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Investigation of the donor and acceptor range for chiral carboligation catalyzed by the E1 component of the 2-oxoglutarate dehydrogenase complex

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Abstract

The potential of thiamin diphosphate (ThDP)-dependent enzymes to catalyze C-C bond forming (carboligase) reactions with high enantiomeric excess has been recognized for many years. Here we report the application of the E1 component of the *Escherichia coli* 2-oxoglutarate dehydrogenase multienzyme complex in the synthesis of chiral compounds with multiple functional groups in good yield and high enantiomeric excess, by varying both the donor substrate (different 2-oxo acids) and the acceptor substrate (glyoxylate, ethyl glyoxylate and methyl glyoxal). Major findings include the demonstration that the enzyme can accept 2-oxovalerate and 2-oxoisovalerate in addition to its natural substrate 2-oxoglutarate, and that the tested acceptors are also acceptable in the carboligation reaction, thereby very much expanding the repertoire of the enzyme in chiral synthesis.

Keywords

C-C bond formation; circular dichroism; enantioselectivity; chiral enzymatic synthesis; thiamin diphosphate

1. Introduction

While carboligation (C-C bond formation) is catalyzed by a number of important ThDP enzymes[1] (including transketolases,[2] glyoxylate carboligase[3] and 1-deoxy-D-xylulose-5-phosphate synthase[4], benzaldehyde lyase[5]) as the main reaction, it is a side reaction for nearly all ThDP-dependent 2-oxoacid decarboxylases. This property of ThDP-dependent enzymes has been exploited for purposes of chiral synthesis for a number of years.[1] We have explored for such purposes the E1 component (E1o) of the *Escherichia coli* 2-oxoglutarate dehydrogenase multienzyme complex (OGDHc): 2-oxoglutarate undergoes E1o-catalyzed decarboxylation to the nucleophilic enamine, which then adds to an aldehyde acceptor, analogously to the reaction mechanism of a number of ThDP

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enzymes. Our synthetic program was initiated by making substitutions of the enzyme at the putative binding site of the α -carboxyl group of the substrate so that the enzyme would accept substrate analogues lacking the charged α -carboxyl group.[8] The Rutgers group has previously constructed several active site variants in yeast pyruvate decarboxylase (YPDC) from *Saccharomyces cerevisiae* and in the E1 component of the *Escherichia coli* pyruvate dehydrogenase complex (E1p) which were capable of catalyzing such reactions.[9] The E477Q YPDC variant was an effective acetoin synthase, while the D28A or D28N YPDC variants catalyze acetolactate formation.[10] On the other hand, the E636Q and E636A E1p active site variants also became acetolactate synthases[11]. Note, YPDC and E1p produced the opposite enantiomers of acetoin in excess.

The E1o component of OGDHc also catalyzes carboligation reactions. The central ThDP-bound enamine intermediate reacts with the electrophilic acceptor substrate, typically an aldehyde, which results in the formation of acetoin-like or acetolactate-like ligated products (Scheme 1). In our initial report on this topic, we observed that E1o has a broad substrate range, making it an excellent candidate for protein engineering. Indeed, saturation mutagenesis experiments carried out at histidine-260 and histidine-298 [selected on the basis of the X-ray structure which suggested that these residues are near the α -carboxylate binding site of 2-oxoglutarate (2-OG)] revealed that while H260 is important for catalysis, H298 could be substituted by a number of hydrophobic residues with little loss of activity.[8] We here report important extensions of the carboligation studies with E1o, where both the 2-oxoacid and the acceptor aldehyde could be varied over a wide range of reactivity, greatly adding to the versatility of E1o for carboligase reactions (Figure 1). The products and enantiomeric excess (ee) were confirmed by circular dichroism (CD), ^1H nuclear magnetic resonance (NMR), and chiral gas chromatography (GC). This work adds to the power of E1o as a chiral synthetic tool by demonstrating that (a) this enzyme can also accept 2-oxovalerate (2-OV) and 2-oxoisovalerate (2-OiV) as substrates, in addition to its natural substrate 2-OG, and (b) that ethyl glyoxylate and surprisingly methylglyoxal can also serve as aldehyde acceptors, in addition to glyoxylate and other straight chain aldehydes.

2. Experimental Section

2.1. Materials

2-Oxoglutarate, 2-oxovalerate, 2-oxoisovalerate, glyoxylate, ethyl glyoxylate, and methylglyoxal were from Sigma-Aldrich. *E. coli* strain JW0715 containing the plasmid pCA24N encoding the OGDHc-E1 (E1o) component [ASKA clone (-)] was obtained from National Bio Resource Project (NIG, Japan). Amicon® Ultra-4 Centrifugal Filter Units are purchased from EMD Millipore. The enzyme E1o was purified as reported previously. [8]

2.2. Methods

CD spectroscopy—CD experiments were carried out on a Chirascan CD spectrometer (Applied Photophysics, Leatherhead, UK).

