Use of a Robust Dehydrogenase from an Archael Hyperthermophile in Asymmetric Catalysis: Use of Ethanol as a "Chiral Biorenewable Reductant"

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Introduction

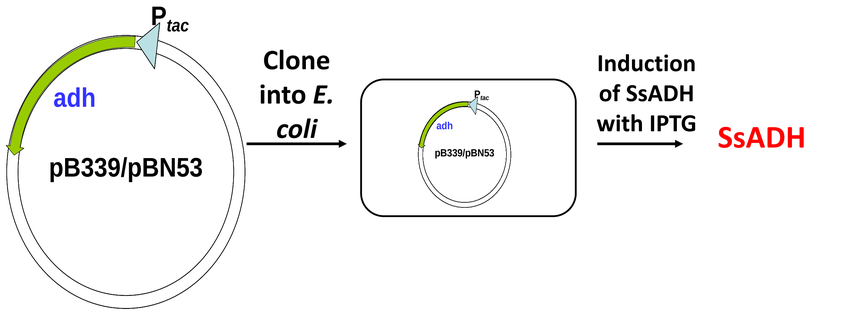
Microorganisms that grow better at elevated temperatures, loosely described as thermophiles, have generated great interest over the years owing to their remarkable properties. These organisms are not only able to withstand high temperatures but also harsh conditions such as extremes of pH, pressure, salinity and redox potentials. The enzymes that are then available from these organism may offer opportunities to the synthetic community, complementary to approaches with mesophilic enzymes. However, perhaps due to a bias that, despite their stability, many hyperthermophilic enzymes have "narrow substrate specificities," archael extremeophiles remain a largely untapped resource in asymmetric synthesis and process chemistry.

Annotation suggests that *Sulfolobus* may possess a number of dehydrogenases, most uncharacterized and of unknown function. However, the limited data available for SsADH-10 substrate specificity and enantioselectivity, suggest that genome mining prospects in *Sulfolobus* for synthetically useful NAD(P)-linked DH's are good.

Collaboration between the Blum lab and the Berkowitz lab has identified a thermophilic archaebacterial enzyme (SsADH-10) that selectively reduces the (S)-antipode of α-methyl aryl acetaldehydes. Moreover, conditions have been found whereby racemization is fast relative to aldehyde reduction, permitting high yields, as well as high enantiopurity. Though the enzyme is a nicotinamide dependent dehydrogenase, we are able to regenerate the cofactor with ethanol demonstrating that a cheap alcohol can serve as the stoichiometric biorenewable reducing agent for this value-added asymmetric reduction.

Sulfolobus sulfataricus ADH Expression

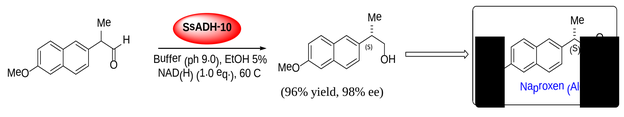
Protein phylogeny of the SsADH proteins. A consensus neighbor joining distance tree is shown of all SsADHs and homologues of highest sequence identity in related taxa. Distances are indicated by the bar (lower left corner) and represent 10 substitutions per 100 residues. Percent occurrence among 100 trees was greater than 50% for all nodes except those indicated with an asterisk.

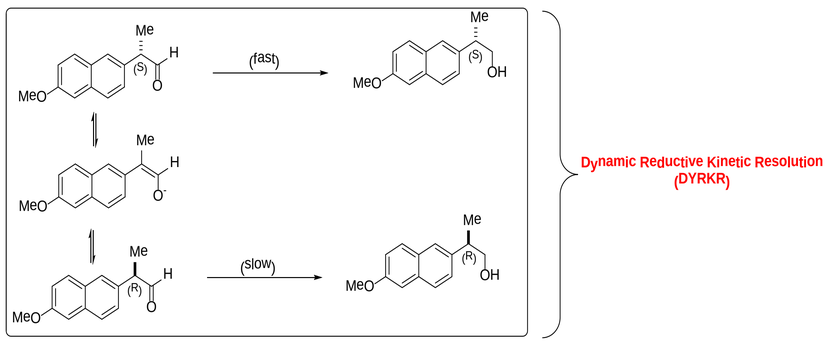


Over expression of SsADH. *adh* gene from *S. solfataricus* was inserted into vector pB339/pBN53 at EcoRI restriction site and cloned into *E. coli* host strain. The SsADH protein is expressed using a strong IPTG inducible promoter (P_{tac}). *E. coli* cells were grown at 37°C, and then SsADH protein was induced by adding IPTG (Isopropyl β-D-1-thiogalactopyranoside) . The SsADH protein was purified by sonication, and then by heat treatment at 80°C (to kill all the *E. coli* protein).

