Expression, Characterization and Use of a New Bacterial Dehydrogenase in Asymmetric Synthesis – Glucose as a "Chiral, Biorenewable Reductant"

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Abstract: This work focuses on the expression of a previously uncharacterized alcohol dehydrogenase (ADH) enzyme and its use in asymmetric carbonyl reduction chemistry. Initially, we describe the expression system and purification of the protein. Homogeneous protein has been examined for substrate specificity across a battery of carbonyl compounds, of variable structures. Finally, we describe the application of the enzyme to asymmetric carbonyl reduction, to produce highly enantiomerically enriched synthetic building blocks of note. For this application, conditions are described in which glucose serves as the terminal reductant, thermodynamically driving the process. In this way, one is harnessing biorenewable redox equivalents for asymmetric synthesis.

Structure Activity Relationship for *CaADH*: Revealing a Preference for Aromatic Carbonyls

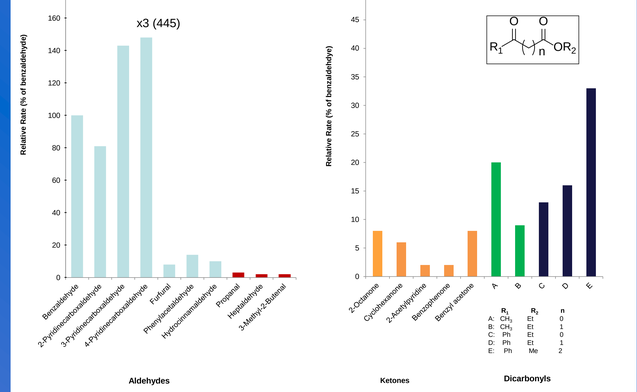
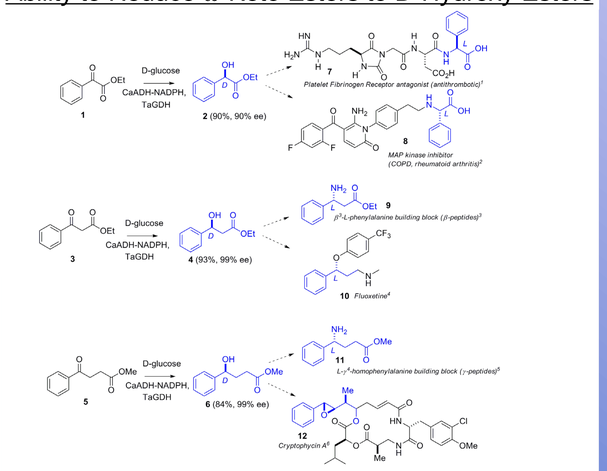


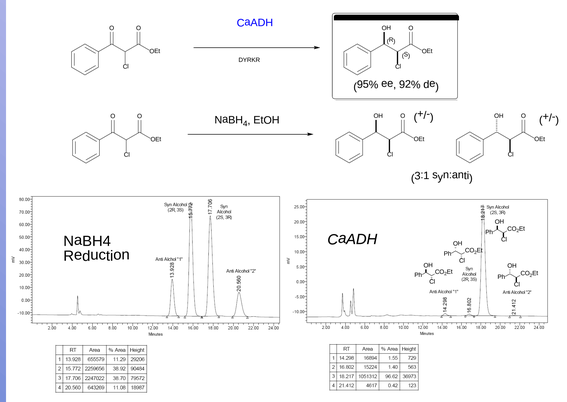
Fig 2. Relative rates of reduction for various substrates tested on *CaADH*. Rates were determined at pH 7.0 and at a substrate concentration of 10 mM. 5% DMSO was present to solubilize substrates. Rates are reported as a percentage relative to the reduction rate of benzaldehyde.

