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An Approach for the Generation of Secondary Structure Specific Abzymes

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Abstract

Secondary structure specific recognition of substrates by enzymes is ubiquitous. In this letter,we describe an approach to incorporate this recognition element into abzymes.

Immunoglobulins capable of catalyzing a wide range of reactions have been generated.[1] Monoclonal antibodies have been raised against specific transition state analogs corresponding to several prototypical enzymatic reactions. These antibodies can increase the rate of ester, carbonate[2,3] and nitroanilide[4] hydrolysis. Hydrolysis of peptide bonds has been reported utilizing metal cofactors[5] and by catalytic autoantibodies.[6] Proteolytic antibodies which would cleave peptides at selected sites have tremendous potential.[1] To realize this potential the reactivity and selectivity of these abzymes would have to rival those of enzymes.

We have been investigating the role of secondary structure in proteolytic processing.[7] β-turns appear to constitute a critical element of specificity in a variety of proteolytic events.[7,8,9,10] In this letter, we wish to report the use of a hapten which incorporates our previously described β-turn mimetic framework[11,12] to elicit catalytic antibodies with primary and secondary structure specific depsipeptidase activity.

The hapten that we designed and synthesized **1** incorporates a difluorostatine transition state analog[13]. The synthesis of **1** is outlined in Scheme **1.** Coupling of the racemic turn mimetic **2**[12] with **3**[14] and subsequent saponfication provided **4.** Removal of the Boc group and coupling to a maleimide linker provided the requisite hapten **1.** Monoclonal antibodies were prepared and selected using previously described protocols[3]. Nine antibodies were screened for their ability to effect the hydrolysis of 5. Three antibodies showed quantifiable levels of activity.[15] The 3 antibodies (2E11.2E7, 26G11.3B12, and 27H1.3H10) exhibited the following kinetic [illegible] 5.39mM, $k_{cat} = 2.8 \times 10^{-3}$ sec^{-1}; respectively .

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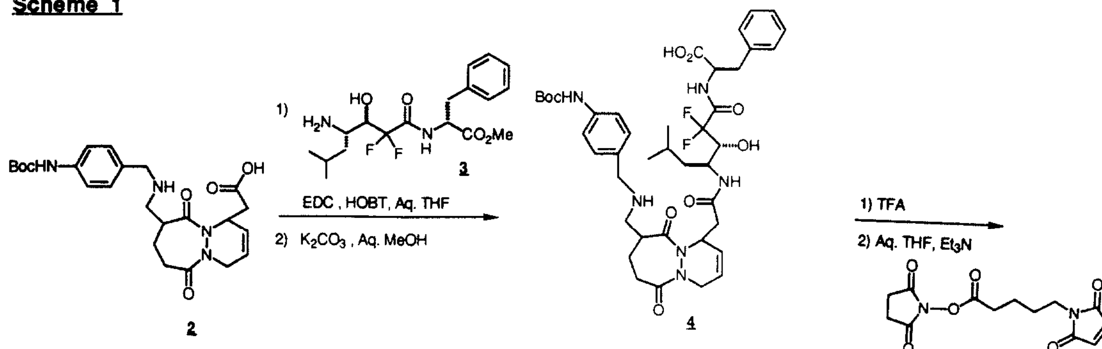
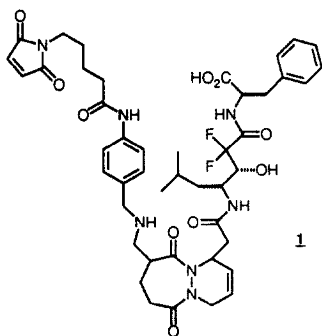
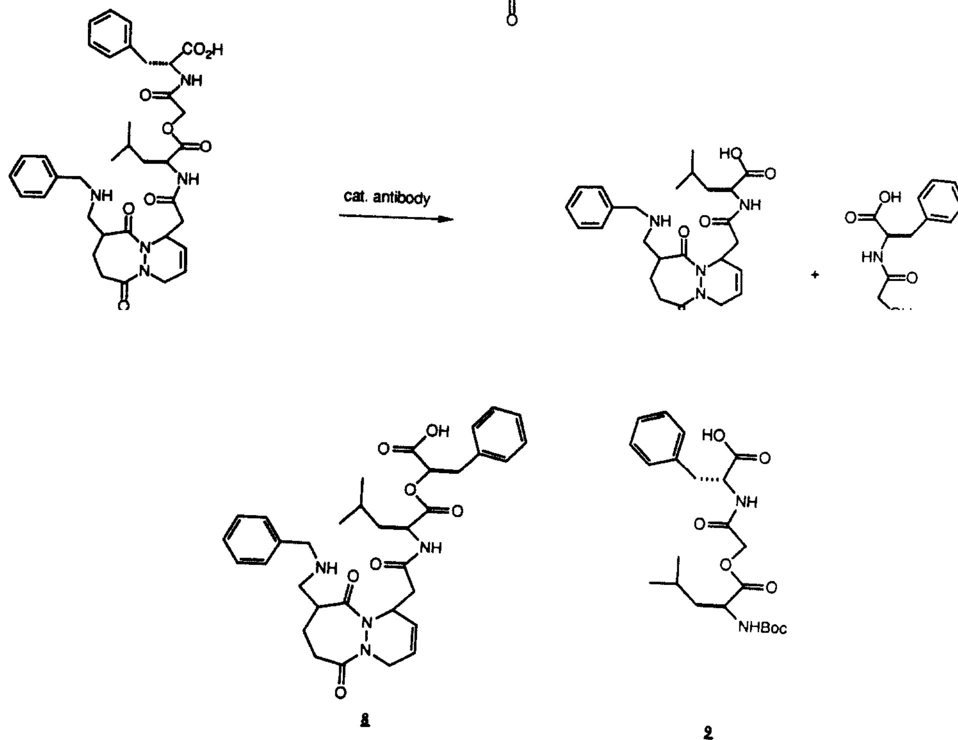
Scheme 1





