An antibody transesterase derived from reactive immunization that utilizes a wide variety of alcohol substrates

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A monoclonal antibody obtained from reactive immunization catalyzes transesterifications of alcohol substrates with both broad and unusual specificity.

Reactive immunization has emerged as a new tool for the study of catalysis at the interface of chemistry and biology.[1] Unlike traditional immunization methods that invoke noncovalent interactions to elicit an antibody repertoire, reactive immunization makes use of a chemical reaction to direct the course of the immune response. Powerful applications have resulted in catalytic antibodies that use an enamine mechanism for myriad aldol reactions,[2,3] an alternative pathway for the allylic rearrangement exemplified by Δ^5-3-ketosteroid isomerase,[4] and the enantioselective hydrolysis of a naproxen ester.[5] A tenet which has emerged is that broad substrate specificities, ascribed to the special ontogeny of antibodies induced by immunogens that form covalent bonds within the binding pocket during induction, may be a feature in catalysts derived from reactive immunization.

We discovered that one of our earlier antibodies[1] SPO50C1, derived from immunization with a reactive bis(4-methylsulfonylphenyl) phosphonate, catalyzed the transesterification of *p*-sulfonylphenyl ester **1** by a large number of different alcohols **2** under aqueous conditions (Fig. 1). Therein, our results required the elaboration of the above hypothesis to include not only broad specificity, but also unanticipated or unusual characteristics with regard to substrate tolerance. Notably, alkyl alcohols as substrates in antibody catalysis have not been previously reported. The binding of these alcohols was unexpected, in light of the aromatic and immunogenic nature of the phenolic leaving group, as was their associated reactivities.

The mechanism of the SPO50C1 reaction to yield esters **3** proceeded by way of a covalent acyl–antibody intermediate (species F in the ping-pong notation of Cleland[6]) as substantiated quantitatively by pre-steady-state kinetic analysis of the observed stoichiometric 'burst' formation of phenol **4** upon antibody acylation (k_{obs} = 1.8 s^{-1}).‡ Therefore, as judged from rates of product ester formation (second product Q), deacylation was entirely the rate-determining step. In that SPO50C1 was also a relatively fast esterase with **1** (k_{cat} = 0.56 min^{-1}), alcohols competed effectively with water to partition the acyl intermediate.

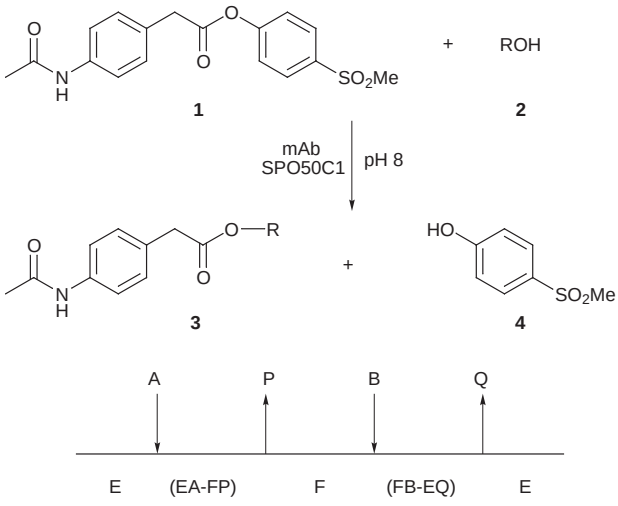

Fig. 1 Reaction catalyzed by SPO50C1

All alcohols were true substrates for the antibody and afforded observed saturation kinetics (Table 1). Significantly, it was shown that even at very high concentrations of some alcohols (*i.e.* 2.43 M for MeOH) the plateau in maximum rates resulted from saturation and not a detrimental 'organic solvent effect' on antibody activity.§ The trend in kinetic data cannot be explained merely in terms of the chemical reactivity of the alcohols, but most likely by a subtle interplay of nucleophilicity, hydrophobic effects and steric interactions.

