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Antibody-catalyzed aminolysis of a chloropyrimidine derivative†

Fujie Tanaka,* Fabiola Flores, Diane Kubitz, Richard A. Lerner* and Carlos F. Barbas, III*
The Skaggs Institute for Chemical Biology and the Departments of Chemistry and Molecular Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, USA.
E-mail: carlos@scripps.edu, ftanaka@scripps.edu; Fax: +1-858-784-2583

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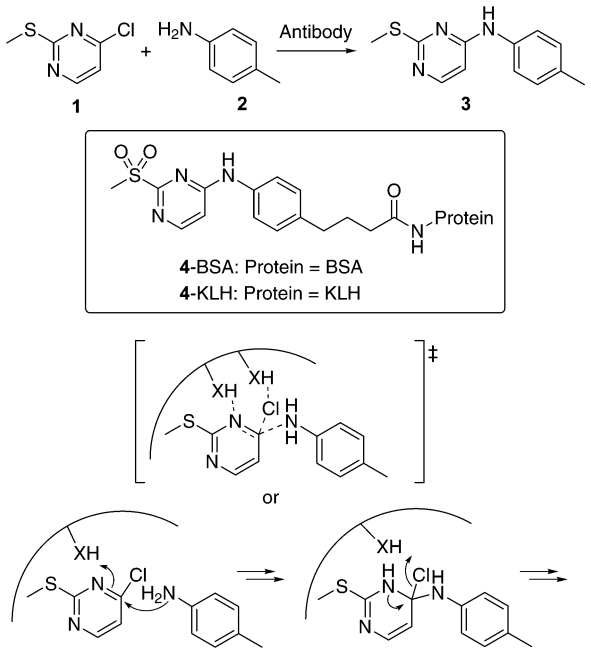
We have generated antibody FTB8E9 by immunization with the designed hapten to catalyze the aminolysis reaction of a chloropyrimidine derivative.

Amino group-substituted pyrimidine derivatives are important as enzyme inhibitors, receptor ligands, and their precursors.[1] Aminolysis of chloropyrimidine derivatives is a key reaction for accessing these compounds. Recently, small molecules were used to accelerate the aminolysis of 6-chloropurine derivatives through hydrogen bond formation.[2] This novel type of aminolysis mechanism has attracted interest and theoretical calculations concerning these types of reactions have been reported.[3] Here we report antibody-catalyzed[4] aminolysis of a 4-chloropyrimidine derivative and characterize the reaction in water in the absence of antibody to provide insights into the mechanism of this type of aminolysis reaction.

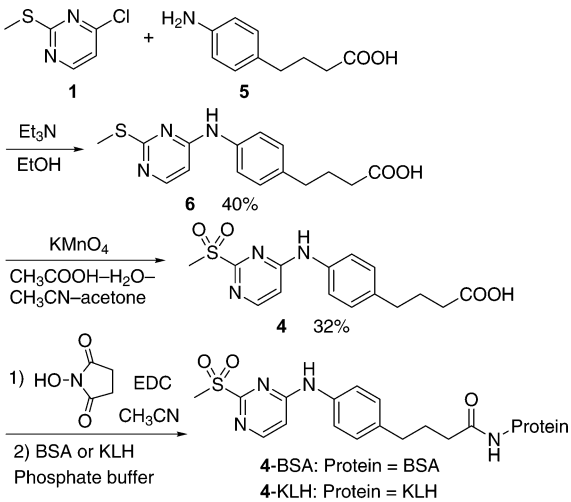
In order to generate antibodies that catalyze the aminolysis of 4-chloro-2-methylthiopyrimidine (**1**) with *p*-toluidine (**2**) to give **3**, hapten **4** was designed (Scheme 1). The hapten was expected to induce antibodies that form hydrogen bonds with the pyrimidine derivative thus accelerating the rate of the reaction. It was expected that the sulfone oxygens of the hapten would induce hydrogen bonding interactions with the antibody during antibody induction. The transition state of the reaction would then be stabilized with the aid of hydrogen bonds to the pyrimidine nitrogen and/or chloride in the catalyzed reaction of sulfide substrate **1**. Hydrogen bonding with the pyrimidine nitrogen of **1** would lower the transition state energy of the amino group attack, and hydrogen bonds with chloride would accelerate chloride leaving. We expected that use of the sulfone in the hapten would also prevent tight binding of the sulfide-containing product in the active site that might otherwise lead to product inhibition in the catalyst.



