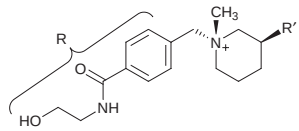
# Enantioselectivity *vs.* kinetic resolution in antibody catalysis: formation of the (*S*) product despite preferential binding of the (*R*) intermediate

**Doron Shabat,[a,b] Hagit Shulman,[b] Harel Itzhaky,[b] Jean-Louis Reymond*[c] and Ehud Keinan*[a,b]†‡**

[a] *The Scripps Research Institute, Department of Molecular Biology and the Skaggs Institute for Chemical Biology, 10550 North Torrey Pines Road, La Jolla, California 92037, USA*
[b] *Department of Chemistry, Technion - Israel Institute of Technology, Technion City, Haifa 32000, Israel*
[c] *Department of Chemistry and Biochemistry, University of Bern, Freiestrasse 3, 3012 Bern, Switzerland*

**Antibody 14D9, which catalyzes the stereoselective transformation of achiral enol ethers into the corresponding (*S*)-ketals, resolves a racemic mixture of structurally similar chiral enol ethers by selective conversion of the (*R*)-enol ether into the (*R*)-ketal, raising the possibility that the (*S*) transition state is preferencially stabilized by the antibody despite a better binding of the (*R*) intermediate.**

Catalytic antibodies, which are produced by immunization against stable transition state analogs of chemical reactions,[1] offer unique opportunities, not only in expanding the repertoire of synthetic tools available to the organic chemist,[2] but also in studying fundamental aspects of enzymatic catalysis.[3] For example, antibody 14D9, which was raised against the quaternary ammonium hapten **1b**, has taught us a great deal about

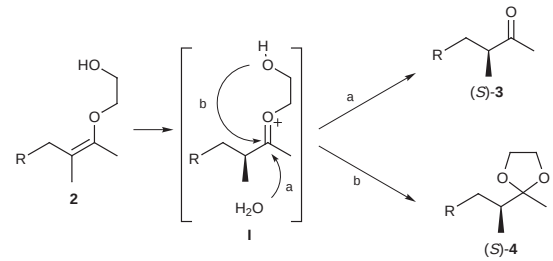



**1a** R′ = H
**b** R′ = $CH_2NHCO(CH_2)_3CONH$-carrier protein

synthetic opportunities using catalytic antibodies and also about mechanistic aspects of biocatalysis.[4] This proficient catalyst might be mechanistically related to primordial glycosidase enzymes.[5] Herein we report on a unique property of this catalyst. The antibody catalyzes the enantioselective protonolysis of achiral enol ethers to give the (*S*) product. Yet, evidence from kinetic resolution of chiral enol ethers shows that 14D9 binds the (*R*) oxocarbenium ion intermediate more strongly than the (*S*) intermediate.

The 14D9-catalyzed conversion of enol ether **2** into a mixture of ketone **3** and ketal **4**, which both have an (*S*) configuration (Scheme 1), goes through an intermediate oxocarbenium ion **I**, which is produced in the rate-limiting protonation of enol ether **2**.[6] Partitioning of this intermediate to give the final products **3** and **4** depends on the availability of water molecules in the medium. Ketal **4** does not form in aqueous solutions and is produced exclusively within the antibody binding site. Therefore, the optical purity of **4** provides a direct measure of the enantioselectivity in the antibody-catalyzed reaction. Indeed, the 14D9-catalyzed protonolysis of **2** was found to be highly stereoselective, producing (*S*)-**4** in 99.5% ee.[6]

There is an intriguing question related to the origin of the enantioselectivity in the rate-determining protonation step. Intuitively, one would expect that (*S*) selectivity arises from preferential binding of the antibody to both the (*S*) transition state, (*S*)-TS and the structurally similar (*S*) intermediate, (*S*)-**I** [Fig. 1A]. Nevertheless, we cannot rule out *a priori* the alternative possibility, in which the antibody still binds selectively to (*S*)-TS but binds preferentially to the opposite enantiomeric intermediate (*R*)-**I** [Fig. 1B].




