Acta Chemica Scandinavica, 1996: 50: 333–336
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ACTA
CHEMICA SCANDINAVICA
ISSN 0904–213X

Antibody Catalysis via Strategic Use of Haptenic Charge†

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Kikuchi, K. and Hilvert, D., 1996. Antibody Catalysis via Strategic Use of Haptenic Charge. – Acta Chem. Scand. 50: 333–336. © Acta Chemica Scandinavica 1996.

General acid–base catalysis contributes substantially to the efficacy of many enzymes. Similar effects can be exploited in antibody catalysis by taking advantage of charge complementarity between immunoglobulin and hapten (the template used to induce the antibody) to elicit functional groups in the combining site. This strategy has proved useful in the catalysis of a diverse set of chemical transformations, including elimination reactions. Provided that hapten design is optimized and the immune response is screened extensively, the efficiency of the resulting antibody catalysts can rival that of analogous natural enzymes.

Following an original suggestion of Jencks,[1] the immune system's capacity to produce high-affinity binding sites for synthetic ligands has been successfully exploited over the last 10 years to create antibodies with a wide range of tailored catalytic activities.[2–4] Immunization with template molecules that mimic the short-lived transition-state of a chemical reaction yields catalysts that exhibit many of the properties of natural enzymes. From a practical standpoint, the exacting control of reaction pathway and absolute stereochemistry that can be achieved with these agents is particularly notable.

Nevertheless, the efficiency of catalytic antibodies is generally considerably lower than that of analogous enzymes. There are several reasons this may be so. First, the antibody molecule's distinctive architecture may place intrinsic limits on the activities and reaction pathways that can be accommodated within the immunoglobulin binding pocket. Second, there are practical difficulties accessing the full diversity of the immunological repertoire with traditional methods of producing monoclonal antibodies. Third, and perhaps most importantly, stable molecules are inherently imperfect mimics of short-lived transition states, and antibodies raised against them are unlikely to provide optimal microenvironments for the corresponding chemical transformations.

The design of effective haptens is made even more difficult by the fact that many reactions require the participation of precisely positioned functional groups. Enzymes, for example, utilize an extensive array of acids, bases and nucleophiles to increase their catalytic efficiency.[5] In some instances, their catalytic capabilities are further augmented with metal ions and organic cofactors.[5] The difficulty of exploiting similar effects in designed catalysts like catalytic antibodies is that of positioning the functional groups for maximal efficacy. The use of charge complementarity between the antibody and its hapten to elicit catalytic residues in the binding pocket represents one promising solution to this problem.

Structural studies of many antibody–hapten complexes have revealed superb shape and chemical complementarity between the protein and its ligand.[6,7] Consequently, charged groups and π-systems can be incorporated into the hapten to induce complementary charged and aromatic residues, respectively. This approach has successfully produced antibody catalysts for a variety of reactions requiring acids, bases, and nucleophiles.[8–14] Here we briefly review the strategic use of haptenic charge to promote chemical transformations involving proton transfer from carbon acids. This elementary process is of great importance in chemistry and biology for addition–elimination reactions, racemizations, and the formation of carbon–carbon bonds.

Antibody-catalyzed elimination reactions

One of the first attempts to use antibody–hapten complementarity for catalysis targeted the β-elimination of hydrogen fluoride from β-fluoro ketone **1**.[8] Positively charged hapten **3**, in which an ammonium ion mimics a methylene group with an acidic proton, was designed to induce a negatively charged carboxylate which could

† Contribution presented at the Nobel Symposium on *Catalytic Asymmetric Synthesis*, September 3–7, 1995 at Tammsvik, Bro, Sweden.