Correlation analyses[7] of catalysis with Hansch hydrophobicity constants (π), Taft steric constants (E_s) or a multiparameter equation were nonlinear. While decreased K_m values roughly paralleled increased hydrophobicity [$\Delta\log(1/K_m)/\Delta\pi \sim 1$], k_{cat} showed the opposite bias together with breaks and plateaus. The specificity (k_{cat}/K_m) across the entire series of primary alcohols was remarkably similar with K_m becoming dominant and elevating k_{cat}/K_m about 10-fold only at high π values (π = 3.0, 3.5) (entries 10, 11) or with aromatic structures (entries 12, 13). MeOH, as might be predicted due to a low π (0.5), had the highest K_m, and perhaps because of its high nucleophilicity also gave the best turnover. The secondary alcohols were expectedly slower substrates than their primary congeners, however the differences did not reflect the solution reactivities of these alcohols (compare ratios of entries 3 and 4; k_{cat} = 4.4, k_{uncat} = 78.6). Although Bu^nOH (entry 5) and the other primary alcohols could be considered more efficient substrates (k_{cat}/K_m) than Pr^iOH, the latter was comparable or faster under saturating conditions [*i.e.* k_{cat} (entry 4)/k_{cat} (entry 5) = 5.6]. The k_{cat} for Pr^iOH even approached that of BnOH which was 128 times more reactive in solution. Furthermore, Pr^iOH was 10-fold and 100-fold faster than the other secondary alcohols (entries 7, 14), respectively; in addition cyclohexanol (not depicted) showed no detectable activity. Clearly, there was unique behavior associated with Pr^iOH as a substrate for SPO50C1.

The catalysis by SPO50C1 contrasts with the transesterification of some acyl trypsins[8] and esterases[9] where the more hydrophobic alcohols are often better substrates than lower homologs, except MeOH, and secondary alcohols show little or no activity. Also, Bu^nOH (entry 5) was particularly unusual and showed a tangible downward break in both k_{cat} and K_m. It is possible that nonproductive binding modes, as proposed for special substrates of chymotrypsin,[10] were operative. Aromaticity and steric constraints were contributing factors in the SPO50C1 reactions evidenced by the surprising lack of reactivity of cyclohexylmethanol (not depicted) (compare entries 6, 12). Interestingly, phenethyl alcohol was the most specific substrate which might reflect a favorable combination of hydrophobic and/or aromatic interactions and flexibility of the side-chain hydroxy functionality.

Table 1 Kinetic constants[a] for catalytic antibody SPO50Cl transesterification experiments

Entry	Substrate	k_{cat}/min^{-1}	K_m/mM	(k_{cat}/K_m)/ M^{-1} min^{-1}	k_{uncat}/ M^{-1} min^{-1}	Effective molarity/M
1	MeOH	1.4	8.7×10^2	1.7	2.9×10^{-3}	4.8×10^2
2	EtOH	0.19	3.1×10^2	0.60	2.8×10^{-4}	6.8×10^2
3	Pr^{n}OH	0.22	1.2×10^2	1.8	1.1×10^{-3}	2.0×10^2
4	Pr^{i}OH	0.050	5.1×10^2	0.090	1.4×10^{-5}	3.6×10^3
5	Bu^{n}OH	9.0×10^{-3}	19	0.48	1.5×10^{-4}	60
6	Bu^{i}OH	0.050	48	1.1	2.8×10^{-5}	1.8×10^3
7	Bu^{s}OH	1.4×10^{-3}	65	0.022	nd	nd
8	$C_5H_{11}OH$	0.020	7.3	2.9	3.7×10^{-5}	5.4×10^2
9	PrCH(Me)OH	2.0×10^{-3}	9.1	0.22	1.9×10^{-5}	1.1×10^2
10	$C_6H_{13}OH$	0.020	1.1	19	nd	nd
11	$C_7H_{15}OH$	0.020	1.2	17	nd	nd
12	BnOH	0.060	3.9	15	1.8×10^{-3}	33
13	$PhCH_2CH_2OH$	0.13	5.0	26	2.0×10^{-3}	65
14	MeCH(Ph)OH	4.0×10^{-4}	18	0.023	3.7×10^{-6}	1.1×10^2

[a] Determined at 22 °C in 100 mM bicine, pH 8.0 with 2% MeCN as cosolvent in the presence or absence of antibody. Antibody-catalyzed reactions used 10 μM SPO50Cl and varying concentrations of alcohol in the presence of substrate **1** fixed at 200 μM (close to both the solubility limit and saturation). The mAb has one functional active site (ref. 1). Assays were conducted using reverse-phase (C-18) HPLC (MeCN–H_2O–0.1% TFA eluent) by observing the formation of the ester product at 254 nm. The background rates for entries 4, 6, 8, 9 and 14 were determined at 60 °C and extrapolated to 22 °C by assuming an activation energy of 25 kcal mol^{-1}. The half-life for the spontaneous hydrolysis of **1** at 22 °C under the reaction conditions was 99 min. nd = Not determined (no ester formation was observed at 60 °C). All values have an estimated experimental error of ±10%.

