Regio- and Stereoselective Deprotection of Acylated Carbohydrates via Catalytic Antibodies

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Considering the inherent high binding specificity of antibodies, catalytic antibodies in which the substrate specificity and the reaction pathway can be programmed by the hapten could provide powerful tools for regio- and stereoselective organic synthesis.[1] In particular, regioselectively protected and/or deprotected carbohydrates in oligosaccharide synthesis are interesting targets for testing the potential of catalytic antibodies. Although many efforts have been mounted to develop regio- and stereoselective syntheses of oligosaccharides, the problem of differential protection of potentially competing functional groups is still formidable.[2] For instance, conventional means for selection of just one of the five hydroxyl groups of glucose to function as a glycosyl acceptor or donor typically requires several steps of protection and deprotection, followed by tedious chromatographic purification.[3] Herein, we report the generation of catalytic antibodies that are capable of discriminating between chemically identical functional groups in the same molecule, catalyzing regioselective deprotection of acylated carbohydrates.

To extend catalytic antibody technology for practical use, we focused on the generation of catalytic antibodies that possess both the desired specificity and applicability to a wide range of substrates. Broad applicability is particularly important as antibody-catalyzed reactions are frequently too specific toward their substrates.[4] In contrast, natural enzymes, such as serine proteases, which also perform specific reactions, are able to do so on a wide range of substrates.[5] X-ray structural analyses have shown a negative relationship between the size of a hapten and the percentage of its surface area buried upon binding to the antigen-combining site.[6] For example, a small hapten such as fluorescein buries 94% of its surface area upon binding, while relatively large haptens use only a limited surface area. Thus, the unburied surface area of a large hapten should allow binding

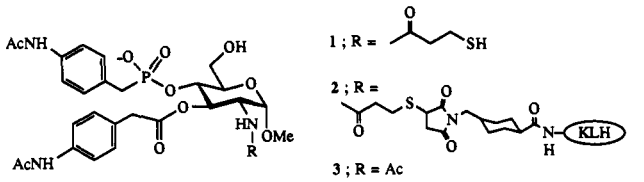
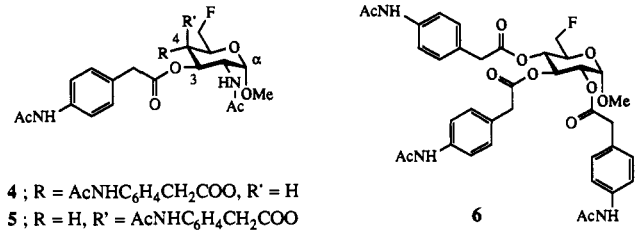


Figure 1.



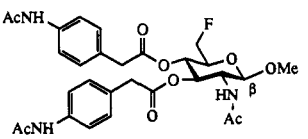


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Figure 2.

to a variety of substrates. For immunization, therefore, we decided to use a phosphonate transition-state analog **1** with two 4-(*N*-acetylamino)phenylacetyl groups at C-3 and C-4. The combined size of the two ester groups is enough to occupy the antigen-combining site,[7] leaving the remaining moiety free. Such catalytic antibodies should be capable of recognizing the regio- and stereochemistry at C-3 and C-4. Thus, the ester group at C-4 should be hydrolyzed exclusively in acylated carbohydrates possessing a variety of substituents at C-1, C-2, and C-6.

Phosphonate **1**[8] was coupled to the carrier proteins KLH (keyhole limpet hemocyanin) and BSA (bovine serum albumin) (Figure 1).[9] Mice were immunized with the KLH conjugate **2**, and antibodies were generated by standard protocols.[10] Ascites fluid was purified via cation-exchange chromatography (Mono S, Pharmacia), followed by affinity chromatography (Protein G) as described previously.[11] The 12 monoclonal antibodies that bound to the hapten on ELISA were then screened for their ability to catalyze the hydrolysis of diesters **4** (Figure 2).[12] The reaction rates were followed by monitoring the production of 4-(*N*-acetylamino)phenylacetic acid via high-performance liquid chromatography (HPLC). Two antibodies, 17E11 and 20C6, were found to catalyze the hydrolysis at a rate significantly above the uncatalyzed background reaction. One antibody, 17E11, was characterized in more detail.

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(3) In some cases, enzymes offer the opportunity of conducting one-step preparations in a regioselective manner: (a) Drueckhammer, D. G.; Hennen, W. J.; Pederson, R. L.; Barbas, C. F.; Gautheron C. M.; Krach, T.; Wong, C.-H. *Synthesis* **1991**, 499–525. (b) Sweers, H. M.; Wong, C.-H. *J. Am. Chem. Soc.* **1986**, *108*, 6421–6422.

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(5) Fersht, A. *Enzyme Structure and Mechanism*; Freeman: New York, 1985; pp 405–426.

(6) (a) Padlan, E. A.; Cohen, G. H.; Davis, D. R. *Ann. Inst. Pasteur/Immunol.* **1985**, *136C*, 259–294. (b) Horron, J. N.; He, X.-M.; Mason, M. L.; Voss, E. W., Jr.; Edmundson, A. B. *Proteins* **1989**, *5*, 271–280. (c) Stanfield, R. L.; Fieser, T. M.; Lerner, L. A.; Wilson, I. A. *Science* **1990**, *248*, 712–719. (d) Sheriff, S.; Silverton, E. W.; Padlan, E. A.; Cohen, G. H.; Smith-Gill, S. J.; Fizel, B. C.; Davies, D. R. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 8075–8079. (e) Turip, W. R.; Varghese, J. N.; Webster, R. G.; Air, G. M.; Laver, W. G.; Colman, P. M. *Cold Spring Harbor Symp. on Quant. Biol* **1989**, *54*, 257–263.

