Molecular Imprinting of a Transition State Analogue Leads to a Polymer Exhibiting Esterolytic Activity

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Molecular imprinting of *p*-nitrophenyl methylphosphonate, a transition state analogue, in poly[4(5)-vinylimidazole] leads to a polymer which hydrolyses *p*-nitrophenol acetate at an increased rate and which can then be inhibited by addition of the *p*-nitrophenol methylphosphonate.

The formation of a polymer around a print, or template, molecule, a technique referred to as 'molecular imprinting,' has been utilized to create chiral binding cavities for the separation of racemates by either covalent[1] or non-covalent interactions.[2] We have previously shown that this recognition capacity may be combined with the catalytic activity of functional polymers to yield an esterolytic catalyst which distinguishes between different amino acid ester analogues.[3] Here, we report that a polymer exhibiting substrate-specific binding and increased activity can be formed in a manner analogous to that used for catalytic antibodies[4] but based on the approach of molecular imprinting.

As shown in Figure 1, the print molecule, *p*-nitrophenol methylphosphonate (**1**) is structurally similar to the substrate molecule *p*-nitrophenyl acetate (**2**), but contains a tetrahedral phosphoryl group in place of the carboxy group. Therefore, as with other phosphonate esters[4] (**1**), is expected to serve as a transition state analogue for the hydrolysis of (**2**). An imprint of (**1**) in the functionally active polymer[6]† poly [4(5)-vinylimidazole] (**3**) was formed by a modification of a method previously used to create metal binding sites in polymers.[7] A solution of cobalt(II) ions (1 mmol) and (**1**) (1 mmol) in deaerated methanol (2 ml) and a bifunctional cross-linker, 1,4-dibromobutane (2 mmol), was added with vigorous agitation to (**3**) (20 mmol imidazole units in 20 ml of deaerated

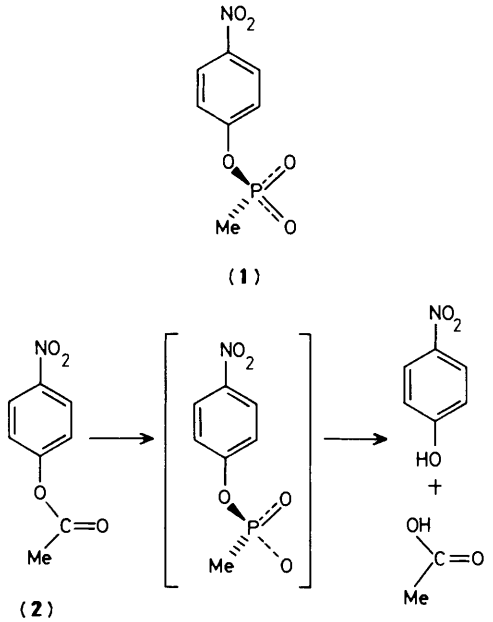

Figure 1. *p*-Nitrophenol methylphosphonate (**1**) was synthesised as described in the literature[5] and is expected to serve as a transition state analogue for the hydrolysis of *p*-nitrophenyl acetate (**2**).

† Cobalt forms a complex with (**3**). Spectroscopic data indicate that cobalt also forms a complex with (**1**) (data not shown). As such, cobalt could be essential for the proper imprinting of (**1**) and, later, the binding of (**2**). It is not yet clear whether the cobalt also functions catalytically in the observed hydrolysis of (**2**).

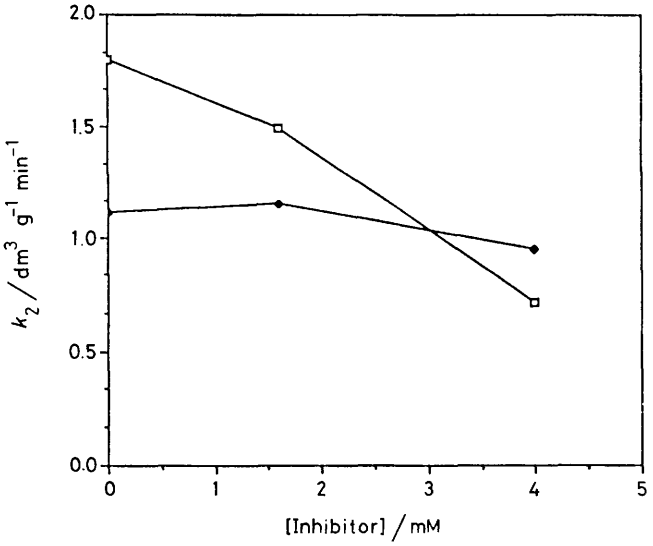

Figure 2. Inhibition of the hydrolysis of (**2**) by addition of the transition state analogue used as the print molecule: second-order rate constant *vs.* inhibitor concentration. The rate of hydrolysis of *p*-nitrophenol acetate by the non-print control polymer (◆) and the polymer imprinted with the transition state analogue (**1**) (□) was measured using (**1**) as an inhibitor. The hydrolysis of (**2**) was monitored as an increase in absorbance at 400 nm. Print and non-print polymers were suspended in 10% methanol : Tris, 0.05 M, pH 8.0, 0.5 M NaCl buffer at 25 °C. Kinetic constants were determined as given by ref. 5 and are based on a dry polymer concentration of 2 g l^{-1}.

methanol). After heating for 5 days at 65 °C (note that polymerisation conditions were not optimised), the resultant solid, blue cross-linked polymer, referred to as the 'print polymer,' was air-dried. The print molecule (**1**) was removed by extensive washing with phosphate buffer (100 mM, pH 7) and methanol. As a control, polymers were prepared in the presence of the cobalt but without (**1**) and are referred to as the 'non-print polymers.'†

Imidazole-containing polymers are known to catalyse the general hydrolysis of activated esters[6] and, as expected, both the print and non-print polymers catalysed the hydrolysis of (**2**) (Figure 2). However, the polymer imprinted with the transition state analogue (**1**) was 60% more active in hydrolysing the appropriate substrate (**2**) than was the non-print, control polymer. In order to show that this rate enhancement resulted from the molecular imprinting of (**1**), the hydrolytic activities of both polymers were determined using (**1**) as an inhibitor [owing to its higher stability, (**1**) was not hydrolysed by the polymers; data not shown]. As shown in Figure 1, as the concentration of (**1**) in the reaction mixture was increased, the hydrolytic activity of the print polymer towards (**2**) decreased steadily, while the activity of the non-print control was essentially unaffected.

In the control polymer, the randomly oriented imidazole moieties were present in excess. As such, their activity was not expected to be inhibited by interaction with (**1**). In the print polymer, on the other hand, binding cavities formed by molecular imprinting of (**1**) would not be available in excess. The observation that the increased hydrolytic activity of the print polymer was inhibited by interaction with (**1**) indicates that this increased activity was not a function of the bulk characteristics of the polymer, that is porosity or particle size. Rather, inhibition of the hydrolytic activity suggests that specific cavities of the transition state analogue have been obtained and that binding of the substrate molecules within these sites accounts for the measured rate enhancement.

Thus, molecular imprinting of a functionally active polymer (**3**) using a transition state analogue (**1**) as a template led to enhanced rates of ester hydrolysis. Most significantly, this rate enhancement was specifically inhibited by the transition state analogue. This provides strong evidence that the catalysis achieved is a function of providing specific binding sites within the functional polymer by molecular imprinting.

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