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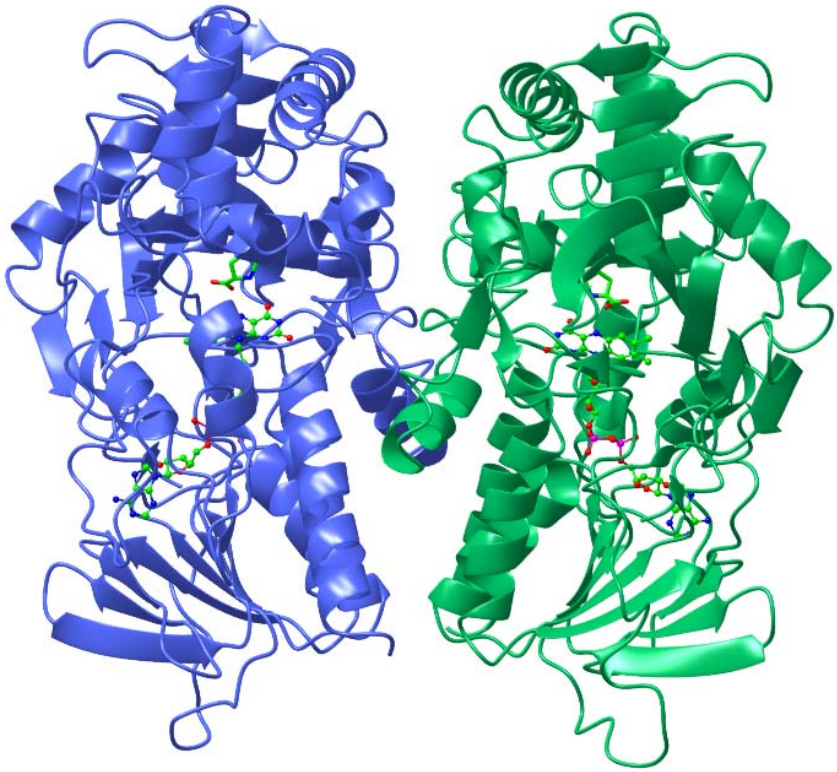

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COMMUNICATION

A new dehydrogenase from *Clostridium acetobutylicum* for asymmetric synthesis: dynamic reductive kinetic resolution entry into the Taxotère side chain†‡

Gregory A. Applegate, Ross W. Cheloha, David L. Nelson and David B. Berkowitz*

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An NADP-dependent alcohol dehydrogenase from *Clostridium acetobutylicum* (CaADH) has been expressed and characterized. CaADH enantioselectively reduces aromatic α-, β- and γ-keto esters to the corresponding D-hydroxy esters and provides a building block for the Taxotère side chain (95% yield, 95% de, 99% ee) by dynamic reductive kinetic resolution (DYRKR).

Biocatalytic synthesis with purified enzymes, including aldolases,[1] epoxide hydrolases,[2] Baeyer-Villigerases,[3] amine oxidases,[4] lipases,[5] and alcohol dehydrogenases,[6] is emerging as a viable alternative to traditional asymmetric synthesis. The recent transaminase-based asymmetric synthesis of Januvia™ highlights the viability of such approaches.[7]

This strategem tracks with a long-standing interest in our group in using enzymes in asymmetric synthesis.[8,9] Of late, that focus has been especially on alcohol dehydrogenases (ADHs), both for asymmetric carbonyl reduction[10] and for installation of a center alpha- to a carbonyl, *via* dynamic reductive kinetic resolution (DYRKR), using a hyperthermophilic archael dehydrogenase.[11]

In seeking other sources of synthetically useful DH's, we were struck by properties of *Clostridium acetobutylicum*. The organism has both acidogenic and solventogentic growth phases,[12] and is known to produce high titres of organic solvent in the latter, leading to interest in *Clostridial* strain fermentation,[13] and importation of *Clostridial* genes into other hosts toward bio-butanol production.[14] Particularly notable in this latter endeavor (yeast host) are the efforts of Keasling.[15] This suggested to us that *Clostridia* likely possess organic carbonyl redox enzymes.

Indeed, early studies at UMIST revealed alicyclic/aromatic carbonyl reduction activity in *C. acetobutylicum*.[16] Now the entire *C. acetobutylicum* genome has been sequenced,[17] and consists of a 3.94 MB chromosome and a 192 KB megaplasmid (Fig. 1) that encodes six different genes annotated as DHs, one of which (GI: 15004706) has 40% homology to a β-ketoacyl reductase from *Ralstonia eutropha*, an enantioselective carbonyl reductase.[18] Henceforth, this enzyme will be labeled simply as *Clostridium acetobutylicum* ADH (*CaADH*—251 AAs, 28.5 KD—calcd MW and 9.5—calcd pI). This short chain NADPH-dependent DH is clearly distinct from the established NADH-dependent butanol DH that has been well characterized for some time.[19] CaADH was expressed in recombinant form in *E. coli* and purified *via* a hexahistidine-tag.