(7) Based on a calculation using Insight II with a 1.4-Å probe sphere and van der Waals radii, the total solvent accessible area of the two-ester moiety on the extended structure was estimated to be 576 Å^2.

(8) Phosphonate **1** was prepared from methyl 2-((benzyloxycarbonyl)-amino)-2-deoxy-α-D-glucopyranoside in six steps: (i) 4-methoxybenzaldehyde dimethyl acetal, *p*-TsOH, DMF (98%); (ii) 4-(*N*-acetylamino)phenylacetic acid, DCC, pyridine (70%); (iii) $NaBH_3CN$, CF_3COOH, 3-Å molecular sieves, DMF (75%); (iv) 4-(*N*-acetylamino)benzylphosphonic acid, DCC, pyridine (34%); (v) H_2, $Pd(OH)_2$, MeOH then *N*-succinimidyl 3-(2-pyridyldithio)-propionate, phosphate buffer, pH 8.0 (83%); (vi) dithiothreitol, phosphate buffer, pH 8.0.

(9) Maleimide-activated carrier protein (Imject, PIERCE) was used for the conjugation. The degree of conjugation per mole of carrier protein was estimated by titrating the thiol group of the phosphonate **1** remaining unreacted after the conjugation with Ellman's reagent: 11 haptens/KLH (calculated as a molecular weight of 100 000) and 17 haptens/BSA .

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(12) For assays of hydrolytic activity, fluoride derivatives at C-6 were used to prevent complex acyl migrations involved in hydrolyses of acylated carbohydrates.

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Antibody 17E11 catalyzed the hydrolysis of **4** in a manner consistent with Michaelis–Menten kinetics.[13] A Lineweaver–Burk plot of the steady-state data afforded a k_{cat} of 0.182 min^{-1} and a K_m of 6.6 μM. Comparison of the k_{cat} value with the rate constant for the uncatalyzed reaction gives a 2.74×10^3-fold rate acceleration.[14] The antibody-catalyzed reaction was competitively inhibited by phosphonate **3**, demonstrating that catalysis takes place against diester **4** in the antibody combining site. A Dixon analysis with hapten **1** afforded a K_i of 0.026 μM. No rate enhancement of hydrolysis toward either monosubstituted ester (4-OH or 3-OH) was observed.

In order to investigate the regio- and stereoselectivity of antibody 17E11, the reaction was assayed using 2 μM highly purified antibody and 150 μM diesters **4** in 10% DMSO/50 mM Tris buffer, pH 8.2, 20 °C. The hydrolysis of diester **4** involves deacylation followed by acyl migration between C-4 and C-3. In a spontaneous reaction, the rate of acyl migration was much faster than that of deacylation, giving an equilibrium mixture (4:1) of 4-OH and 3-OH in the hydrolysis.[15] On the other hand, antibody 17E11 catalyzed the hydrolysis of **4** at C-4 with high regioselectivity, affording 4-OH and 3-OH in a ratio of 20:1 (60 min, 26% conversion), despite the accompanying acyl migration. Furthermore, using 30 μM of 17E11, the 4-OH was produced exclusively with no detectable 3-OH, even after the starting material had been completely consumed (20 min). In addition to exhibiting regioselectivity, the antibody-catalyzed reaction demonstrated a strong ability to discern the stereochemistry at C-4 by hydrolyzing a glucopyranoside **4** but not a galactopyranoside **5** bearing an axial ester group at C-4.

(13) Fersht, A. *Enzyme Structure and Mechanism*; Freeman: New York, 1985; pp 98–118.

(14) The background rate of the hydrolysis without antibodies was determined to be 6.64×10^{-5} min^{-1}.

(15) The appearance of the products, 4-OH and 3-OH, was followed by RP-HPLC on a C-18 column eluted with water/acetonitrile (75/25)/0.1% TFA at a flow rate of 1 mL/min, with UV detection at 254 nm. Retention times: **4**, 21.6 min; 4-OH, 5.4 min; 3-OH, 6.9 min.

We next examined the substrate specificity of this antibody-catalyzed reaction. Using the triester **6** as a substrate, the spontaneous reaction in 50 mM Tris (pH 8.2) afforded a mixture of regioisomers (2-OH:3-OH:4-OH = 1:0.07:0.2).[16] In contrast, antibody 17E11 (1.0 μM) catalyzed the hydrolysis of the triester **6** (50 μM) with high regioselectivity, giving the 4-OH as the major product (2-OH:3-OH:4-OH = 1:0.8:13; 90 min, 18% conversion). This indicates a 91-fold enhancement in the rate (v_{cat}/v_{uncat}) of catalyzed vs uncatalyzed hydrolysis of the ester at C-4. No rate enhancement of hydrolysis of the ester at C-2 was observed. Further analysis revealed that regioselective deacylation of substrate **7**, possessing the glycosyl moiety in a β-configuration, was also catalyzed by 17E11. Thus, antibody 17E11 may be suitable for regioselective hydrolysis of nonreducing termini in a variety of oligosaccharides.

In this work, we demonstrate a simple strategy for the generation of catalytic antibodies possessing both regio- and stereoselectivity, as well as their suitability for deprotection of acylated carbohydrates in a wide range of substrates. These antibody catalysts are potentially useful reagents for the synthesis of complex oligosaccharides.

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Supplementary Material Available: Experimental details of the preparation and characterization of substrates **4–7** and their products (9 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(16) The reaction rates were followed by monitoring the production of 4-(*N*-acetylamino)phenylacetic acid by RP-HPLC on a C-18 column eluted with water/acetonitrile (65/35)/0.1% TFA at a flow rate of 1 mL/min, with UV detection at 254 nm. Retention times: **6**, 19.0 min; 4-OH, 7.7 min; 3-OH, 8.7 min; 2-OH 6.8 min.