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Enzyme promiscuity: first protein-catalyzed Morita–Baylis–Hillman reaction

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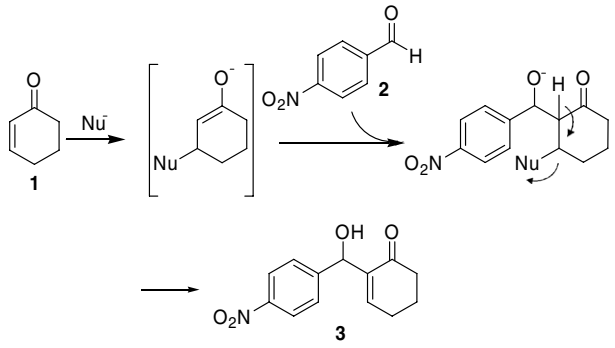
Abstract—The Morita–Baylis–Hillman reaction of cyclohexenone and *p*-nitro benzaldehyde is catalyzed by carrier proteins such as serum albumins or enzymes such as certain lipases, conversion of up to 35% and enantioselectivities of up to 19% being observed.

In recent years a new frontier in biocatalysis, termed enzyme promiscuity, has emerged.[1] It concerns the ability of a given enzyme or mutant thereof to catalyze more than one type of chemical transformation. From a synthetic organic point of view enzyme promiscuity is especially interesting when the type of reaction being catalyzed does not occur in nature. Such a development enlarges the repertoire of methods available to organic chemists. For example, it has been demonstrated that lipases (wild-type)[2] and rationally designed mutants[3] catalyze the Michael addition of amines, thiols, and β-ketoesters to various acceptors such as acrylonitrile and α,β-unsaturated carbonyl compounds, thus opening the door for many future applications in synthetic organic chemistry. It has even been observed that some proteins which have no natural catalytic function themselves are capable of catalyzing certain synthetic reactions, although such cases are rare.[1] An interesting example of this phenomenon is the Kemp elimination[4] and related processes[5] which were found to be catalyzed by bovine serum albumin (BSA). This is actually a carrier protein present in blood plasma with no natural catalytic function.[6] In the Kemp elimination catalyzed by BSA, it was shown that the amino group present in the side chain of lysine acts as the base,[4] and such a mechanism is also likely in the case of related elimination reactions.[5] In the present Letter we report that BSA and various other serum albumins as well as certain lipases catalyze a transformation important in synthetic organic chemistry, namely the Morita–Baylis–Hillman reaction (MBH).[7] The gross mechanism involves Michael addition of a neutral or charged nucleophile with formation of an enolate which undergoes an aldol addition, followed by a proton shift and elimination of the nucleophile.[7] However, the details of the mechanism are more complicated and appear to depend on the type of nucleophilic catalyst and on the reaction conditions.[8] Along synthetic lines, numerous examples of asymmetric variations (including aza MBH reactions) are continuing to appear.[7–9]

As a model transformation we chose the reaction of cyclohexenone (**1**) and *p*-nitro benzaldehyde (**2**) with the formation of **3**, a standard reaction in MBH chemistry (Scheme 1).[7–9] It is well known that MBH reactions are catalyzed by amino compounds such as DABCO[7,10] or imidazole derivatives,[7,10] phosphines,[7] or alcohols.[11] In the latter case, basic conditions need to be chosen



Scheme 1. Morita–Baylis–Hillman reaction of **1** and **2**.

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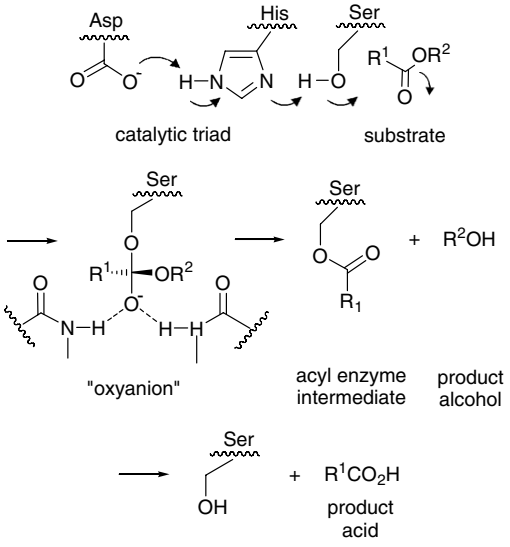
which ensure the presence of catalytically active alkoxides.[11] The MBH reaction is an example of an organocatalyzed process.[12]

