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Polyclonal antibody-catalysed hydrolysis of a β-lactam

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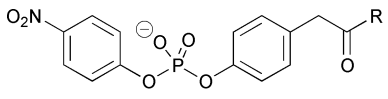
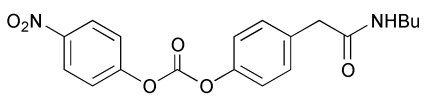
We report the first example of antibody-catalysed hydrolysis of a β-lactam where the antibodies were generated by a simple transition-state analogue; in this example the antibodies are polyclonal.

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 previously[13] 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 purified[13] from sheep antisera (*via* salt fractionation, and chromatography over protein G then Sephadex), and our most recent report[16] established that a polyclonal catalytic antibody preparation contained at most 8% catalytic antibodies, and probably less than 1%.



1a R = NH-Protein
1b R = OH



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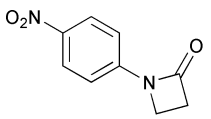
Current work involves studies with a variety of 4-nitroanilides and 4-nitrophenyl esters and carbonates, with the aim of determining the minimum recognition features required for catalysis by these antibody preparations. One such compound is *N*-(4-nitrophenyl)azetidin-2-one **3**.[17] This β-lactam 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.



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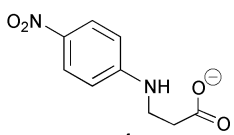

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We here report that hydrolysis of the β-lactam **3** is accelerated by the anti-nitrophenylphosphate antibodies at pH values between 8 and 10. This rate acceleration is completely inhibited by the phosphate transition state analogue **1b**, demonstrating 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 1.5μM). For the antibody preparation 270-26,[13] (2 μM 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 μM and k_{cat} = 1.3×10^{-5} s^{-1}, on the assumption that the concentration of catalytic sites = 2 × [IgG]. If the proportion of catalytic antibodies is assumed to be 8%[16] then k_{cat} = 1.6×10^{-4} 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

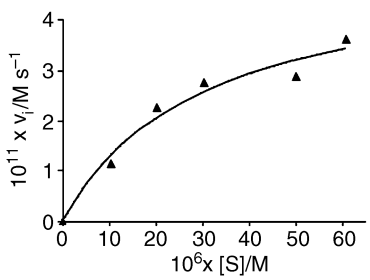

Fig. 1 Plot of initial rate against concentration of substrate **3**. The points represent values obtained in initial rate experiments corrected for background hydrolysis ($k_{non\text{-}cat}$ = 6.4×10^{-6} s^{-1}), and the curve represents best fit values of V_{max} ($5.11 \pm 0.78 \times 10^{-11}$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

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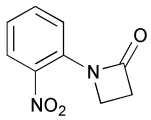
be a different binding mode in the case of the formanilide such that catalysis does not occur. These data demonstrate that the catalysis is both specific for the 4-nitro substituent and is extremely limited in tolerance for alternative reaction centres, characteristics consistent with antibody catalysis.



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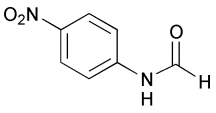

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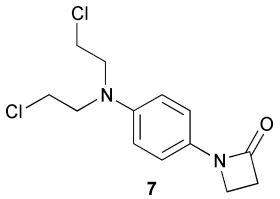
We have demonstrated antibody catalysis of the hydrolysis of an *N*-aryl-β-lactam by antibodies raised to an immunogen that was not designed for this purpose. There have been only two other reports of antibody-catalysed hydrolysis of β-lactams. One of these antibodies was produced[10] by an antiidiotypic approach, and the other[11] *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 ($t_{0.5}$ ~ 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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