interact with **1** through $\pi-\pi$ stacking and van der Waals interactions to aid in defining the recognition site topography in the resultant polymer.

An empirical evaluation of polymer−substrate selectivity was carried out using the polymer as an HPLC stationary phase, see Table 1. Retention characteristics of the reaction substrates, product, and the imprint species were compared using a DBM-Co^{2+} imprinted polymer and a series of reference polymers imprinted with either Co^{2+}, DBM (**1**), or nothing. Recognition was shown to be dependent upon the presence of Co^{2+} in the elution buffer, with a substantial reduction in affinity for **1** observed in the absence of the ion, most notable in the case of the DBM-Co^{2+} MIP. Optimal recognition of **1**, the reactive intermediate analogue, was dependent upon both

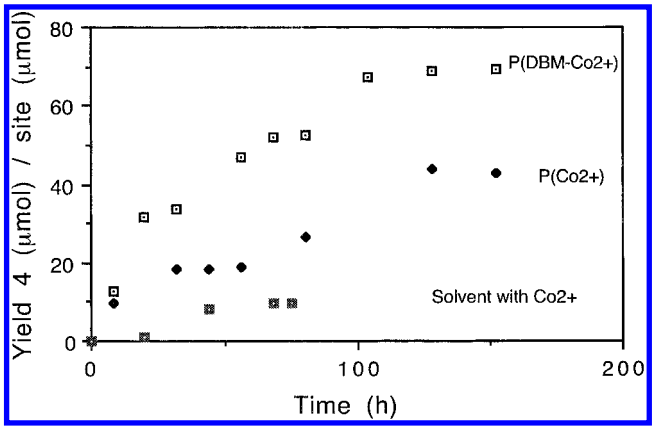

Figure 3. Production of chalcone (**4**) using DBM-Co^{2+} and Co^{2+} MIPs and solvent containing cobalt(II) acetate. Yields were calculated from the average of triplicate determinations of duplicate sets of reactions.

the presence of the coordinating metal and the complementary imprint site interactions, consistent with the assumptions made in the polymer design. Significantly, **1** showed a much higher affinity for the DBM-Co^{2+} MIP than either of the substrates or the reaction product. Moreover, the affinity of **1** for the DBM-Co^{2+} MIP was much higher than in any of the reference polymer systems.

To evaluate the role of the DBM-Co^{2+} recognition sites in mediating this aldol condensation, reactions were conducted in the presence of the DBM-Co^{2+} and the Co^{2+} MIPs and in solution in the presence of pyridine and cobalt(II) ion, Figure 2B. The rate of the DBM-Co^{2+} MIP-mediated reaction was eight-fold higher than the solution reaction and twice that of the Co^{2+} MIP reaction, Figure 3. Product yields were determined by reversed phase high performance liquid chromatographic analysis of the reaction mixtures. The ratios between substrate and product peak areas were compared to standard curves prepared using authentic product samples. HPLC based stability studies of reactants and products revealed negligible breakdown of materials under the reaction conditions and over the time courses employed in these studies. It is notable that products from these reactions were obtained on sufficient scale to permit direct spectroscopic analysis (mass spectrometry and ^{1}H-NMR) to further confirm their identity.

The presence of Co^{2+}, or another suitable cation, is a necessary requirement for catalysis of this aldol condensation under the conditions employed here.[21] As imprinted polymer recognition sites are of a heterogeneous nature,[22] not all the DBM-Co^{2+} sites will necessarily be active, nor are they all accessible to solvent and substrates. The contribution of inextricably bound Co^{2+} to the MIP reactivity was examined using reactions run with the washed DBM-Co^{2+} polymer only as the cobalt source. A very low background activity was observed, some 0.1% of that of the Co^{2+} incubated DBM-Co^{2+} polymer. The concentration of Co^{2+} present in MIP assay samples was determined by the spectrophotometric analysis of incubation mixture supernatants. This data provides a theoretical maximum number of "active" sites in the assayed polymers, *i.e.* assuming that all available Co^{2+} ions present in the polymer are active.

Table 2. Substrate Selectivity

ketone substrate	product	reactivity ratio[a]
2	**4**	2.0
8	**10**	1.3
9	**11**	1.4

[a] Reactivity ratios were determined from product yields (yield from DBM-Co^{2+} MIP/yield from Co^{2+} MIP) after 75 h of reaction.