As can be seen in the SDS-PAGE overview (Fig. 2), abundant expression of the target protein was seen in the *E. coli* host, and the $(his)_6$-tag, provided a useful handle for purification *via* Co^{2+}-NTA chromatography. Even though the growth temperature was reduced to 25 °C, considerable protein (likely aggregated CaADH) was seen in the pellet. That said, we were delighted to obtain over 5 mg of soluble, homogeneous protein, of high specific activity (58 U per mg-benzaldehyde reduction) per litre of culture.

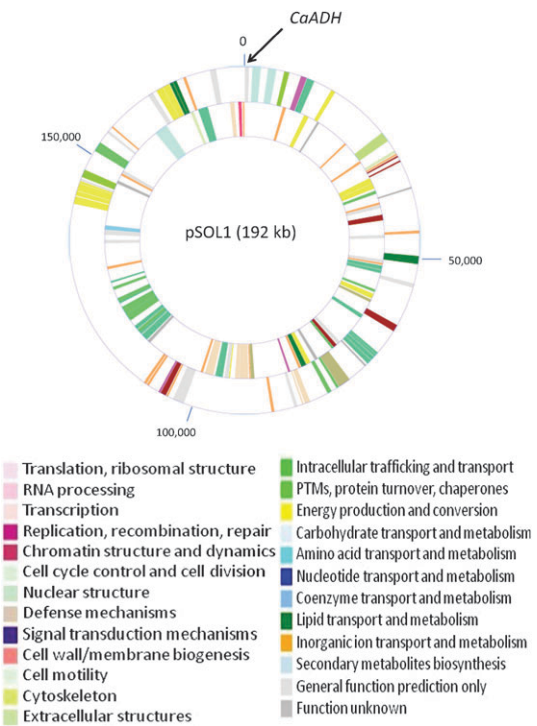

Fig. 1 Map of the *C. acetobutylicum* 192 KB megaplasmid, generated with Microbial Gene Viewer[20] (arrow indicates CaADH gene locus).

Department of Chemistry & Nebraska Center for Energy Sciences Research (NCESR), University of Nebraska, Lincoln, NE, USA. E-mail: dbb@unlserve.unl.edu; Fax: 01 402 472 9402; Tel: 01 402 472 2738

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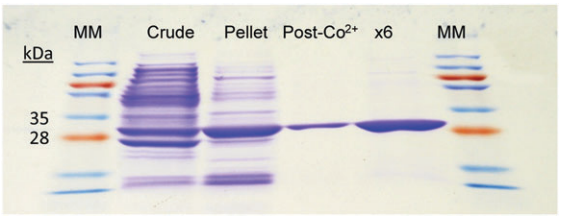

Fig. 2 SDS-PAGE gel illustrating the purification of CaADH: outside lanes = molecular markers (MM); then l to *r*: (i) crude supernatant; (ii) crude pellet; (iii) eluent from Co^{2+}-column; (iv) 6× loading of (iii).

Next, substrate specificity was evaluated, by screening against batteries of aldehyde (Fig. 3) and ketone (Fig. 4) candidates. Several clear trends emerged. Aromatic aldehydes and ketones possessing a carbonyl group directly conjugated with a benzenoid ring are quite good substrates. Simple aliphatic aldehydes are much poorer substrates. Insertion of one or two methylenes between the aromatic ring and the aldehyde carbonyl, *i.e.* as in phenylacetaldehyde and hydrocinnamaldehye, respectively, greatly depletes activity.

Within the apparently preferred Ar–C═O substructure, there appears to be great sensitivity to aryl ring electronics. At the extremes, the electronic rich aromatic aldehyde, furfural, shows a full order of magnitude slower reduction than benzaldehyde, whereas 4-pyridine carboxaldehyde is the fastest substrate yet identified for this ADH. Interestingly, however, movement of the ring nitrogen to the 3-position leads to a better substrate, than its placement at the 2-position, clearly indicating that the inherent electronics of the substrate can be overridden in the active site. One possible explanation is that an active site general acid is present capable of protonating a ring N when it is presented at the 3- or 4-position, relative to the carbonyl, but incapable of doing so at the 2-position.

Retaining the well-tolerated aroyl group, we next appended a $(CH_2)_nCO_2R$ moiety, to scan for the ability of this CaADH to handle ω-keto esters (Fig. 4, violet bars). Good activity was seen with the set of α-, β- and γ-keto esters (n = 0–2). Moreover, all three of these biocatalytic reductions proceed with excellent facial selectivity, providing an efficient (84–93% yields) and enantioselective (90–99% ee; D-configuration—see

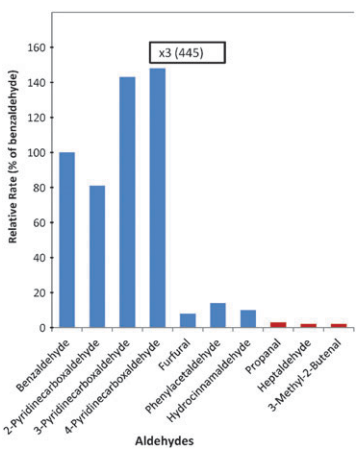

Fig. 3 SAR of aldehyde substrates for CaADH (all at 10 mM in 100 mM K_2PO_4,/KH_2PO_4 pH 7). Rates are normalized to that for benzaldehyde (100%). The rate for 4-pyridine carboxaldehyde is off scale.