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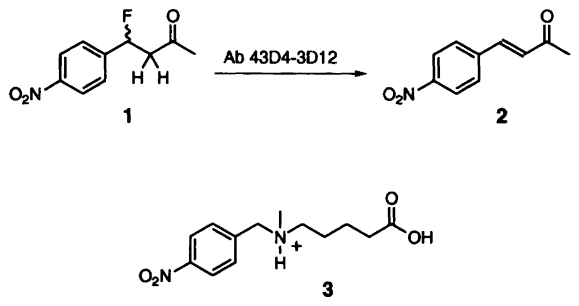
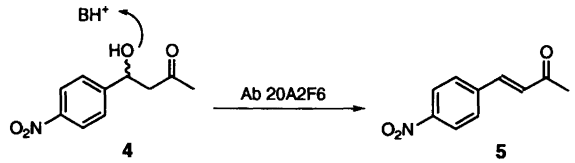
serve as a catalytic base. Four of the six antibodies raised against this compound were found to accelerate the desired reaction.

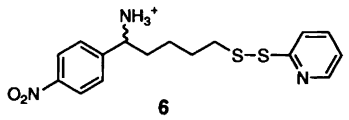


In accord with the hapten design, the reaction catalyzed by antibody 43D4 is pH-dependent, requiring ionization of a single titratable group. The pK_a of the latter is 6.2, consistent with a carboxylate base located in a relatively apolar microenvironment. Selective modification of heavy chain residue GluH46 in affinity labeling experiments supports this conclusion.[15] The efficiency of the antibody catalyzed reaction can consequently be estimated through comparison with the analogous acetate-promoted elimination. For 43D4, the apparent bimolecular rate constant (k_{cat}/K_m) exceeds k_{AcO^-} by a factor of 9×10^4 and the effective molarity of the active site carboxylate is 18 M (uncorrected for the difference in pK_a values of acetate and the antibody carboxylate).[8] Thus, despite the fact that hapten **3** bears little resemblance to the transition-state for conversion of **1** into **2**, substantial catalytic effects could be achieved simply by eliciting a base in the substrate binding pocket.

Although the precise mechanism of 43D4 (E2 vs. E1cb) has yet to be established, an E1 elimination pathway has been ruled out on the basis of a primary kinetic isotope effect (k_H/k_D) of 2.35.[15] In addition, experiments with selectively deuteriated substrates show that 43D4 favors an *anti* over a *syn* elimination process but exhibits little stereofacial selectivity, abstracting either the *pro-R* or *pro-S* proton from C-3 of **1**.[15] Hapten **3** is a relatively flexible molecule, and the latter results suggest that the complementary antibody active site is able to bind substrate **1** in multiple conformations. Conformationally restricted haptens (*vide infra*) might therefore increase both chemical efficiency and control of the reaction pathway.

The properties of 43D4 validate the use of an ammonium ion to induce a general base positioned for proton abstraction. Ammonium ions have also been used to mimic the oxonium ion formed by protonation of an alcohol to make it a better leaving group. For example, antibodies raised against hapten **6** promote the dehydration of β-hydroxy ketone **4** to give enone **5** with rate accelerations (k_{cat}/k'_{H_2O}) of $>10^3$-fold.[11] The results of isotope effect studies and chemical modification of the catalyst 20A2F6 are consistent with a mechanism involving a transition-state with oxonium ion character, but an antibody carboxylate/carboxylic acid apparently does not play a role in this case. Interestingly, 20A2F6 also catalyzes the stereoselective addition of hydroxylamine to *p*-nitroacetophenone, favoring formation of the *syn* oxime rather than the *anti* isomer by 9:1.[16] The pH dependence of this second reaction indicates that an active site group with a pK_a of approximately 7.5 may stabilize the protonated transition-state or serve as a general acid in catalysis.