Adamantyl methyl ketone (**8**) and 9-acetylanthracene (**9**) were substituted for **2** in a set of parallel experiments to investigate the degree of MIP substrate selectivity, see Table 2. Lower relative rate enhancements were observed for reaction of the bulkier substrates **8** and **9** with benzaldehyde (**3**) to form their respective aldol condensation products **10** and **11**. The greater nonspecific reactivity of **9** than that of **8** was attributed to its planar aromatic structure which allows for easier access into and greater affinity for the MIP through aromatic stacking. The lower selectivity for the alternate substrates lend support to the shape recognition effects previously observed for recognition in styrene−divinylbenzene MIPs.[23]

Long term experiments, of three weeks duration, afforded up to 80% conversion of starting materials to product, necessitating some 138 turnovers per theoretical active site.

To confirm that the imprint recognition site was also the catalytic center, **1** was tested for its ability to inhibit the polymer-catalyzed reaction ($V_{max} = 0.61 \pm 0.06$ μmol h^{-1}; Michaelis constant $K_m = 1.23 \pm 0.04$ μM), Figure 4. The concentration-dependent inhibition of chalcone (**3**) production by **1** ($K_i = 60 \pm 10$ μM) implies the presence of a specific reaction center in the polymer matrix. This is in accord with the chromatographic data, which demonstrated a higher affinity for DBM (**1**) by the DBM-Co^{2+}-imprinted polymer recognition sites, relative to the reaction substrates **2** and **3**. Substrate (1.42 ± 10 mmol g (polymer)$^{-1}$) was required to saturate high affinity nonspecific binding sites in the bulk polymer before evidence of reaction onset; this necessitated calculation of an apparate substrate concentration, S*, for use in kinetic analyses. We propose that the high K_i and low turnover result from the template structure (**1**) being a better product analogue than true transition state analogue for this reaction.

A number of polymer samples were recovered from reaction mixtures, exhaustively reextracted and reused for similar reactions. These materials retained 80−95% of their previous activity. No doubt, the reaction conditions employed here, 100 °C over several days, led to the gradual decomposition of the polymeric matrix, a plausable explanation for the reduction in activity.

Conclusions

The results presented demonstrate the use of molecular imprinting for preparing synthetic nonbiological macromolecules capable of enzymelike catalytic turnover, substrate selectivity, and rate enhancement. Furthermore, this is the first report of true enzymelike catalysis of C−C bond formation using MIPs. The simple preparation, high mechanical, thermal, and chemical stability, and the lack of need for biologically based protocols make molecularly imprinted polymer "artificial enzymes" interesting alternatives to their biological counterparts.

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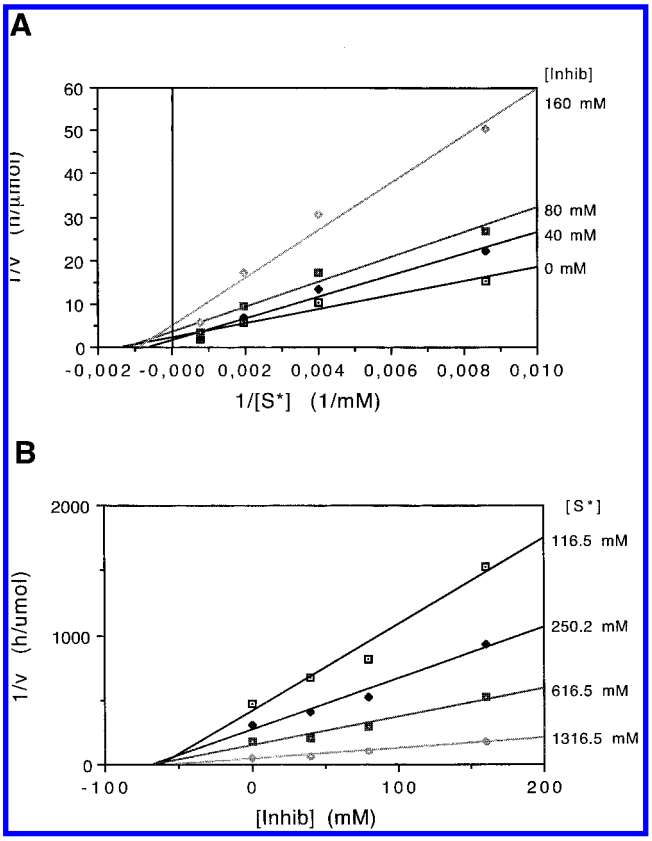