We speculated that several types of nucleophilic moieties in the side chains of amino acids present in proteins could serve as catalytically active centers. One possibility is lysine, because the amino group in the side chain could perform as a nucleophile in the MBH reaction, analogous to its performance in the Kemp elimination catalyzed by BSA.[4] Other candidates are the imidazole moiety of histidine and/or the alcohol function of serine. The latter could be of particular interest in the case of lipases,[13] because these enzymes, catalyzing the hydrolysis of esters, are characterized by a catalytic triad composed of aspartate, histidine, and serine (Scheme 2). As can be seen, these undergo a proton shuttle which leads to partial deprotonation and thus activation of the alcohol function of serine, which adds to the carbonyl function of the substrate nucleophilically with formation of the so-called oxy-anion.[13] A serine in such a constellation could function as the catalytically active nucleophile in MBH reactions in the presence of other serine members of the protein which are not activated, similar to simple alkoxides.[11]



Scheme 2. Mechanism of lipase-catalyzed hydrolysis of esters.

We first determined conditions under which there is no detectable background reaction by varying the nature of the buffer, the pH, and the reaction time. It turned out that a phosphate buffer at pH 7.0 alone at a temperature of 30 °C does not lead to any product formation even after five days. These conditions were then applied in the process of screening various carrier proteins and enzymes as potential catalysts in the model MBH reaction (Table 1).

Table 1 reveals that BSA and several other serum albumins are more or less active catalysts in the MBH reaction (e.g., entries 1–8). These results are noteworthy because, as delineated above, serum albumins are carrier proteins which bind and transport a variety of compounds in blood plasma without acting as catalysts. Several standard lipases such as CALB show no activity (Table 1, entry 10) while other lipases (Table 1, entries 12–16) result in low but detectable conversion. It is currently unclear why some lipases are active, while others show no activity at all under the same reaction conditions. Interestingly, in some cases a small but appreciable degree of enantioselectivity is observed (up to 19%, entry 2).

Table 1. Morita–Baylis–Hillman reaction of cyclohexenone (**1**) and *p*-nitro benzaldehyde (**2**) catalyzed by proteins/enzymes (1 mL of 100 mM phosphate butter; pH 7.0; 20 μL of each substrate **1**/**2**/200 mM in CH_3CN; 30 mg enzyme/protein; 30 °C)

Entry	Protein	Reaction time (d)	Conversion (%)	ee (%)
1	Rabbit serum albumin	2	23	1
2	Bovine serum albumin (fraction V)[a]	2	15	19
3	Bovine serum albumin (fraction V)[a]	2	9	18
4	Bovine serum albumin (fraction V)[a]	2	9	13
3	Bovine serum albumin (98% by electrophoresis) SIGMA	2	10	15
4	Bovine serum albumin (fraction V)[b]	2	35	9
5	Sheep serum albumin	2	11	10
6	Human serum albumin[c]	2	5	13
7	Chicken white egg albumin	2	4	0
8	Porcine serum albumin	2	4	0
9	Streptavidin	2	2	9
10	CALB lipase	5	0	—
11	Porcine liver esterase	5	10	0
12	Hog pancreas lipase	5	7	(−)2
13	Wheat germ lipase	5	6	0
14	Porcine pancreas lipase	5	6	0
15	Calf pregastric lipase	5	6	0
16	*Aspergillus niger* lipase	5	4	
17	*Rhodococcus rhodochrous* epoxide hydrolase	5	0	—
18	Blank 100 mM phosphate buffer pH 7.0	5	0	—

[a] Different commercial samples of BSA (fraction V) were assayed.
[b] In this case 40 °C.
[c] 8 mg Protein.