R = $HO(CH_2)_2NHCOC_6H_4$

**Scheme 1**

This mechanistic issue could be resolved if the affinity of 14D9 to each of the two enantiomeric forms of **I** could be compared. These enantiomeric intermediates occur not only along the reaction pathway leading from **2** to ketal **4**, but also along the similar conversion of the isomeric enol ether **5** to **4** (Scheme 2). Therefore, one could obtain the desired information about the relative stability of (*S*)-**I** and (*R*)-**I** by studying the kinetic resolution of **5** by 14D9. Conversion of (*S*)-**5** and (*R*)-**5** into ketals (*S*)-**4** and (*R*)-**4**, respectively, proceeds *via* the enantiomeric intermediates (*S*)-**I** and (*R*)-**I**. If the antibody catalyzed the protonolysis of (*S*)-**5** preferentially over (*R*)-**5** this would imply that 14D9 binds (*S*)-**I** more tightly than (*R*)-**I**. This would be consistent with the energy diagram shown in Fig. 1(A) for enol ether **2**. Conversely, if catalytic protonolysis of (*R*)-**5** was faster than that of (*S*)-**5**, this would support the alternative energy profile described in Fig. 1(B).

Substrate **5** was prepared from methyl 4-bromomethylbenzoate and ethyl 2-methyl-3-oxobutanoate.[6,7] The initial alkylation product was decarboxylated and the resultant ketone was converted to the corresponding 1,3-dioxolane. The latter was opened with $(Me_3Si)_2NH$ and TMSI to produce a mixture of three isomeric enol ethers in almost equal proportions. These isomers were separated by column chromatography and each was subjected to aminolysis with ethanolamine. The two

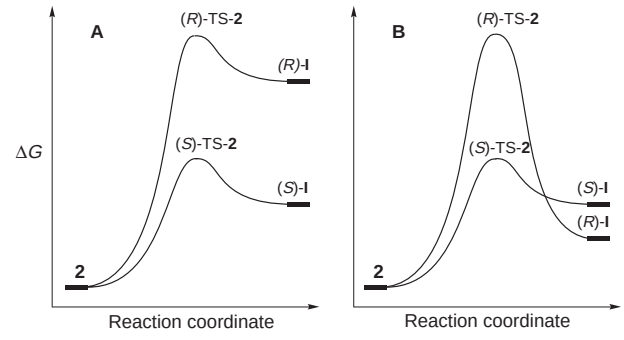
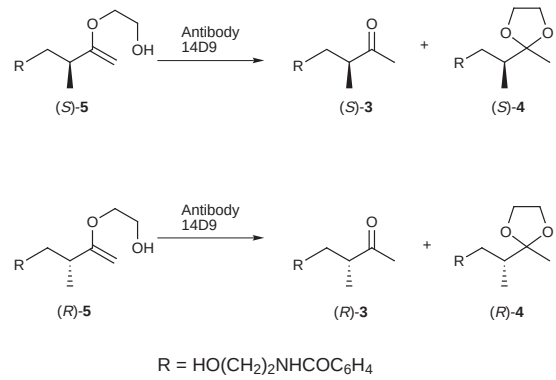



**Fig. 1** Alternative free energy diagrams for the antibody-catalyzed enantioselective protonation of enol ether **2**




**Scheme 2**

**Table 1** Kinetic parameters for the antibody 14D9-catalyzed hydrolysis of **5**[a]

| Substrate | $K_M$/μM | $k_{cat}$/min$^{-1}$ | $k_{un}$/min$^{-1}$ | $k_{cat}/k_{un}$ | $K_{TS}$/μM |
|---|---|---|---|---|---|
| *rac*-**5** | 480 ± 200 | (4.3 ±1.9)× 10$^{-2}$ | 1.55× 10$^{-5}$ | 2760 | 0.17 |
| (*R*)-**5** | 210 ± 70 | (4.6 ± 1.5)× 10$^{-2}$ | 1.55× 10$^{-5}$ | 2990 | 0.07 |
| (*S*)-**5** | 470 ± 230 | (3.4 ± 1.7)× 10$^{-3}$ | 1.55× 10$^{-5}$ | 220 | 2.14 |

[a] Reactions were carried out in 100 mM NaCl and 50 mM 1,3-bis[tris-(hydroxymethyl)methylamino]propane (bistris), pH 8.0, 25 °C. Ketal **4** (*ca.* 20%) was formed in all cases.