Figure 4. (A) Lineweaver-Burk plot for the production of chalcone (**4**) over a range of inhibitor concentrations [active site concentration 5.76 μmol g (polymer)$^{-1}$; maximum velocity $V_{max} = 0.61 \pm 0.06$ μmol h^{-1}; Michaelis constant $K_m = 1.23 \pm 0.04$ μM]. (B) Dixon plot ($K_i = 60 \pm 10$ μM].

Finally, the technique offers potential for developing tailor made catalysts, perhaps with catalytic functionalities not utilized in biology.

Experimental Section

General. All solvents (Labscan) and chemicals (Aldrich) were of HPLC or analytical grade. Monomers were distilled immediately prior to use.

MIP Preparation. DBM-Co^{2+} MIP: vinylpyridine (**5**) (420 mg), styrene (**6**) (4.20 g), and divinylbenzene (**7**) (5.20 g) were mixed with the imprint molecule, dibenzoylmethane (DBM) (**1**) (448 mg), 2,2′-azobis(2,4-dimethylvaleronitrile) (100 mg) and cobalt(II) acetate (498 mg) in anhydrous methanol (2.5 mL) and chloroform (6.7 mL), briefly sonicated under vacuum then sparged with dry nitrogen (5 min) at 0 °C. Polymerization was carried out at 45 °C (24 h). The bulk polymer was ground in a mechanical mortar and wet sieved (water/ethanol) through a 25 μm sieve. The *fines* were removed by repeated sedimentation from acetonitrile, and the sediment was collected on a 15 μm filter. The print molecule complex (DBM-Co^{2+}) was removed by packing the polymer in an HPLC column and washing with methanol/acetic acid (7/3, v/v) for 24 h at 1.0 mL min^{-1} and then methanol. The reference polymers were prepared and treated identically, except for the exclusion of one or both of DBM and cobalt(II) acetate. BET surface area measurements (Micromeritics Flowsorb II 2300 instrument, 30% N_2 in Ar) found areas of 3.67 and 2.30 m^2 g^{-1} for the DBM-Co^{2+} and Co^{2+} MIPs, respectively.

MIP Assays. Polymer samples were typically incubated at room temperature for 16 h with cobalt(II) acetate (10 mg/gram of polymer (dry weight)) in methanol (5.0 mL g (polymer)$^{-1}$). The polymers were collected on a 15 μm filter and then dried under vacuum. [Co^{2+} determinations were conducted in duplicate on triplicate polymer samples. The Co^{2+} present in the polymers were determined by the quantitative spectrophotometric (520 nm) analysis of the residual Co^{2+} in the filtrate: 5.76 (DBM-Co^{2+} MIP) and 5.44 (Co^{2+} MIP) μmol (site) g (polymer)$^{-1}$.] Co^{2+}-treated MIP samples (200 mg) were incubated with ketone (200 μmol) and benzaldehyde (200 μmol) in dry *N*,*N*′-dimethylformamide (1.0 mL). Solution reactions were carried out as above with pyridine (0.01 mL) and cobalt(II) acetate (8 μmol). Sealed reaction mixtures were heated in a thermostated oil bath at 100 °C. Aliquots (10 μL) were taken directly from reaction mixtures, and no significant difference was observed with results obtained by total extraction of the polymer matrix. Samples were analyzed by RP-HPLC using a Serva C-18 column (300 × 2 mm). Samples were serially diluted 100-fold in buffer before filtering and were run isocratically using methanol/water (75:25) as eluent at 0.35 mL min^{-1}. The production of **4** and **10** were monitored at 280 nm and **11** at 254 nm. Standard curves of concentration versus peak area and substrate/product peak ratios were prepared over the concentration ranges used in the studies for yield calculation. The reaction products **10**[24] and **11**[25] were prepared as described previously.

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