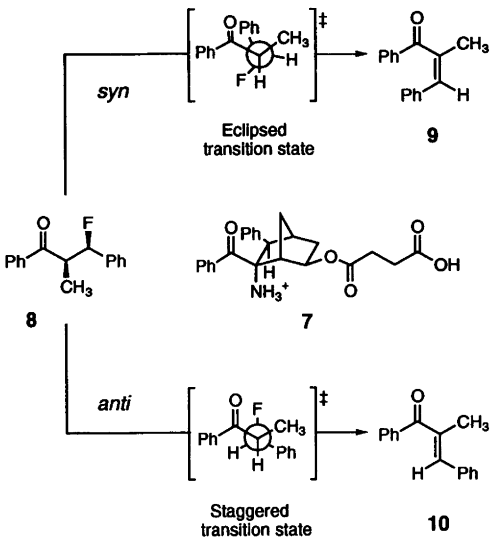
syn Elimination to give *cis* olefins

Chemical transformations that are difficult to control via existing chemical methods present an exciting opportunity for the field of antibody catalysis.[2] In the present context, for example, catalytic antibodies can be envisioned that favor *syn* over *anti* elimination pathways. *syn* Eliminations of acyclic substrates to give *cis* (*Z*) olefins are often highly disfavored as a consequence of the formidable steric and torsional strain that results when the substrate is forced to adopt the requisite eclipsed conformation.[17,18]

While haptens **3** and **6** were successful in inducing catalytic groups, they are conformationally flexible molecules and the stereochemical preferences exhibited by the induced antibodies reflect serendipity rather than design. By synthesizing conformationally restricted templates, it should be possible to predetermine the stereochemical course of the catalyzed elimination reaction.

This approach is illustrated by hapten **7**, which was designed to produce antibodies that bind and lock β-fluoro ketone **8** in an energetically unfavorable eclipsed conformation.[13] As in the previous examples, the ammonium group of **7** was introduced to elicit an appropriately oriented catalytic base. The position of this moiety corresponds to the substrate's α-keto proton, however, so its N–H bond is not congruent with the substrate's acidic C–H (in contrast with the ammonium group in haptens **3** and **6**). Nevertheless, hapten **7** produced an immunoglobulin with the desired properties: antibody 1D4 converts substrate **8** exclusively into *cis* product **9** with a k_{cat} value of 3×10^{-3} min^{-1}.[13] For comparison, only *trans* product **10** was obtained in the absence of the catalyst ($k_{un} = 2.48 \times 10^{-4}$ min^{-1}). Although antibody

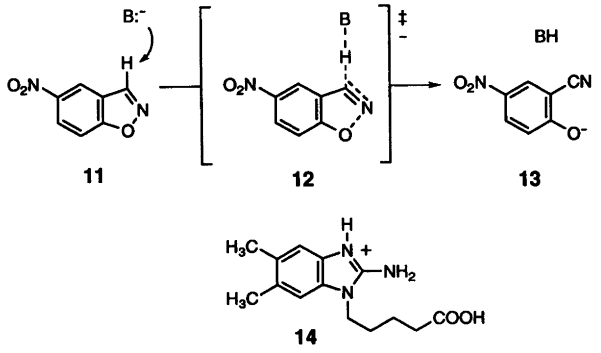
1D4 is not yet synthetically useful (the background elimination via the *anti* pathway actually produces more product than the antibody under the reported assay conditions), the ability of the catalyst to produce the *syn* isomer at all is remarkable in light of the estimated 5 kcal mol^{-1} difference in energy between the *syn* and *anti* transition states.[13]



High catalytic efficiency

The theoretical underpinnings of antibody catalysis predict that improvements in hapten design, coupled with more extensive screening of the immune response, will yield more active catalysts. Highly efficient antibody catalysis of benzisoxazole decomposition[14] provides some support for these notions.

The base-promoted breakdown of substituted benzisoxazoles (**11**) to give salicylonitriles (**13**) is an ideal system to probe the factors that contribute to efficient base catalysis in proteins. This reaction occurs by a concerted E2 mechanism.[19] Moreover, it is irreversible, highly exothermic, and sensitive to both base strength and solvent microenvironment.[19–21] More importantly, and in contrast with the examples discussed above, the geometry of all reacting bonds is well defined by the planarity of the heterocyclic substrate. To elicit antibody catalysts for this elimination reaction, the cationic benzimidazolium hapten **14** was designed.[14] It is appropriately sized and partially protonated under physiological conditions ($pK_a = 7.8$). An antibody carboxylate elicited in response to the cation and capable of hydrogen bonding to the hapten's N-3 proton would be perfectly positioned to abstract the corresponding proton from bound substrate. Aromatic amino acids induced in response to the π system of **14** could further stabilize the polarizable transition-state through dispersion interactions. Furthermore, because the hapten bears little resemblance to the reaction product, minimal product inhibition is anticipated.