Scheme 1 Antibody-catalyzed aminolysis of 4-chloropyrimidine derivative **1** with amine **2** to afford **3**, the hapten used to generate the catalytic antibody (**4**), and schematic representations of the antibody active site.

The hapten was synthesized by the amination of **1** with 4-(4-aminophenyl)butyric acid (**5**), followed by oxidation of sulfide **6** to sulfone hapten **4** (Scheme 2).[5] Hapten **4** was conjugated to bovine serum albumin (BSA) and keyhole limpet hemocyanin (KLH). Mice (129G1X$^+$) were immunized with **4**–KLH and monoclonal antibodies were generated using standard protocols.[6] Hybridoma supernatants were screened for antibodies with affinity for **4**–BSA using an enzyme-linked immunosorbent assay (ELISA). Seventeen monoclonal antibodies were purified from the hybridoma supernatants by DEAE anion exchange chromatography and Protein G affinity chromatography. The binding of **4**–BSA to ten of these antibody preparations was inhibited by free hapten **4**. These ten antibodies were screened for their ability to catalyze the amination of **1** by **2** as discussed below.

Although many examples of aminolysis of chloropyrimidine derivatives have been reported, little information on these reactions as they occur in water is available.[7] Therefore, before the antibodies were analyzed, several solvent/buffer systems were studied. The reaction shown in Scheme 1 was performed by using **1** (250 μM) and **2** (2 mM) in the solvents shown in Table 1 at 25 °C and the formation of **3** was analyzed by HPLC. When 5% CH_3CN/95% H_2O was used as the solvent (the pH was 7 ~ 7.5), the reaction was about 5-fold faster than the reaction in MeOH, CH_3CN, or 50% CH_3CN/50% H_2O.[8] Next, the pH effect on the reaction rate was examined in the pH range compatible with antibody catalysis. The velocities of the reaction in buffer composed of 5% CH_3CN/50% PBS[9]/45% 50 mM Na phosphate (pH 6.6, 7.0, 7.5, and 8.0) were evaluated. (The resulting pH values varied within ±0.1 from 6.6, 7.0, 7.5, and 8.0, respectively.) The relative velocities of the reaction under these conditions are shown in Fig. 1. The reaction



Scheme 2 Synthesis of hapten and conjugates.

† Electronic supplementary information (ESI) available: (1) synthesis of the hapten, (2) preparation of hapten conjugate, and (3) HPLC assay and kinetics. See http://www.rsc.org/suppdata/cc/b4/b403672g/

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was found to be most rapid at the lowest pH. Since the pK_as of pyrimidine and **2** are 1.23 and 5.08, respectively,[10] both **1** and **2** are predominantly neutral at pH 7. The results in Fig. 1 indicate that general acid catalysis or protonation of one or both substrates is key for rate acceleration. According to the pK_a values of the substrates, substrate **2** is more likely protonated than **1** at pH 6.6–8.0. Protonated **2** may form a hydrogen bond with **1**, and this activated complex may react with another molecule of unprotonated **2**, or the proton of protonated **2** may transfer to the pyrimidine nitrogen when the reaction proceeds. These results support our idea that antibodies providing proton(s) for hydrogen bond(s) with the transition state should accelerate the reaction. The solvent effects in Table 1 can be explained by the stabilization of a charged transition state in polar solvents.[8,11,12]

Antibody-catalyzed reactions were analyzed at pH 6.6. The reaction was performed by using antibody (20 μM active site), **1** (250 μM), and **2** (500 μM) in 5% CH_3CN/50% PBS/45% 50 mM Na phosphate (pH 6.6) at 25 °C, and the initial velocity of the generation of product **3** was determined by HPLC analyses. Antibody FTB8E9 had the highest initial velocity of the ten antibodies tested, and this antibody was further analyzed.