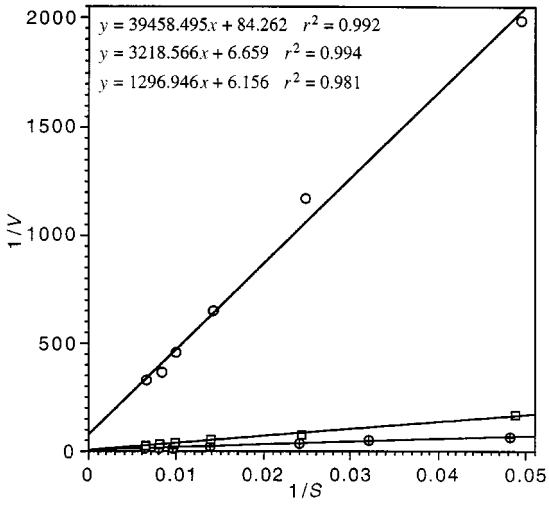



**Fig. 2** Lineweaver–Burk plot of reaction rates for the formation of ketone **3** from (□) racemic enol ether **5**, (○) (*S*)-**5** and (⊕) (*R*)-**5**. For the reactions conditions, see Table 1.

enantiomers of **5** were separated by HPLC using a chiral-phase column.[8] Their absolute configurations were determined by converting them to the corresponding ketals and comparing these ketals with authentic samples of (*R*)-**4** and (*S*)-**4**.[6]

Antibody 14D9 catalyzes the protonolysis of racemic **5**, (*R*)-**5** and (*S*)-**5**. In each case catalysis is fully inhibited by the addition of the hapten **1a**, confirming that the reaction occurs within the antibody's combining site. The observation that ketal **4** (approximately 10–20% of the product) is obtained from all three substrates, **2**, (*R*)-**5** and (*S*)-**5**, is consistent with the concept that all of these reactions proceed *via* intermediate **I** within the antibody's combining site.

Interestingly, (*R*)-**5** was found to be a better substrate than (*S*)-**5**, with both a lower $K_M$ and a higher $k_{cat}$ (Table 1 and Fig. 2). A preparative scale experiment with antibody 14D9 using saturating concentrations of racemic **5** (pH 8, 5 μM catalyst and 1 mM racemic **5**) lead to the formation of (*R*)-**4**. Measuring the optical purity of **4**, which is formed exclusively in the antibody-catalyzed process with no background reaction, should allow an unequivocal determination of the degree of kinetic resolution of racemic **5** by 14D9. Under these saturating conditions the enantiomeric purity (63% ee) is consistent with the observed ratio of $k_{cat}$ for each enantiomer of **5**. We calculated the dissociation constants of the transition states,[9] using the equation $K_{TS} = K_M/(k_{cat}/k_{un})$, and found that antibody 14D9 binds the transition state leading from (*R*)-**5** to intermediate (*R*)-**I** ($K_{TS\text{-}5}$ = 7× 10$^{-8}$ M) 31 times more strongly than the transition state leading from (*S*)-**5** to intermediate (*S*)-**I** ($K_{TS\text{-}5}$ = 2.14× 10$^{-6}$ M).

Although this experiment does not measure directly the binding constant of 14D9 to the oxocarbenium ions (*R*)-**I** and (*S*)-**I**, the transition states that lead from (*S*)-**5** to (*S*)-**I** and from (*R*)-**5** to (*R*)-**I** are very closely related to these intermediates. The 31-fold selectivity in the kinetic resolution of **5** suggests that the natural binding selectivity of antibody 14D9 favors intermediate (*R*)-**I**. The (*R*) selectivity in the hydrolysis of **5** stands in stark contrast to the (*S*) selectivity observed in the 14D9-catalyzed hydrolysis of several enol ethers such as **2**, and supports the mechanistic option shown in Scheme 2(*b*).