Scheme 2



The importance of both the proper primary sequence and appositive presentation to the antibody combining site is highlighted by the inability of depsipeptides **8** and **9** to be hydrolyzed by any of the three antibodies.

The lack of proteolytic activity of any of these species was not particularly surprising in light of earlier efforts[1], however, the low esterolytic activity was less anticipated. Two potential rationalizations have been contemplated. The most obvious being the choice of transition state analog, however, use of the corresponding phosphonamidate did not lead to enhanced hydrolysis.[16] Alternatively, the requirement for binding a large hapten, thereby diminishing the percentage of TS stabilization could be a concern.

The use of elements of secondary structure by natural enzymes to enhance their specific recognition of substrates is apparently widespread.[7-10,17-19] Catalytic antibodies offer the potential to generate catalysts with nonnatural specificities. The ability to control both primary and secondary structure specificity in these tailor made catalysts could provide exciting new applications for abzymes. We have described an approach to accomplish this goal. Further developments in the generation of catalytic antibodies[20] and the synthesis of secondary structure mimetics[21,22] should provide exciting new opportunities in this arena.

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References

1) Schultz, P.F., Lerner, R.A. and Benkovic, S.J. *Chem Eng. News* **68**, 26 (1990).
2) Pollack, S.J., Jacobs, J.W. and Schultz, P.G. *Science* **234**, 1570 (1986); Tramontano, A., Janda, K. and Lerner, R. *Science* **234**, 2566 (1986).
3) Jacobs, J., Schultz, P.G., Sugasawara, R., Powell, M. *J.Amer.Chem.Soc.*, 2174 (1987); Tramontano, A., Amann, A.A., Lerner, R.A. *J.Amer.Chem.Soc* **110** 2282 (1988).
4) Janda, K.D., Schloeder, D., Benkovic, S.J. and Lerner, R.A. *Science* **241**, 1188 (1988).
5) Iverson, B.L. and Lerner, R.A. *Science* **243**,1184 (1989).
6) Paul, S., Volle, D.J., Bench, C.M., Johnson, D.R., Powell, M.J., and Massey, R.J.*Science* **244**, 1158 (1989).
7) Nakanishi, H., Chrusciel, R. A., Shen, R., Bertenshaw, S., Johnson, M.E., Rydel, T.J., Tulinsky, A., and Kahn, M. *Proc. Natl. Acad. Sci. USA* **89** 1705, (1992).
8) Rholam, M., Nicolas, P. and Cohen, P. *FEBS Letters* **207**, 1 (1986).
9) Rholam, M., Cohen, P., Brakch, N., Paolillo, L., Scatturin, A. and DiBello, C. *Biochem.Biophys.Res.Commun.***168**, 1066 (1990).
10) Bek, E. and Berry. R. *Biochemistry* **29**, 178 (1990).
11) Kahn, M., Wilke, S., Chen, B. and Fujita, K. *J.Am.Chem.Soc.* **110**,1638 (1988).
12) Kahn, M. and Bertenshaw, S. *Tetrahedron Lett.* **30**, 2317 (1989).