Catalysis by antibody FTB8E9 (20 μM active site) was evaluated over a range of concentrations of **2** (0.5–4 mM) at a fixed concentration of **1** (250 μM) in 5% CH_3CN/50% PBS/45% 50 mM Na phosphate (pH 6.6). The S–V plot showed a moderate saturation of the velocity according to the increase in the concentration of **2** (see supplementary information). The plot was fitted to the Michaelis–Menten equation: antibody FTB8E9 catalyzed the reaction with a K_m of 11 mM and a k_{cat} of 1.2×10^{-3} min^{-1}. The rate acceleration of the antibody-catalyzed reaction above the background (the reaction without antibody) k_{cat}/k_{uncat} was 1300 ($k_{uncat} = 9.5 \times 10^{-7}$ min^{-1}). When the reaction was performed with FTB8E9 (20 μM, active site), **1** (250 μM), and **2** (2 mM) in the presence of hapten **4** (300 μM) in the buffer described above, the FTB8E9-catalyzed reaction was completely inhibited. Therefore, the antigen combining site of antibody FTB8E9 is the catalytic active site. The apparent dissociation constant of antibody FTB8E9 to hapten **4** was 0.2 μM as determined by competitive ELISA.[13] Inhibition by product **3** in competitive ELISA was not detected even at 4 mM of **3**. These results indicate the effectiveness of our hapten design in inducing antibodies not prone to product inhibition.

Table 1 Solvent effect on the reaction of **1** and **2** to form **3**[a]

Solvent	Relative velocity
5% CH_3CN/95% MeOH	0.19
CH_3CN	0.19
50% CH_3CN/50% H_2O	0.22
5% CH_3CN/95% H_2O	1.00

[a] Conditions: [**1**] 250 μM and [**2**] 2 mM in the solvent indicated at 25 °C. The rate of the reaction was determined by HPLC.

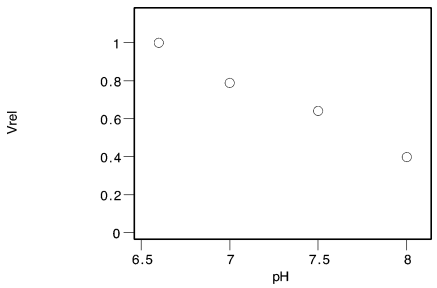

Fig. 1 The pH effect on the reaction of **1** with **2** to form **3**. Vrel is the velocity at indicated pH divided by the velocity at pH 6.6. Conditions: [**1**] 250 μM and [**2**] 2 mM in the buffer composed of 5% CH_3CN/50% PBS/45% 50 mM Na phosphate (pH 6.6, 7.0, 7.5, or 8.0) at 25 °C. Velocity at pH 6.6 was 1.9×10^{-3} μM min^{-1}.

We have demonstrated the first example for antibody-catalyzed aminolysis of a chloropyrimidine derivative. In addition, we showed that aminolysis without antibody is accelerated in water relative to MeOH or CH_3CN. Within the antibody-relevant pH range of 6.6–8.0, the reaction is most rapid at low pH, implicating protonation of substrate in acceleration of the reaction. Further investigation of the reaction in water and of the reaction with catalytic antibodies may provide more detailed information on the mechanism of aminolysis of chloropyrimidine derivatives.

