

Polyclonal antibody-catalysed hydrolysis of a β -lactam

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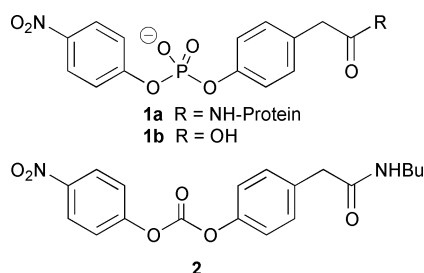
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We report the first example of antibody-catalysed hydrolysis of a β -lactam where the antibodies were generated by a simple transition-state analogue; in this example the antibodies are polyclonal.

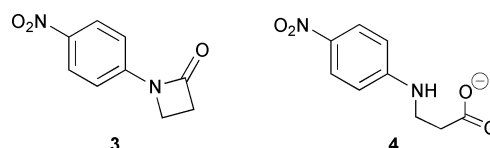
Since the first demonstration^{1,2} of catalytic antibodies, the field has grown rapidly with large numbers of catalytic antibodies being described^{3,4} for a range of chemical reactions. However, although there is a plethora of antibodies that catalyse the hydrolysis of esters and carbonates, only a few^{5–11} have been generated that catalyse the hydrolysis of amides. Consequently the production of catalytic antibodies for amide hydrolysis is the focus of considerable current effort^{3,4,12} and success promises a wealth of applications in medicine and biotechnology.

Here we report that antibodies that were designed to catalyse the hydrolysis of a carbonate have now been found to catalyse also the hydrolysis of a structurally related amide, a β -lactam. This is the first indication that it is possible to obtain catalytic antibodies with β -lactamase activity by immunisation with a simple transition-state analogue. We have shown previously¹³ that polyclonal antibodies generated by the transition state analogue **1a** catalyse the hydrolysis of the carbonate **2**, and these catalytic antibodies have been the subject of extensive investigation.^{14,15} We work with total IgG purified¹³ from sheep antisera (*via* salt fractionation, and chromatography over protein G then Sephadex), and our most recent report¹⁶ established that a polyclonal catalytic antibody preparation contained at most 8% catalytic antibodies, and probably less than 1%.



Current work involves studies with a variety of 4-nitroanilides and 4-nitrophenyl esters and carbonates, with the aim of determining the minimum recognition features required for catalysis by these antibody preparations. One such compound is *N*-(4-nitrophenyl)azetidin-2-one **3**.¹⁷ This β -lactam has the following advantages as a substrate for catalytic antibody investigations: (i) it is a reactive amide whose hydrolysis is activated by both ring strain and electronic effects, (ii) the product of hydrolysis, the amino acid carboxylate **4**, contains a nitroaniline chromophore which facilitates kinetic measurements, (iii) base promoted hydrolysis, in the absence of catalyst, is well characterised,^{12,18} (iv) the nitrophenyl group provides recognition and binding in antibody studies, (v)

product inhibition is unlikely because the ring-opened product is very different from both the substrate and the phosphate transition state analogue, and lastly (vi) it is intermediate in reactivity between a nitrophenyl ester and a nitroanilide. Given these advantages it is surprising that this structure has not been the subject of catalytic antibody investigations until now.



We here report that hydrolysis of the β -lactam **3** is accelerated by the anti-nitrophenylphosphate antibodies at pH values between 8 and 10. This rate acceleration is completely inhibited by the phosphate transition state analogue **1b**, demonstrating that the observed activity is due to a binding site specific for the transition state analogue **1b** (preliminary experiments give a value for K_i of 1.5 μ M). For the antibody preparation 270-26,¹³ (2 μ M IgG, pH 9, 37 °C) a plot of initial rate for the catalysed reaction against concentration of substrate **3** is shown in Fig. 1. At this pH the catalysed reaction has $K_M = 30 \mu$ M and $k_{cat} = 1.3 \times 10^{-5} \text{ s}^{-1}$, on the assumption that the concentration of catalytic sites = $2 \times [\text{IgG}]$. If the proportion of catalytic antibodies is assumed to be 8%¹⁶ then $k_{cat} = 1.6 \times 10^{-4} \text{ s}^{-1}$. This antibody activity is sustained for multiple turnovers (the catalytic reaction was followed for more than 6 turnovers).

Further evidence that the catalysed hydrolysis of the β -lactam is indeed antibody-mediated was provided by investigations of substrate specificity. The hydrolyses of *N*-(2-nitrophenyl)azetidin-2-one **5**¹⁷ (the 2-substituted analogue) and 4-nitrophenylformanilide **6** were not catalysed by the catalytic antibody preparation 270-26. It is of particular interest that the formanilide **6** is more labile towards hydrolysis than the substrate β -lactam **3**. One possible explanation for this observation might

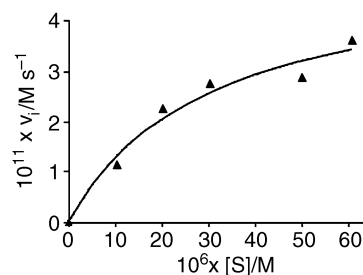
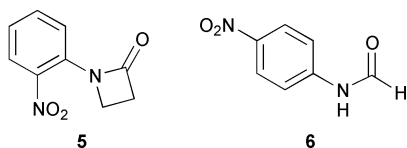
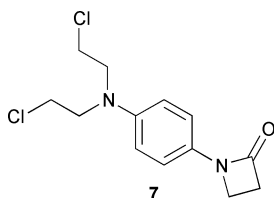


Fig. 1 Plot of initial rate against concentration of substrate **3**. The points represent values obtained in initial rate experiments corrected for background hydrolysis ($k_{\text{non-cat}} = 6.4 \times 10^{-6} \text{ s}^{-1}$), and the curve represents best fit values of V_{max} ($5.11 \pm 0.78 \times 10^{-11} \text{ M s}^{-1}$) and K_M ($30 \pm 10 \mu\text{M}$) obtained by fitting the data to the Michaelis-Menten equation by non-linear regression using an unweighted least squares analysis ($r^2 = 0.974$).

be a different binding mode in the case of the formanilide such that catalysis does not occur. These data demonstrate that the catalysis is both specific for the 4-nitro substituent and is extremely limited in tolerance for alternative reaction centres, characteristics consistent with antibody catalysis.



We have demonstrated antibody catalysis of the hydrolysis of an *N*-aryl- β -lactam by antibodies raised to an immunogen that was not designed for this purpose. There have been only two other reports of antibody-catalysed hydrolysis of β -lactams. One of these antibodies was produced¹⁰ by an antiidiotypic approach, and the other¹¹ *via* reactive immunisation. Therefore, the example described above is the first report of antibodies with β -lactamase activity generated *via* immunisation with a simple transition-state analogue. The use of immunogens more closely related to the substrate structure (*e.g.* cyclic phosphonates) would be expected to produce antibodies with improved β -lactamase activity. The combination of stability ($t_{0.5} \sim 2$ weeks at pH 8) and susceptibility to antibody-catalysed hydrolysis makes *N*-aryl- β -lactams attractive targets for the development of prodrugs that can be activated by catalytic antibodies. For example, we are now investigating the β -lactam **7** as a potential nitrogen mustard prodrug for use in Antibody-Directed Abzyme Prodrug Therapy (ADAPT).¹⁹



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