Structure of the Michaelis Complex of an Efficient Antibody Acyl Transferase Determined by Transferred Nuclear Overhauser Enhancement Spectroscopy

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The use of phosphonates and phosphonamidates as transition-state analogues has proven a powerful approach for generating antibodies that catalyze a variety of acyl group transfer reactions, including ester[1] and amide[2] bond hydrolysis as well as transesterification[3] and peptide bond forming reactions.[4] A number of these antibodies are highly efficient catalysts, including antibody 13D6.1, which was elicited against phosphonate diester **4** and catalyzes the transesterification of ester **2a** to alcohol **1** with an effective molarity (k_{cat}/k_{uncat}) of 27 000 M (Figure 1).[3b] Moreover, the antibody does not catalyze the hydrolysis of **2a**. To better understand the mechanistic basis for the high selectivity and catalytic efficiency of this antibody, we have determined the structure of substrates **1** and **2b** bound simultaneously in the 13D6.1 combining site using transferred nuclear Overhauser enhancement spectroscopy (tr-NOESY).[5] The bimolecular Michaelis structure reveals that the antibody orients the two substrates in an optimal stereoelectronic configuration for the acyl transfer reaction.

Solutions of substrate and protein for tr-NOESY experiments were prepared in the manner previously described.[6] A methyl ester (substrate **2b**) was used in place of the activated cyanomethyl ester **2a** to slow turnover for the spectroscopy experiments. Because antibody 13D6.1 has been previously shown to efficiently catalyze acyl transfer reactions using a wide variety of activated esters,[3b] the relatively subtle substitution of **2b** for **2a** was not expected to measurably affect the structure of the Michaelis complex. NMR samples contained 10 mM $NaHPO_4$ or H_3BO_3, 50 mM NaCl, 2 mM each of **1** and **2b**, 10% CD_3OD, and 50 μM antibody 13D6.1 at pH 7.0, in either D_2O or H_2O. NMR spectra for analysis of the Michaelis complex were acquired at 500 MHz using standard ^{1}H-NOESY pulse sequences with 1-s presaturation or WATERGATE[17] for solvent suppression, at nine mixing times ranging from 50 to 600 ms (18 °C). Distances were determined from initial buildup rates.[7] Rapid chemical exchange was confirmed by titration experiments, and introduction of hapten **4** eliminated large molecule NOEs for substrates **1** and **2b** in the presence of antibody 13D6.1.

A total of 38 inter- and intramolecular NOEs were used to generate distance constraints[8] for Monte Carlo conformational searches and constrained minimizations (Table 1). Intramolecular constraints alone were sufficient to determine the bound conformation of substrate **1** from conformational searches.[9] The lowest energy conformer of **1** was then fixed and used in subsequent Monte Carlo searches with substrate **2b** to determine the relative orientation of the two bound substrates, according to both intermolecular and **2b** intramolecular constraints.[10] The resulting low-energy Monte Carlo conformations were found to have a hydrogen bond between the carbonyl oxygen and the 3′-hydroxyl proton. To remove any artificial bias in this region of the structure due to the hydrogen bond, carbonyl rotamers (about the ψ torsion) were tested at 30° increments over a full 360° in separate minimization runs.[9b] Final structures were lower in energy by roughly 24 kcal mol^{-1} than those prior to ψ rotation, and those lowest in