In summary, we have discovered that the Morita–Baylis–Hillman reaction can be catalyzed by various proteins, including some which themselves are not enzymes (serum albumins). Although the details of the mechanism of catalysis remain to be uncovered, the present observations extend the phenomenon of enzyme promiscuity.[1] This is of special significance because the MBH reaction does not occur in nature. Enantioselectivity and activity are not very high. However, this may not be surprising because only the wild-type proteins were considered here. Indeed, the present results set the stage for possible genetic optimization by means of directed evolution.[14]

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References and notes

1. Reviews of enzyme promiscuity and moonlighting: (a) Copley, S. D. *Curr. Opin. Chem. Biol.* **2003**, *7*, 265–272; (b) James, L. C.; Tawfik, D. S. *Trends Biochem. Sci.* **2003**, *28*, 361–368; (c) Bornscheuer, U. T.; Kazlauskas, R. J. *Angew. Chem.* **2004**, *116*, 6156–6165; *Angew. Chem., Int. Ed.* **2004**, *43*, 6032–6040; (d) Jeffery, C. J. *Curr. Opin. Struct. Biol.* **2004**, *14*, 663–668; (e) Tompa, P.; Szász, C.; Buday, L. *Trends Biochem. Sci.* **2005**, *30*, 484–489; (f) Aharoni, A.; Gaidukov, L.; Khersonsky, O.; McQ Gould, S.; Roodveldt, C.; Tawfik, D. S. *Nat. Genet.* **2005**, *37*, 73–76.
2. Torre, O.; Alfonso, I.; Gotor, V. *Chem. Commun.* **2004**, 1724–1725.
3. (a) Carlqvist, P.; Svedendahl, M.; Branneby, C.; Hult, K.; Brinck, T.; Berglund, P. *ChemBioChem* **2004**, *5*, 1–6; (b) Svedendahl, M.; Hult, K.; Berglund, P. *J. Am. Chem. Soc.* **2005**, *127*, 17988–17989.
4. Hollfelder, F.; Kirby, A. J.; Tawfik, D. S. *Nature* **1996**, *283*, 60–62.
5. Klein, G.; Reymond, J.-L. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1113–1116.
6. Peters, T. *All about Albumin: Biochemistry, Genetics and Medical Applications*; Academic Press: San Diego, 1996.
7. Reviews of the Morita–Baylis–Hillman reaction: (a) Drewes, S. E.; Roos, G. H. P. *Tetrahedron* **1988**, *44*, 4653–4670; (b) Ciganek, E. *Org. React.* **1997**, *51*, 201–350; (c) Basavaiah, D.; Rao, A. J.; Satyanarayana, T. *Chem. Rev.* **2003**, *103*, 811–891; (d) Langer, P. *Angew. Chem.* **2000**, *112*, 3177–3180; *Angew. Chem., Int. Ed.* **2000**, *39*, 3049–3052.
8. Discussions regarding mechanistic details of the MBH reaction: (a) Santos, L. S.; Pavam, C. H.; Almeida, W. P.; Coelho, F.; Eberlin, M. N. *Angew. Chem.* **2004**, *116*, 4430–4433; *Angew. Chem., Int. Ed.* **2004**, *43*, 4330–4333; (b) Price, K. E.; Broadwater, S. J.; Walker, B. J.; McQuade, D. T. *J. Org. Chem.* **2005**, *70*, 3980–3987; (c) Aggarwal, V. K.; Fulford, S. Y.; Lloyd-Jones, G. C. *Angew. Chem.* **2005**, *117*, 1734–1736; *Angew. Chem., Int. Ed.* **2005**, *44*, 1706–1708; (d) Krafft, M. E.; Haxell, T. F. N.; Seibert, K. A.; Abboud, K. A. *J. Am. Chem. Soc.* **2006**, *128*, 4174–4175; (e) Shi, M.; Chen, L.-H.; Li, C.-Q. *J. Am. Chem. Soc.* **2005**, *127*, 3790–3800.