The parameter of 'effective molarity'[11] was used as a measure of the catalytic power of SPO50C1. Although the mechanisms of the antibody-catalyzed and the one-step, base-catalyzed background transesterifications are different, an operational estimate can be made by comparing k_{cat} (min^{-1}) (k_{cat} represents the number of moles of ester product produced per mole of antibody per minute) to k_{uncat} (M^{-1} min^{-1}), the second-order rate constant for alcoholysis. Thus, for example, to achieve the same rate of product synthesis in the uncatalyzed transesterification of **1** by Pr^{i}OH, the concentration of Pr^{i}OH must approach 3600 M (0.050 min^{-1}/1.4×10^{-5} M^{-1} min^{-1}) (Table 1, entry 4). While our previous antibody transesterase, PCP21H3, derived from a transition-state analogue, attained rate enhancements of $>10^6$ M, the alcohol acceptors were restricted to a class structurally congruent to the hapten wherein small changes in binding energy were important.[12]

Our findings here indicated that the influence of structural and electronic factors upon catalysis were not overwhelming. The results refine our view that traditional, inert haptens program complementary binding pockets of somewhat limited scope, whereas reactive immunogens lead to active sites that are more unusual in their substrate tolerance and reactivity. This approach potentially allows one to procure antibodies with the intricate mechanisms of enzymes along with other features useful for biologcal and chemical applications.

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Notes and References

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‡ Reactions were performed in a Hi-Tech stopped-flow spectrophotometer by observing the increase in absorbance due to the formation of **4** (ε_{266} = 13 360 M^{-1} cm^{-1}); 0.2 ml cell, 1 cm path length, 0.2 ml stop volume, 33 ms filter time. The conditions were as described in Table 1 using 1.0 μM antibody.

§ None of the alcohols had any detrimental effect on activity as judged from time-dependent assays of free antibody incubated with alcohols at concentrations above the observed saturation limit.

1 P. Wirsching, J. A. Ashley, C.-H. L. Lo, K. D. Janda and R. A. Lerner, *Science*, 1995, **270**, 1775.
2 J. Wagner, R. A. Lerner and C. F. Barbas III, *Science*, 1995, **270**, 1797.
3 C. F. Barbas III, A. Heine, G. Zhong, T. Hoffmann, S. Gramatikova, R. Björnestedt, B. List, J. Anderson, E. A. Stura, I. A. Wilson and R. A. Lerner, *Science*, 1997, **278**, 2085.
4 C.-H. Lin, T. Z. Hoffman, P. Wirsching, C. F. Barbas III, K. D. Janda and R. A. Lerner, *Proc. Natl. Acad. Sci. USA*, 1997, **94**, 11 773.
5 C.-H. L. Lo, P. Wentworth Jr., K. W. Jung, J. Yoon, J. A. Ashley and K. D. Janda, *J. Am. Chem. Soc*., 1997, **119**, 10 251.
6 W. W. Cleland, *Biochim. Biophys. Acta*, 1963, **67**, 104.
7 C. Hansch and A. Leo, *Substituent Constants for Correlation Analysis in Chemistry and Biology*, Wiley, New York, 1979.
8 F. Seydoux, J. Yon and G. Némethy, *Biochim. Biophys. Acta*, 1969, **171**, 145.
9 P. Barton, A. P. Laws and M. I. Page, *J. Chem. Soc., Perkin Trans. 2*, 1994, 2021; D. Wynne and Y. Shalitin, *Eur. J. Biochem*., 1972, **31**, 554; P. Greenzaid and W. P. Jencks, *Biochemistry*, 1971, **10**, 1210.
10 M. I. Page, *Chem. Soc. Rev*., 1973, **2**, 295; M. I. Page and W. P. Jencks, *Proc. Natl. Acad. Sci. USA*, 1971, **68**, 1678.
11 W. P. Jencks, *Catalysis in Chemistry and Enzymology*, Dover, New York, 1969, ch. 5.
12 P. Wirsching, J. A. Ashley, S. J. Benkovic, K. D. Janda and R. A. Lerner, *Science*, 1991, **252**, 680.

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