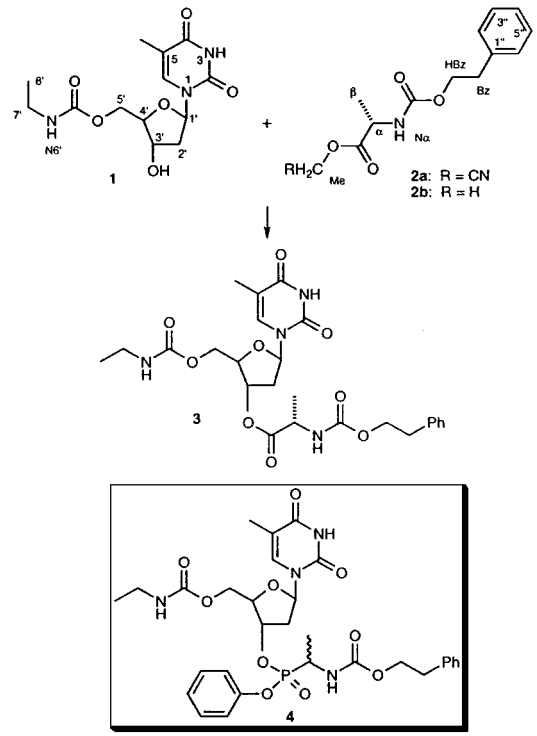

Figure 1. Antibody-catalyzed transesterification reaction. Substrates **1** and **2b** were used for structure determination; compound **4** is the hapten.

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(1) (a) Tramontano, A.; Janda, K. D.; Lerner, R. A. *Science* **1986**, *234*, 1566−1570. (b) Pollack, S. J.; Jacobs, J. W.; Schultz, P. G. *Science* **1986**, *234*, 1570−1573. (c) Jacobs, J.; Schultz, P. G.; Sugasawara, R.; Powell, M. *J. Am. Chem. Soc.* **1987**, *109*, 2174−2176.

(2) Janda, K. D.; Schloeder, D.; Benkovic, S. J.; Lerner, R. A. *Science* **1988**, *241*, 1188−1191.

(3) (a) Wirsching, P.; Ashley, J. A.; Benkovic, S. J.; Janda, K. D.; Lerner, R. A. *Science* **1994**, *252*, 680−685. (b) Jacobsen, J. R.; Prudent, J. R.; Kochersperger, L.; Yonkovich, S.; Schultz, P. G. *Science* **1992**, *256*, 365−367.

(4) Jacobsen, J. R.; Schultz, P. G. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 5888−5892.

(5) Ni, F. *Prog. NMR Spectrosc.* **1994**, *26*, 517−606.

(6) Driggers, E. M.; Cho, H. S.; Liu, C. W.; Braisted, A. C.; Katzka, C. P.; Ulrich, H. D.; Wemmer, D. E.; Schultz, P. G. *J. Am. Chem. Soc.* **1998**, *120*, 1945−1958.

(7) The reference distance, r_{ref}, is 2.97 Å, measured from H6 to the geometric pseudoatom position of the C5-methyl group. Interproton distances, r_{ij}, were calculated from linear initial NOE buildups (σ) using the two-spin approximation, $r_{ij} = r_{ref}(\sigma_{ref}/\sigma_{ij})^{1/6}$. All NOEs were scaled for multiplicity (Yip, P. F. *J. Magn. Reson.* **1990**, *90*, 382−383).

(8) Lower bounds = 1.80 Å, upper bounds = calculated distances + 20% to account for spectral signal-to-noise. Diastereotopically indistinguisable protons and nonlinear NOE buildups were treated according to the following: Williamson, M. P.; Havel, T. F.; Wüthrich, K. *J. Mol. Biol.* **1985**, *182*, 295−315. Clore, G. M.; Gronenborn, A. M.; Brünger, A. T.; Karplus, M. *J. Mol. Biol.* **1985**, *186*, 435−455. These are similar to approaches used by the following: Ni, F.; Meinwald, Y. C.; Vásquez, M.; Scheraga, H. A. *Biochem.* **1989**, *28*, 3094−3105. Wakamatsu, K.; Okada, A.; Miyazawa, T.; Ohya, M.; Higashijima, T. *Biochemistry* **1992**, *31*, 5654−5660.

(9) (a) Conformational searches of substrate **1** were performed using MacroModel v6.0 (Clark Still group, Columbia University) with AMBER* (McDonald, D. Q.; Still, W. C. *Tetrahedron Lett.* **1992**, *33*, 7743−7746) in bulk water solvent with 10 000 Monte Carlo and 2000 minimization steps (conjugate gradient with maximum derivative of <1 kJ Å^{-1}). (b) The lowest energy converged conformations were subjected to a final minimization with the same distance constraints using Insight II 95.0/Discover (MSI), allowing explicit pseudo-atom approximations for diastereotopically indistinguishable protons (AMBER; 2000 conjugate gradient steps with maximum derivative of <0.01 kcal Å^{-1}).