9. Selected examples including mechanistic aspects: (a) Iwabuchi, Y.; Nakatani, M.; Yokoyama, N.; Hatakeyama, S. *J. Am. Chem. Soc.* **1999**, *121*, 10219–10220; (b) McDougal, N. T.; Schaus, S. E. *J. Am. Chem. Soc.* **2003**, *125*, 12094–12095; (c) Imbriglio, J. E.; Vasbinder, M. M.; Miller, S. J. *Org. Lett.* **2003**, *5*, 3741–3743; (d) Raheem, I. T.; Jacobsen, E. N. *Adv. Synth. Catal.* **2005**, *347*, 1701–1708; (e) Berkessel, A.; Roland, K.; Neudörfl, J. M. *Org. Lett.* **2006**, *8*, 4195–4198; (f) Shi, M.; Liu, Y.-H. *Org. Biomol. Chem.* **2006**, *4*, 1468–1470; (g) Rodgen, S. A.; Schaus, S. E. *Angew. Chem.* **2006**, *118*, 5051–5054; *Angew. Chem., Int. Ed.* **2006**, *45*, 4929–4932; (h) Buskens, P.; Klankermayer, J.; Leitner, W. *J. Am. Chem. Soc.* **2005**, *127*, 16762–16763; (i) Gausepohl, R.; Buskens, P.; Kleinen, J.; Bruckmann, A.; Lehmann, C. W.; Klankermayer, J.; Leitner, W. *Angew. Chem.* **2006**, *118*, 3772–3775; *Angew. Chem., Int. Ed.* **2006**, *45*, 3689–3692; (j) Nakano, A.; Takahashi, K.; Ishihara, J.; Hatakeyama, S. *Org. Lett.* **2006**, *8*, 5357–5360; (k) Matsui, K.; Takizawa, S.; Sasai, H. *Synlett* **2006**, 761–765; (l) Shi, M.; Liu, Y.-H.; Chen, L.-H. *Chirality* **2007**, *19*, 124–128.
10. (a) Aggarwal, V. K.; Mereu, A. *Chem. Commun.* **1999**, 2311–2312; (b) Shi, M.; Jiang, J.-K.; Li, C.-Q. *Tetrahedron Lett.* **2002**, *43*, 127–130; (c) Gatri, R.; Gaied, M. M. E. *Tetrahedron Lett.* **2002**, *43*, 7835–7836; (d) Luo, S.; Zhang, B.; He, J.; Janczuk, A.; Wang, P. G.; Cheng, J.-P. *Tetrahedron Lett.* **2002**, *43*, 7369–7371.
11. Luo, S.; Mi, X.; Xu, H.; Wang, P. G.; Cheng, J.-P. *J. Org. Chem.* **2004**, *69*, 8413–8422.
12. Reviews of organocatalytic reactions: (a) Seayad, J.; List, B. *Org. Biomol. Chem.* **2005**, *3*, 719–724; (b) List, B. *Tetrahedron* **2002**, *58*, 5573–5590; (c) *Asymmetric Organocatalysis*; Berkessel, A., Gröger, H., Eds.; Wiley-VCH: Weinheim, 2004; (d) Dalko, P. I.; Moisan, L. *Angew. Chem.* **2001**, *113*, 3840–3864; *Angew. Chem., Int. Ed.* **2001**, *40*, 3726–3748.
13. (a) Schmid, R. D.; Verger, R. *Angew. Chem.* **1998**, *110*, 1694–1720; *Angew. Chem., Int. Ed.* **1998**, *37*, 1608–1633; (b) Krishna, S. H.; Karanth, N. G. *Catal. Rev.* **2002**, *44*, 499–591; (c) Reetz, M. T. *Curr. Opin. Chem. Biol.* **2002**, *6*, 145–150.
14. (a) *Directed Enzyme Evolution: Screening and Selection Methods*; Arnold, F. H., Georgiou, G., Eds.; Humana Press: Totowa, NJ, 2003; Vol. 230; (b) *Directed Molecular Evolution of Proteins (or How to Improve Enzymes for Biocatalysis)*; Brakmann, S., Johnsson, K., Eds.; Wiley-VCH: Weinheim, 2002; (c) *Enzyme Functionality—Design, Engineering, and Screening*; Svendsen, A., Ed.; Marcel Dekker: New York, 2004; (d) *Evolutionary Methods in Biotechnology (Clever Tricks for Directed Evolution)*; Brakmann, S., Schwienhorst, A., Eds.; Wiley-VCH: Weinheim, 2004; For a historical account of directed evolution, see: (e) Koltermann, A.; Kettling, U. *Biophys. Chem.* **1997**, *66*, 159–177; Reviews of directed evolution of enantioselective enzymes: (f) Reetz, M. T. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 5716–5722; (g) Reetz, M. T. In *Directed Evolution of Enantioselective Enzymes as Catalysts for Organic Synthesis*; Gates, B. C., Knözinger, K., Eds.; Elsevier: San Diego, CA, 2006; pp 1–69; (h) Reetz, M. T. *Tetrahedron* **2002**, *58*, 6595–6602; See also: (i) Turner, N. J. *Trends Biotechnol.* **2003**, *21*, 474–478.