Several highly active catalysts for the decomposition of **11** were identified from a panel of $\approx 10^3$ hapten binders. One antibody (34E4) catalyzes the elimination of 5-nitrobenzisoxazole with $k_{cat} = 0.66\ s^{-1}$ and $K_m = 120\ \mu M$. Combined kinetic and chemical modification studies implicate a carboxylate with an elevated pK_a (6.0) as the active site base. Comparison of k_{cat}/K_m with k_{AcO^-} gives a rate acceleration of 3.4×10^8, and the effective molarity (relative to acetate) for this catalyst is 41 000 M. Correcting the latter value for the difference in basicity between the active site carboxylate and acetate still gives an effective molarity of 5000 M. By way of contrast, effective molarities rarely exceed 10 M for intramolecular general base catalysis in model systems[22] or for general catalysis in other antibodies.[8–12] Furthermore, although strong product inhibition limits the effectiveness of many catalytic antibodies, $> 10^3$ turnovers per active site were achieved with 34E4. These data indicate that 34E4 is one of the most efficient catalytic antibodies described to date. We believe its high activity is attributable to three factors: (1) an apolar active site that increases the reactivity of the antibody carboxylate through desolvation, (2) a microenvironment that favors delocalization of the carboxylate's negative charge to the nascent phenolate anion of the transition-state **12**, and (3) the entropic advantage of correctly aligning the active site base with the 3-hydrogen of benzisoxazole.

These experiments convincingly show that very large rate effects can be achieved in antibody catalysis. They also illustrate the importance of careful hapten design and efficient screening of the immune response for identifying antibodies with optimally positioned catalytic residues.

Conclusions

The generation of effective constellations of functional groups will be crucial to future attempts to catalyze many transformations of interest in chemistry and biology, including eliminations, isomerizations, racemizations, hydrolyses and carbon–carbon bond forming reactions. The examples cited above show that the stra-

tegic use of haptenic charge is a powerful strategy for eliciting such residues within antibody combining sites.

Extension of this approach to the simultaneous induction of acid–base pairs capable of additive bifunctional catalysis is likely to afford even greater efficiency and selectivity. Even so, it could prove difficult for a single hapten to generate arrays of residues as sophisticated as the catalytic triad in serine proteases. Heterologous immunization with two different but structurally related haptens, each containing a different charged group, represents one possible solution to the problem of inducing multiple catalytic residues.[23] Better access to and screening of the immune response to a given hapten will be essential for the successful implementation of such innovations, however. In this context, the development of new technologies for producing[24–26] and screening[27–32] large libraries of immunoglobulin fragments is very important.

For specific problems, other strategies – perhaps in combination with haptenic charge – may be required to elicit desirable combinations of functional groups. The use of mechanism-based inhibitors to select directly for specific catalytic mechanisms ('reactive immunization') appears to be one particularly promising avenue of future research.[33,34] As structural information becomes available, engineering catalytic groups into antibody active sites by site-directed mutagenesis will also become increasingly attractive. Finally, where applicable, genetic selection may ultimately be the most effective way of identifying and optimizing antibody catalysts.[27–29]

Acknowledgments. Partial support of research in the authors' laboratory by grants from the National Institutes of Health is gratefully acknowledged.