(10) Identical to ref 9 except that **1** was fixed and **2b** was allowed torsional freedom, translation up to 1 Å, and rotation up to 180° prior to each Monte Carlo step; 5000 minimization iterations were performed following each Monte Carlo step.

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Table 1. Sets of Interproton Contacts Used To Define Constraints for Conformational Searches and Minimizations

intra-**1**		intra-**2b**		inter **1**−**2b**	
C5M-	2′	*β*-	Bz	1′-	Bz
	7′		HBz		2″
	8′		Me	2′-	2″
6-	1′		2″		4″
	2′		4″		Bz
	3′	Bz-	2″		HBz
	5′		4″		*β*
	7′	HBz-	2″	3′-	Bz
	8′		4″		*β*
2′-	7′			5′-	Me
3′-	7′			C5M-	2″
N6′-	C5M				4″
	6				Bz
					HBz
				6-	Bz
					HBz

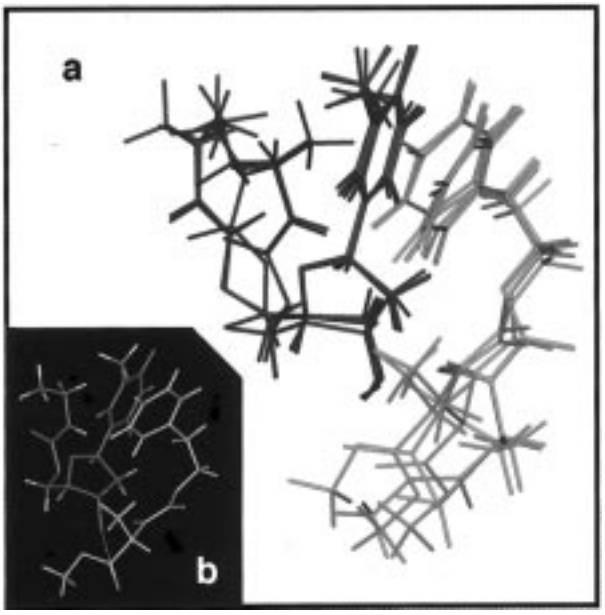

Figure 2. Representations of the Michaelis complex of **1** (blue) and **2b** (orange): (a) family of the five lowest energy structures and (b) single structure indicating the nucleophile approach path.

energy constitute the family of structures analyzed (Figure 2a). The constraints are violated a total of two times in all of the structures, with the sum total of distance violations being <0.07 Å.

In the bound state, the thymine base of substrate **1** is in an *anti* conformation with respect to the deoxyribose ring. The deoxyribose ring itself adopts a $C_{3'}$-*endo* conformation (N-type).[11] The 5′-ethyl carbamate substituent of **1**, analogous to the linker portion of hapten **4**, is held proximal to the C_5-methyl and H6 of the thymine ring in the bound structure. The relative position of the 5′-ethyl carbamate is relatively poorly defined, as indicated by the variety of conformations in the final family of structures; differences in local geometry at the 5′-ethyl carbamate and the linker of hapten **4** probably account for this variability. Consistent with this conformational variability, NOEs between substrate **2b** and the 5′-ethyl carbamate portion of **1** were weak or absent at several mixing times, and not included in the set of intermolecular constraints.

Strong intermolecular contacts between the benzylic, homobenzylic, and aromatic protons of substrate **2b** and the 1′, 2′, and C5-methyl protons of substrate **1** indicate that the phenyl ring and the thymine base are in close proximity in the antibody combining site and tightly define this portion of the structure (Figure 2a). The lowest energy conformations place the two aromatic rings in a π-stacking interaction, although T-stacking cannot be ruled out due to the axis of symmetry in the aromatic ring.