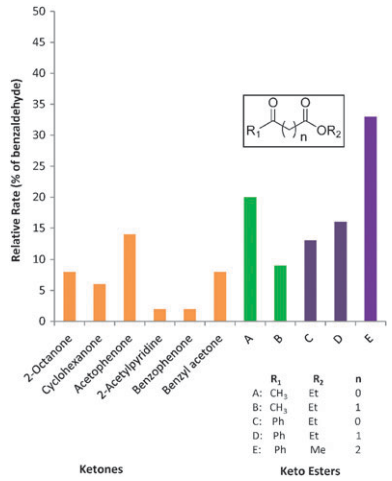

Fig. 4 SAR survey of ketone substrates for CaADH, revealing a predilection for reducing ω-keto ester substrates.

ESI†) entry into the corresponding α- (**2**), β- (**4**) and γ-hydroxy esters (**6**) (Fig. 5). While other biocatalytic entries into **2**,[21,22] **4**,[22,23] and **6**[24] are known,[25] the observed CaADH enantioselectivity is comparable, and the CaADH substrate scope compares favorably to these systems.

Inversion of such D-α- and β-hydroxy esters to the corresponding esters of L-α-phenylglycine[26] and L-β^3-phenylalanine[27] is known. The former amino acid serves as a building block for peptides with anti-thrombotic[28] and anti-arthritic[29] potential (Fig. 5). The L-β^3-phenylalanine building block, in turn, is useful for the assembly of β-peptides,[30] whereas the D-β-hydroxy ester obtained directly from the CaADH reduction serves as a precursor for the important anti-depressant, fluoxetine.[31] Finally, the D-γ-hydroxy ester itself is a valuable chiron for the assembly of cryptophycin,[32] and L-γ^4-homophenylalanine is as useful monomer for γ-peptides.[33]

We next sought to perform a similar reduction on α-halo-β-keto ester **13**, in an effort to install two stereocenters. This strategy, pioneered by Stewart *et al.*[34] (yeast) provides an efficient chemoenzymatic entry into the phenylisoserine side chain of the

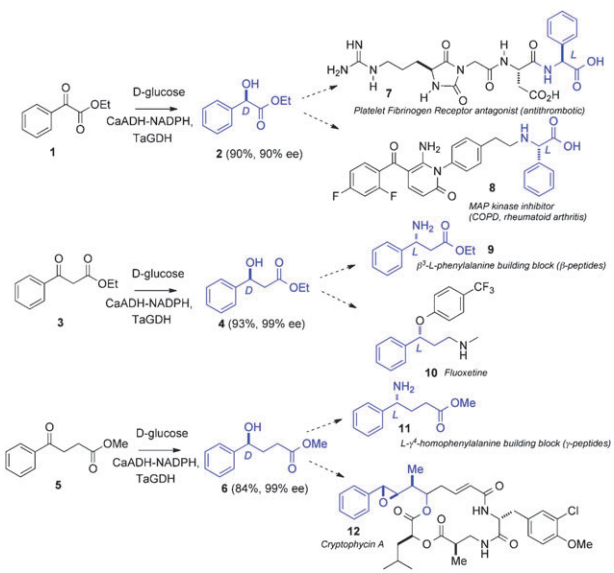

Fig. 5 High facial selectivity observed for the CaADH-mediated reduction of α-,β- and γ-keto esters.

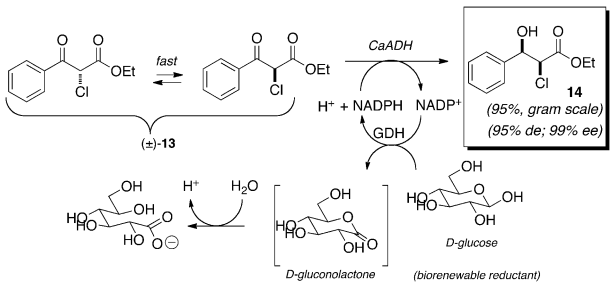

Fig. 6 Efficient DYRKR entry into the side chain for taxoid anti-cancer drugs, including Taxol and Taxotère.

taxoid chemotherapeutics. In the event, CaADH performed exceptionally, delivering the desired α-chloro-β-hydroxy ester, **14**, with proper relative (95% de) and absolute stereochemistry (99% ee) under DYRKR conditions (Fig. 6). Pleasingly, this level of stereochemical control was maintained on a gram scale. D-Glucose serves as a biorenewable reductant regenerating NADPH under the aegis of GDH from *T. acidophilum*. In light of these results, it will be of interest to further mine the genome of this solventogentic bacterium and further explore the potential of this *Clostridium acetobutylicum* ADH.

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