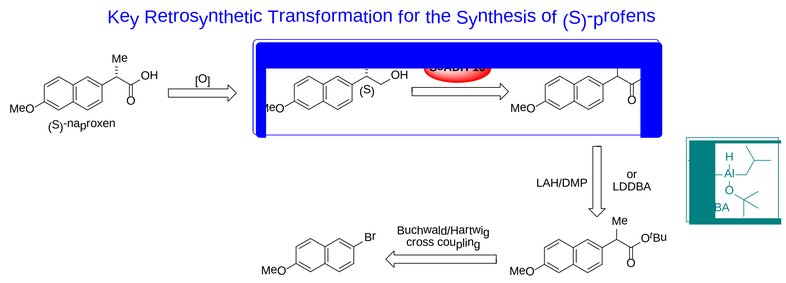
Panning SsADH-10 for pharmaceutically relevant substrates…

Screening in the Berkowitz lab with the Ss-ADH expressed and purified in the Blum lab has led to an exciting result. We are pleased to report here that the Naproxen (aleve)-leading aldehyde is successfully reduced by this unusual recombinant ADH from a hyperthermophilic archae bacterium. The sense of enantioselection is that which is required for synthesis of the pharmaceutical drug.

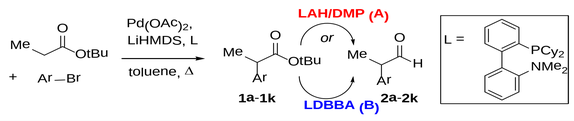


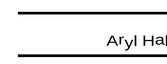
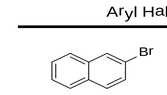
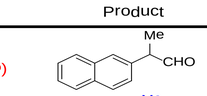
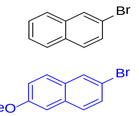
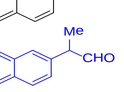
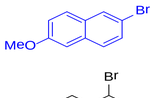
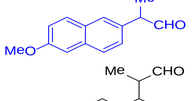
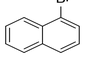
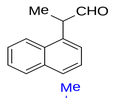
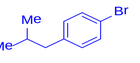
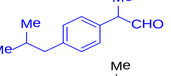
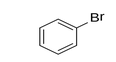
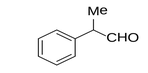
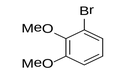
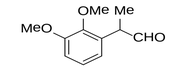
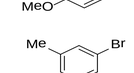
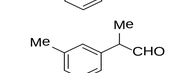
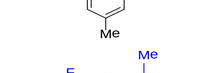
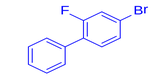
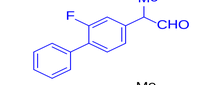


A rapid entry to (S)-profens



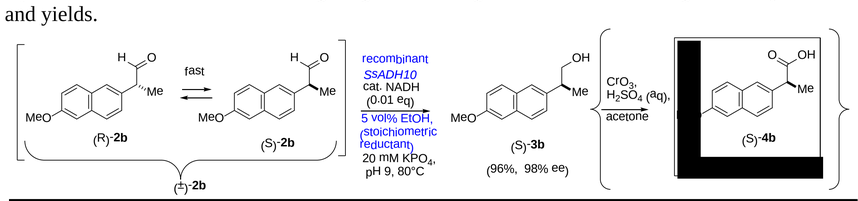
Assembly of requisite racemic 2-aryl propionaldehydes

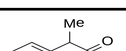
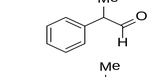
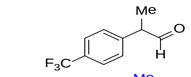
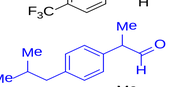
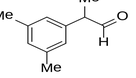
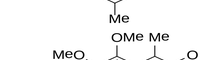
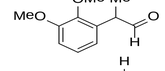
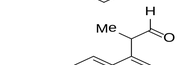
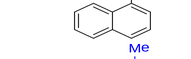
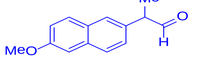