Earlier experiments indicate that the high catalytic efficiency observed with enol ethers such as **2** ($k_{cat}/k_{un}$ = 10$^3$–10$^4$) is caused by a carboxylic acid residue acting as a general acid catalyst within the antibody's binding pocket.[10] Thus, the strong preference for protonation on the *re*-face of enol ether **2** to produce the (*S*) products is the result of the relative positioning of this general acid with respect to the bound substrate.[11] The evidence presented here suggests that 14D9 binds (*R*)-**I** tighter than (*S*)-**I**. It also raises the intriguing possibility that moving the catalytic residue in the antibody binding pocket by mutagenesis could create a new catalyst that will convert prochiral enol ethers to (*R*) products. Moreover, such a modified antibody is expected to be a more efficient catalyst. Future experiments will address this possibility.

This work was supported by the US-Israel Binational Science Foundation (E. K.), the Skaggs Institute for Chemical Biology (E. K.), the Swiss National Science Foundation (J. L. R.) and the Wander Stiftung, Bern, Switzerland (J. L. R.).

## Notes and References

† E-Mail: keinan@tx.Technion.ac.il, keinan@scripps.edu
‡ Incumbent of the Benno Gitter and Ilana Ben-Ami Chair of Biotechnology, Technion.

1 P. G. Schultz and R. A. Lerner, *Science*, 1995, **269**, 1835; E. Keinan and R. A. Lerner, *Isr. J. Chem.*, 1996, **36**, 113; R. A. Lerner, S. J. Benkovic and P. G. Schultz, *Science*, 1991, **252**, 659; N. R. Thomas, *Nat. Prod. Rep.*, 1996, 479.
2 T. L. Li, R. A. Lerner and K. D. Janda, *Acc. Chem. Res.*, 1997, **30**, 115.
3 J.-P. Charbonnier, B. Golinelli-Pimpaneau, B. Gigant, D. S. Tawfik, R. Chap, D. G. Schindler, S.-H. Kim, B. S. Green, Z. Eshhar and M. Knossow, *Science*, 1997, **275**, 1140; G. J. Wedemayer, P. A. Patten, L. H. Wang, P. G. Schultz, R. C. Stevens, *Science*, 1997, **276**, 1665.
4 J.-L. Reymond, K. D. Janda, R. A. Lerner, *Angew. Chem., Int. Ed. Engl.*, 1991, **30**, 1711; S. C. Sinha, E. Keinan and J.-L. Reymond, *Proc. Natl. Acad. Sci. U.S.A.*, 1993, **90**, 11 910; S. C. Sinha, E. Keinan and J.-L. Reymond, *J. Am. Chem. Soc.*, 1993, **115**, 4893; J.-L. Reymond, J.-L. Reber and R. A. Lerner, *Angew. Chem., Int. Ed. Engl.*, 1994, **33**, 475; S. C. Sinha and E. Keinan, *J. Am. Chem. Soc.*, 1995, **117**, 3653.
5 D. Shabat, S. C. Sinha, J.-L. Reymond and E. Keinan, *Angew. Chem.*, 1996, **35**, 2628.
6 D. Shabat, H. Itzaky, J.-L. Reymond and E. Keinan, *Nature*, 1995, **374**, 143.
7 S. C. Sinha and E. Keinan, *Isr. J. Chem.*, 1996, **36**, 185.
8 Separation of the enantiomers was carried out using a normal phase chiral column (Chiralcel OD, Chiral Technologies Inc.) with hexane–Pr$^i$OH (80:20) at a flow rate of 1 ml min$^{-1}$; the retention times of (*S*)-**5** and (*R*)-**5** are 9.13 and 12.23 min, respectively.
9 J. L. Kurz, *J. Am. Chem. Soc.*, 1963, **85**, 987; J. Kraut, *Science*, 1988, **242**, 533; J.-L. Reymond and Y. Chen, *Isr. J. Chem.*, 1996, **36**, 199.
10 J.-L. Reymond, G. K. Jahangiri, C. Stoudt and R. A. Lerner, *J. Am. Chem. Soc.*, 1993, **115**, 3909.
11 G. K. Jahangiri and J.-L. Reymond, *J. Am. Chem. Soc.*, 1994, **116**, 11 264

*Received in Glasgow, UK, 25th March 1998; 8/02374C*