Ability to Reduce ω-Keto Esters to D-Hydroxy Esters



(1) H.U. Stilz; W. Guba; B. Jablonka; M. Just; O. Klingler; W. Konig; W. Wehner; G. Zoller *J. Med. Chem.* 2001, **44** 1158-1176; (2) D.F.C. Moffat; S. Pintat; S. Davies (Chroma Therapeutics Ltd) *PCT Int. Appl.* O 2009060160 (2009), 71 pp; (3a) A.V.R. Rao, M.K. Gurjar; B.R. Nallaganchu; A. Bhandari *Tetrahedron Lett.*, 1993, **34**, 7085-7088; (3b) D. Seebach; A.K. Beck; S. Capone; G. Deniau ; U. Grosel; E. Zass *Synthesis* 2009, 1-32; (4) J.W. Hilborn; Z.H. Lu; A.R. Jurgens; Q.K. Fang; P. Byers; S.A. Wald; C.H. Senanayake *Tetrahedron Lett.* 2001, **42**, 8919-8921; (5) D. Seebach; D.F. Hook, A. Glaettli *Biopolymers (Protein Science)* 2006, 84, 23-27; (6) A.K. Ghosh; L. Swanson. *J. Org. Chem.* 2003, **68**, 9823-9826.

Adding a Stereocenter to the β-Ketoester Motif: A DYRKR Entry into the Taxoid Sidechain



	RT	Area	% Area	Height
1	13.926	655579	11.29	29206
2	16.802	2256656	38.92	90484
3	17.706	2247022	38.70	75672
4	21.412	46117	0.42	123

	RT	Area	% Area	Height
1	14.290	98964	1.55	729
2	16.802	15224	1.40	563
3	18.217	1051312	96.62	36973
4	21.412	4617	0.42	123

Important Note: Substrate decomposition was observed under neutral or basic pH, so acidic conditions must be used to preserve yield (pH 6.5). This dictated a study of cofactor regeneration under acidic pH

C. acetobutylicum contains a 192-kb megaplasmid in addition to a 3.94-Mb chromosome. This megaplasmid (above) contains genes responsible for solventogenic properties that are of interest in biofuel research[1]. Figure was generate using Microbial Genome Viewer[1].

1. R Kerkhoven, F H J van Enckevort, J Boekhorst, D Molenaar, and R J Siezen. *Bioinformatics* 2004. **20,** 1812-1814.

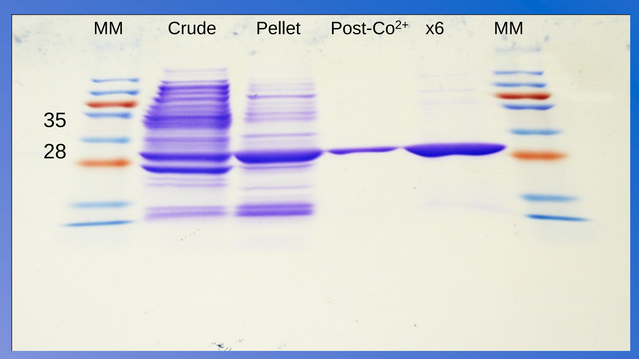


Fig 1. SDS-PAGE (12%) of *CaADH* purification

Fraction	Units/mL*	Total Units	mg/mL	Total mg	Units/mg	Yield	Pur Factor
Crude	38.5	463.1	19.7	236.4	1.95		1
Final	32.1	321.5	0.56	5.6	58.1	70%	30

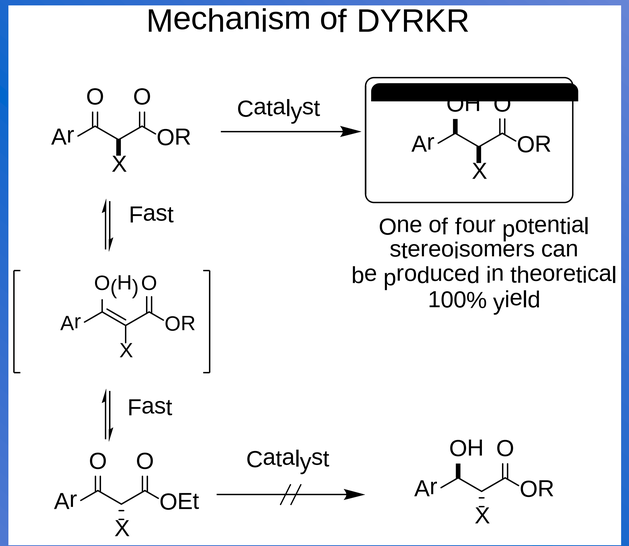
* - Units defined as μmoles of benzaldehyde reduced per minute