Aryl Halide	Coupling Yld (%)	Red'n Yld (%)[a]	Product
	(88%)	A (50/95%)	
	(73%)	A (95/85%) B (67%)	
	(73%)	A (95/92%)	
	(99%)	A (94/86%) B (69%)	
	(70%)	A (53/80%)	
	(52%)	A (60/89%)	
	(81%)	A (99/79%)	
	(90%)	A (88/91%) B (64%)	
	(73%)	A (92/83%)	
	(82%)	A (83/80%) B (72%)	
	(79%)	A (86/84%) B (72%)	

SsADH-10 mediated DYRKR entry to (S)-profenals

Screening of α-methyl aryl acetaldehydes containing an enolizable stereocenter at the alpha position demonstrate that the NADH dependent reduction utilizing the SsADH-10 is under Dynamic Reductive Kinetic control giving the resulting chiral alcohols with high enantiopurities and yields.



Entry	Substrate	Time(h)	yield(%)	ee(%)
1		18	74	98
2		24	55	98
3		18	92	99
4		18	99	90
5		18	recovered SM	-
6		18	90	80
7		18	96	98
8		18	57	94
9		18	99	90
10		12	85	95
11		18	95	61
12		18	85	95

A reiterable "thermal" recycling switch

Cycle	yield (%)	ee(%)
1	99	96
2	99	96
3	95	95
4	78	96
5	64	94

20 mM KPO_4 buffer pH 9, NADH (1.0 eq), 5% ethanol (v/v%),18 h, 80 C.

Molecular dynamics (GROMACS) and docking experiments (Autodock Vina)

Docking experiments were carried out on the relaxed stucture (GROMACS) utilizing Autodock Vina on the (*S*)-antipode of naproxenal, flurbiprofenal, ketoprofenal and fenoprofenal. Importantly, in all four cases, the resulting docked structure places the α-hydrogen rather than the bulkier methyl group in a trajectory pointed toward Trp-95, suggesting that this residue is, indeed, important for the observed (*S*)-selectivity of the enzyme.

Space filling representation of 1R37 (left), and the GROMACS annealed structure (right)

The results of these in silico MD/docking experiments also suggest that four profenals cluster into two distinct binding modes, corresponding to two different approaches to binding the distal aromatic ring:

(A) On the one hand, for (S)-flurbibropenal and (S)-naproxenal, the distal aromatic ring appears to fit into the hydrophobic pocket formed by Leu-295 and Leu-272.

(B) On the other hand, the docking results for (S)-keotprofenal and (S)-fenoprofenal suggest that, in the case of flexible (bis)aryl substrates, Trp-117 plays a critical role in binding. That is, the distal aromatic ring of the profenals adopts a conformation so as to have an edge-to-face π-π interaction with the edge of Trp-117 side chain.

Publications

Jacob A. Friest, Yukari Maezato, Sylvain Broussy, Paul Blum*,†,§ and David B. Berkowitz*,‡,§, Use of a Robust Dehydrogenase from an Archael Hyperthermophile in Asymmetric Catalysis–Dynamic Reductive Kinetic Resolution Entry into (*S*)-Profens. *J. Am. Chem. Soc.* **2010**, 132, 5930-5931.
(Featured in *Chemical and Engineering News*, **2010**, *88* (16), 34- 35; Featured in *SYNFACTS*, **2010**, 8, 861)

Conclusions

•SsADH-10 displays a broad substrate profile for hydrophobic propionaldehydes bearing *m*-, and *p*-substituted aryls, fused bi-aryls, and *m*, *p*-linked (ether or ketone) aryl sidechains.

• (S)-profenols corresponding to the NSAIDs naproxen (scaled to 1 g), ibuprofen, flurbiprofen, ketoprofen, and fenoprofen were obtained in excellent yields (85-99%) and high enantioselectivity (90-99% ee).

• Docking and MD simulations provide a model for how these hydrophobic substrates bind to SsADH-10.

• A reiterable "thermal recycling" as demonstrated with SsADH-10, may be generalized to other hyperthermophilic enzymes (not limited to dehydrogenases) and is likely to find broad application from a practical process point of view.

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