The geometry around the reaction center is defined by intramolecular constraints for substrate **2b**, weak constraints between the β-methyl protons of **2b** and the 3′ protons of substrate **1**, and a weak constraint between the methyl ester protons and H5′. Intermolecular NOEs to Hα are ambiguous due to its degeneracy with H5′ and H4′; thus no Hα NOEs were used to generate constraints. Substrate orientation at the site of reaction is consequently less well defined than other portions of the structure but still sufficiently determined to allow an analysis in the context of known parameters for acyl transfer chemistry. The optimal angle for attack on a formaldehyde carbonyl carbon by hydride anion was determined on the basis of early ab initio modeling to be 109.5°.[12] However, calculations[13] and analysis of crystallographic data[14] from a variety of nucleophiles and electrophilic carbonyl species indicate that the angle of attack can vary up to ≈30°. The final family of Michaelis structures positions the nucleophilic 3′-hydroxyl group along an angle of attack varying from 80° to 117° (average = 98.4°). The closest approach distance for a nucleophile to an acyl carbon prior to van der Waals contact is 3.0 Å.[13b,14] The distance from nucleophile to electrophile in the Michaelis structure varies from 3.1 to 3.5 Å, a value roughly 0.3 Å longer than the contact distance.

These experiments show that binding interactions of substrates **1** and **2b** with antibody 13D6.1 place the 3′-hydroxyl of **1** almost in contact with the carbonyl carbon of **2b**,[15] along an open path for attack which varies up to 29° from the optimum. This favorable orientation likely contributes to the high catalytic efficiency of antibody 13D6.1. *Ab initio* calculations (MP2/6-31+G*) of representative phosphonates indicate that the covalent geometry of hapten **4** positions the 3′-oxygen and phosphorus atoms (the carbonyl group mimic) a distance of 1.8 Å apart at an angle of 114°.[16] The degree to which substrate orientation in 13D6.1 reflects this structure, and as a result follows an optimal trajectory, underscores the power of immunological diversity when combined with proper chemical instruction.

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Supporting Information Available: ^{1}H NMR spectra of substrates with assignments, titration spectra verifying fast exchange conditions, a sample NOESY spectrum (150 ms) of **1** and **2b** in the presence of 13D6.1, and a sampling of NOE buildup curves used to determine distance constraints (5 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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(11) Saenger, W. *Principles of Nucleic Acid Structure*, 1st ed.; Springer-Verlag: New York, 1984.

(12) (a) Burgi, H. B.; Lehn, J. M.; Wipff, G. J. *J. Am. Chem. Soc.* **1974**, *96*, 1956−1957. (b) Burgi, H. B.; Dunitz, J. D.; Shefter, E. *J. Am. Chem. Soc.* **1973**, *95*, 5065−5067. (A 90° angle is defined as normal to the $C_\alpha-C_{CO}-O_{CO}$ plane, with 0° lying along the C═O bond and 180° bisecting its substituents.)

(13) (a) Scheiner, S.; Lipscomb, W. N.; Kleier, D. A. *J. Am. Chem. Soc.* **1976**, *98*, 4770−4777. (b) Lightstone, F. C.; Bruice, T. C. *J. Am. Chem. Soc.* **1996**, *118*, 2595−2605.

(14) Burgi, H. B.; Dunitz, J. D.; Shefter, E. *Acta Crystallogr.* **1974**, *B30*, 1517−1527.

(15) As a control, a Monte Carlo search in which the nucleophile was allowed to approach no closer than 6 Å from the carbonyl C was carried out.[10] The lowest energy structures from this search adopt unreactive orientations, with an obstructed path between the 3′-hydroxyl and the carbonyl C.

(16) Teraishi, K.; Saito, M.; Fujii, I.; Nakamura, H. *Tetrahedron Lett.* **1992**, *47*, 7153−7156.

(17) Piotto, M.; Saudek, V.; Sklenar, V. *J. Biol. NMR* **1992**, *2*, 661−665.