Use of *Clostridium acetobutylicum* ADH *(CaADH)* for value added synthesis using cellulose derived terminal reductants

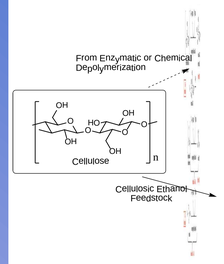
- Biocatalysts are increasingly being used as the asymmetric catalyst of choice for many organic transformations
- Some enzymes (ADH's) require exogeneous cofactor (NAD(P)H) addition for activity
- Cofactor is too expensive to be used in stoichiometric amounts as terminal reductant
- Through coupling reactions, other economical, biological molecules can be used as terminal reductants (see biomass .ethanol. and glucose)

J.C. Moore, D. J. Pollard, B. Kosjek, P. N. Devine, *Acc. Chem. Res.*, 2007, **40,** 1412-1419

Mechanism of DYRKR



Potential Bio-Cellulosic Feeding into Cofactor Regeneration Systems

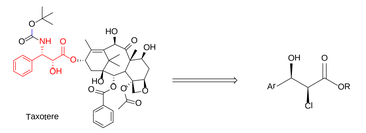


(1) R. Woodyer, H. Zhao, W.A. van der Donk, *FEBS J.* 2005**, *272,*** 3816–27.
(2) C.H. Wong, G.M. Whitesides, *JACS*, 1983**, *105,*** 5012–14.
(3) S. Broussy, R.W. Cheloha, D.B. Berkowitz, *Org Lett.*, 2009, **11**, 305-308.

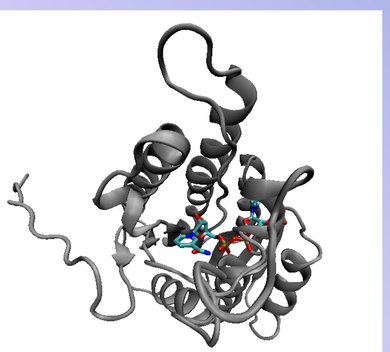
Taxane Targets

Taxanes are a class of therapeutics that display anti-mitotic activity. Taxol, an extract from the Pacific Yew, has the longest running history of Taxane theraperutics. Due to the detrimental effects of bark extraction, semi-synthetic routes to Taxol analogs were employed. Taxotère utilizes the 10-deacetylbaccatin core from the leaves and needles of the Yew family – a process both renewable and readily available[1].

The described *CaADH* has the ability to reduce an α-chloro-β-ketoester to the corresponding hydroxyester (above left). This reduced product can be converted and installed as the phenylisoserine side chain of the taxane class[2].



1. J. N. Denis, A. E. Greene, D. Guenard, F. Gueritte-Voegelein, L. Mangatal, P. Potier. *JACS*, 1988, **110**, 5917-5919.
2. B. Feske, I. Kaluzna, J.D. Stewart, *JOC*, 2005, **70**, 9654-9657.



Homology model of *CaADH*[1]. Model is based off of 1XG5 (Human Putative DH, 2NWQ (*P aeruginosa DH),* and 2NPF (*A aquifex DH).*

(1) N. Eswar, M. A. Marti-Renom, B. Webb, M. S. Madhusudhan, D. Eramian, M. Shen, U. Pieper, A. Sali, *Curr Protocols in Bioinformatics*, John Wiley & Sons, Inc., Supplement 15, 5.6.1-5.6.30, 2006.

Conclusions

Conclusions: A new ADH enzyme from *Clostridium acetobutylicum* (CaADH*)* was expressed, purified and characterized. In addition to displaying high activity for aromatic aldehyde reduction, CaADH, showed a general ability to reduce ω-keto esters, bearing that same aroyl moiety. A general preference for formation of D-hydroxy esters was seen, leading to valuable α-, β- and γ-hydroxy ester building blocks in 90-99% ee. Expanding on this trend, successful DYRKR entry into the syn α-chloro-D-β-hydroxy ester building block for Taxol and Taxotere was achieved (95% de; 99% ee) . When coupled with a biomass derived cofactor regeneration system (e.g. glucose), these transformations represent a highly value added application of biorenewable reducing equivalents.

Support from the NSF (CHE-0911732), NCESR and ACS (SURF award to RWC) is acknowledged.