References

1. Jencks, W.P. *Catalysis in Chemistry and Enzymology*, McGraw Hill, New York 1969, p. 288.
2. Schultz, P.G. and Lerner, R.A. *Acc. Chem. Res. 26* (1993) 391.
3. Stewart, J.D., Liotta, L.J. and Benkovic, S.J. *Acc. Chem. Res. 26* (1993) 396.
4. Hilvert, D. *Acc. Chem. Res. 26* (1993) 552.
5. Fersht, A. *Enzyme Structure and Mechanism*, Freeman, New York 1985.
6. Davies, D.R., Padlan, E.A. and Sheriff, S. *Ann. Rev. Biochem. 59* (1990) 439.
7. Wilson, I.A. and Stanfield, R.L. *Curr. Opinion Struct. Biol. 3* (1993) 113.
8. Shokat, K.M., Leumann, C.J., Sugasawara, R. and Schultz, P.G. *Nature (London) 338* (1989) 269.
9. Janda, K.D., Weinhouse, M.I., Schloeder, D.M., Lerner, R.A. and Benkovic, S.J. *J. Am. Chem. Soc. 112* (1990) 1274.
10. Jackson, D.Y. and Schultz, P.G. *J. Am. Chem. Soc. 113* (1991) 2319.
11. Uno, T. and Schultz, P.G. *J. Am. Chem. Soc. 114* (1992) 6573.
12. Reymond, J.-L., Jahangiri, G.K., Stoudt, C. and Lerner, R.A. *J. Am. Chem. Soc. 115* (1993) 3909.
13. Cravatt, B.F., Ashley, J.A., Janda, K.D., Boger, D.L. and Lerner, R.A. *J. Am. Chem. Soc. 116* (1994) 6013.
14. Thorn, S.N., Daniels, R.G., Auditor, M.-T.M. and Hilvert, D. *Nature (London) 373* (1995) 228.
15. Shokat, K., Uno, T. and Schultz, P.G. *J. Am. Chem. Soc. 116* (1994) 2261.
16. Uno, T., Gong, B. and Schultz, P.G. *J. Am. Chem. Soc. 116* (1994) 1145.
17. Cram, D.J. *J. Am. Chem. Soc. 74* (1952) 2149.
18. Chiao, W.-B. and Saunders, W.H., Jr. *J. Org. Chem. 45* (1980) 1319.
19. Casey, M.L., Kemp, D.S., Paul, K.G. and Cox, D.D. *J. Org. Chem. 38* (1973) 2294.
20. Kemp, D.S. and Casey, M.L. *J. Am. Chem. Soc. 95* (1973) 6670.
21. Kemp, D.S., Cox, D.D. and Paul, K. *J. Am. Chem. Soc. 97* (1975) 7312.
22. Kirby, A. *Adv. Phys. Org. Chem. 17* (1980) 183.
23. Suga, H., Ersoy, O., Williams, S.F., Tsumuraya, T., Margolies, M.N., Sinskey, A.J. and Masamune, S. *J. Am. Chem. Soc. 116* (1994) 6025.
24. Chiswell, D.J. and McCafferty, J. *TIBTECH 10* (1992) 80.
25. Huse, W.D., Sastry, L., Iverson, S.A., Kang, A.S., Alting-Mees, M., Burton, D.R., Benkovic, S.J. and Lerner, R.A. *Science 246* (1989) 1275.
26. Marks, J.D., Hoogenboom, H.R., Griffiths, A.D. and Winter, G. *J. Biol. Chem. 267* (1992) 16007.
27. Tang, Y., Hicks, J.B. and Hilvert, D. *Proc. Natl. Acad. Sci. USA 88* (1991) 8784.
28. Lesley, S.A., Patten, P.A. and Schultz, P.G. *Proc. Natl. Acad. Sci. USA 90* (1993) 1160.
29. Smiley, J.A. and Benkovic, S.J. *Proc. Natl. Acad. Sci. USA 91* (1994) 8319.
30. Lane, J.W., Hong, X. and Schwabacher, A.W. *J. Am. Chem. Soc. 115* (1993) 2078.
31. MacBeath, G. and Hilvert, D. *J. Am. Chem. Soc. 116* (1994) 6101.
32. Tawfik, D.S., Green, B.S., Chap, R., Sela, M. and Eshhar, Z. *Proc. Natl. Acad. Sci. USA 90* (1993) 373.
33. Wirsching, P., Ashley, J.A, Lo, C.-H.L., Janda, K.D. and Lerner, R.A. *Science 270* (1995) 1775.
34. Wagner, J., Lerner, R.A. and Barbas, C.F., III. *Science* 270 (1995) 1797.

Received September 25, 1995.