13) Thaisrivongs, S., Pals, D.T. Harris, D.W., Kati, W.M. and Turner, S.R.*J.Med.Chem.* **29,** 2088 (1986); Shokat, K.M., Ko, M.K., Scanlan, T.S., Kochersperger, L., Yonkavich, S., Thaisrivongs, S. and Schultz, P.G. Angew, *Chem. Int. Ed. Engl.* **29,** 1296 (1990).

14) **3** was prepared by Reformatsky of reaction of ethyl bromodifluoro acetate with leucinal using the procedure of Rich, *J.Org.Chem.* **43**, 3624 (1978). Saponification and coupling to D-phenylalanine methyl ester provided 3 in **27%** yield.

15) The antibody purification scheme was as follows:
   1. Lipid clearing
   2. Ammonium sulfate fractionation; 0-33% ppt
   3. Desalt and equilibrate in binding buffer (see below)
   4. Protein A. Beckman kit; Their binding and elution buffers
   5. DEAE-Sephacel; Buffer A = 200 mM Tris-Ac, pH 8.0
      Buffer B = 20 mM Tris-Ac, 0.3M NaAc; pH 8.0
      After loading and washing with buffer A; linear gradient with from Buffer A to Buffer B. Strip with buffer B.

   The incubations were performed at pH 6.0,6.5 and 7.4 at antibody concentrations of 5×10^{-5}, 5×10^{-6} and 5×10^{-7} M in phosphate buffer at 37°C for up to 7 days. Reactions were monitored by C_{18} reverse phase HPLC (column 25cm x .46 cm, flow rate1.5 ml/min eluted with a linear gradient from 15-70% (V/V) acetonitrile and water monitored at 214nm for the generation of products **6** and **7**, which were synthesized independently. The rate of background hydrolysis was too high at pH 6.0 to monitor the reactions. The rate of background hydrolysis at pH 6.5 was (5×10^{-9} - 1×10^{-8} M.sec^{-1}. The abzyme hydrolysis reaction was inhibited by hapten **1** at micromolar levels.).

16) Described in the Ph.D. thesis of Chetna Patel, U. of Illinois at Chicago, 1991.

17) Bause, E. *Biochem J.* **195**, 639, (1983).

18) Tinker, D.A., Krebs, E., Feltham, I.C., AttukPoku, S.K. and Aranthanarayanan, V.S. *J. Biol.Chem* **263**, 5024 (1988).

19) Fontana, A., Fassina, G. Vita, C., Dalzoppo, D., Zamai, M. and Zambonin, M. *Biochemistry* **25**, 1847 (1986).

20) Huse, W.D., Sastry, L., Iverson, A., Kang, A.S., Alting-Mees, M., Burton R.S., Benkovic, S.J. and Lerner, R.A., *Science,* **245**, 2375, (1989).

21) Saragovi, H.U., Fitzpatrick, D. Raktabutr, A., Nakanishi, Kahn, M. and Greene, M.I. *Science* **253**, 792, (1991).

22) Chen, S., Chrusciel, R.A., Nakanishi, H., Raktabutry, A., Johnson, M.E., Sato, A., Weiner, D., Hoxie, J., Saragovi, H.U., Greene, M.I., and Kahn, M. *Proc.Natl.Acad.Sci. USA, in* press.

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