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Notes and references

1 (*a*) Examples: S. W. Wright, A. A. Carlo, M. D. Carty, D. E. Danley, D. L. Hageman, G. A. Karam, C. B. Levy, M. N. Mansour, A. M. Mathiowetz, L. D. McClure, N. B. Nestor, P. K. McPherson, J. Pandit, L. P. Pustilnik, G. K. Schulte, W. C. Soeller, J. L. Treadway, I.-K. Wang and P. H. Bauer, *J. Med. Chem.*, 2002, **45**, 3865; (*b*) M. Y. Chu-Moyer, W. E. Ballinger, D. A. Beebe, R. Berger, J. B. Coutcher, W. W. Day, J. Li, B. L. Mylari, P. J. Oates and R. M. Weekly, *J. Med. Chem.*, 2002, **45**, 511; (*c*) M. Berger, B. Albrecht, A. Berces, P. Ettmayer, W. Neruda and M. Woisetschlager, *J. Med. Chem.*, 2001, **44**, 3031; (*d*) H. Harada, O. Asano, Y. Hoshino, S. Yoshikawa, M. Matsukura, Y. Kabasawa, J. Niijima, Y. Kotake, N. Watanabe, T. Kawata, T. Inoue, T. Horizoe, N. Yasuda, H. Minami, K. Nagata, M. Murakami, J. Nagaoka, S. Kobayashi, I. Tanaka and S. Abe, *J. Med. Chem.*, 2001, **44**, 170; (*e*) M. Barvian, D. H. Boschelli, J. Cossrow, E. Dobrusin, A. Fattaey, A. Fritsch, D. Fry, P. Harvey, P. Keller, M. Garrett, F. La, W. Leopold, D. McNamara, M. Quin, S. Trumpp-Kallmeyer, P. Toogood, Z. Wu and E. Zhang, *J. Med. Chem.*, 2000, **43**, 4606.

2 M. Tominaga, K. Konishi and T. Aida, *J. Am. Chem. Soc.*, 1999, **121**, 7704.

3 K. N. Rankin, J. W. Gauld and R. J. Boyd, *J. Am. Chem. Soc.*, 2000, **122**, 5384; K. N. Rankin, J. W. Gauld and R. J. Boyd, *J. Am. Chem. Soc.*, 2001, **123**, 2047.

4 Recent reviews on catalytic antibodies: P. G. Schultz, J. Yin and R. A. Lerner, *Angew. Chem., Int. Ed.*, 2002, **41**, 4421; F. Tanaka, *Chem. Rev.*, 2002, **102**, 4885.

5 Sulfone **4** was relatively stable in the buffer. The corresponding sulfoxide readily decomposed in the buffer and could not be used for the immunization.

6 G. Kohler and C. Milsten, *Nature*, 1975, **256**, 495.

7 Reactions with a volatile amine or a water-soluble amine have been performed in water[1*d*,1*e*].

8 The faster aminolysis reaction in water is supported by the calculations described in ref. 3.

9 PBS (pH 7.4) = phosphate buffered saline; 10 mM Na_2HPO_4–1.8 mM KH_2PO_4–137 mM NaCl–2.7 mM KCl (pH 7.4).

10 D. R. Lide, Ed., *CRC Handbook of Chemistry and Physics*, 83rd edn.; CRC Press: New York, 2002.

11 Dielectric constants are: H_2O, 80.1; MeOH, 33.0; CH_3CN, 36.6. D. R. Lide, Ed., *CRC Handbook of Chemistry and Physics*, 83rd edn.; CRC Press: New York, 2002, pp. 8–127. Dimroth–Reichardt *E*(T)N solvent polarity values are as follows: H_2O, 1.00; MeOH, 0.762; CH_3CN, 0.460. C. Reichardt, *Solvents and Solvent Effects in Organic Chemistry*, 3rd edn.; Wiley-VCH: Weinheim, 2003, p. 418 and p. 471.

12 For solvent effects on primary alkylamines (not arylamines) see: F. Tanaka, R. Thayumanavan, N. Mase and C. F. Barbas III, *Tetrahedron Lett.*, 2004, **45**, 325.

13 Competitive ELISA of an antibody often gives higher K_d values (about $10^2 \sim 10^3$) than true binding in solution because of the avidity of the bivalent IgG. For example see: F. Tanaka, K. Kinoshita, R. Tanimura and I. Fujii, *J. Am. Chem. Soc.*, 1996, **118**, 2332.