Carboligase reaction—E1o (2 mg/ml, 19 μM active centers) in 20 mM KH_2PO_4 (pH 7.0) containing 0.2 mM ThDP and 2 mM MgCl_2 was incubated overnight with 2-OG (2 mM) in the presence of the acceptors [glyoxylate (1 mM), ethyl glyoxylate (1 mM), or methyl glyoxal (1 mM)] and CD spectra were recorded in the wavelength range of 260–400 nm at 30 °C. Similar reactions were performed using the other substrates 2-OV (5 mM) or 2-OiV (5 mM) with the above acceptors (10 mM). This was necessitated by the K_m for 2-OV and 2-OiV being greater than for 2-OG. The protein was separated from the carboligase product using an Amicon® Ultra-4 Centrifugal Filter Unit. The filtrate was collected and the CD spectra were recorded between 260 and 400 nm.

NMR spectroscopy—The ^1H NMR was recorded on a Varian 500 MHz INOVA spectrometer at 25 °C. The reactions were carried out at room temperature in 1 ml of 20 mM KH_2PO_4 (pH 7.0) buffer containing 0.5 mM ThDP and 2 mM MgCl_2 , reacting for overnight 2 mg/ml E1o (19 μM active centers), 10 mM donor substrates and 15 mM acceptors. After separation of protein from the reaction mixture by using a centricon unit (Millipore), the pH of the supernatant was adjusted to ~3 and the product(s) was extracted into CDCl_3 to record the ^1H NMR spectrum.

Chiral GC analysis—GC analysis was carried out on a Varian CP-3800 gas chromatograph equipped with a Chiraldex B-DM chiral column (Astec, Advanced Separation Technologies, Inc.) and a flame ionization detector at a flow rate of 1.5 mL/min. Acetoin was extracted from the reaction mixture (after adjustment of pH to 4–5) with chloroform and injected onto the Chiraldex B-DM chiral column. The enantiomers were assigned according to their relative retention time. It is known that the (*S*)-enantiomer interacts more favorably with the matrix than the (*R*)-enantiomer. Hence, the (*S*)-enantiomer has a longer retention time. [9] By using the same method racemic benzoin and (*R*) benzoin were used as standards (data not shown).

3. Results and Discussion

As with all ThDP-dependent decarboxylations, E1o catalyzes the initial formation of a pre-decarboxylation covalent ThDP-bound intermediate by reaction at the C2 thiazolium atom of the enzyme-bound ThDP with the substrate's keto carbon. Decarboxylation of this intermediate leads to a strongly nucleophilic enamine, which, in the absence of the other components of the complex (the E2o-E3 sub-complex), may react with electrophilic compounds leading to the so-called carboligase products (Scheme 1).

The carboligase products with a chiral 2-hydroxyketone are valuable building blocks for organic synthesis. Since the plasmid for the *Escherichia coli* E1o is available from Japan, the methods are ideal for design and synthesis of such chiral products even on an industrial scale. The ThDP-dependent enzyme E1o can catalyze carboligase reactions not only with acid but also with ester as acceptor substrate. First studies of the carboligase activity of the E1o using as substrate 2-OV and the acceptors acetaldehyde and glyoxylate using rat and beef heart particulate fractions were already published in 1970 by the Westerfeld group, however, no *ee* was reported for the products [12]. Just recently, Müller et al. reported the use of E1o from the same source in carboligase reactions with 2-OG and several aliphatic and aromatic aldehyde acceptors with no additional functional groups. [13]

In the present study we used E1o to carry out carboligase reactions using three different 2-oxoacids with three aldehyde acceptors, with very different chemical properties [two of which had not been reported before to lead to carboligase products], which lead to products in good yield with up to 80–90% *ee* (Figures 1 & 2). While the natural substrate 2-OG has a negatively charged carboxylate group, the E1o was found to also catalyze the decarboxylation of 2-OV and 2-OiV with no second charge in the 2-oxoacid, one with a straight chain, the other a branched one (Figure 1).

Remarkably, all three substrates 2-OG, 2-OV and 2-OiV could utilize glyoxylate as acceptor for carboligation, and could also utilize its ethyl ester and methylglyoxal as acceptors, producing compounds with good enantioselectivity. The novel products formed by the carboligase reaction include chiral 2-hydroxy-1,3-diketones and 2-hydroxy- -ketoesters. The 2-OV and 2-OiV when reacted with the acceptors glyoxylate and ethyl glyoxylate produce the (*S*) enantiomers; whereas, 2-OG forms the (*R*) enantiomers. This may be the result of 2-OV and 2-OiV having hydrophobic tails, while that of 2-OG is hydrophilic.

There is no clear enantiomeric preference observed with the methylglyoxal acceptor reacting with the three 2-oxoacids.

The *ee* of the product was determined using chiral GC and circular dichroism (CD) spectroscopy (Figure S1) and ^1H NMR was used to confirm the product (Figure S2). After recording the CD spectrum of the product, protein was separated and the supernatant pH was adjusted to 4–5, conditions under which the product could be extracted into chloroform. This enabled us to record the product's NMR spectrum, and to run out chiral GC separations. Since all products have in common the CH-OH functional group, they all exhibit a ^1H NMR resonance with a proton chemical shift near 5 ppm. CD spectroscopy enabled assessment of the enantiomer being formed: the (*R*) enantiomer displays a negative CD band and the (*S*) enantiomer a positive one, very near 278 nm, reminiscent of the CD spectrum of acetoin enantiomers. All of the products here discussed with incorporation of the C(=O)CH-OH functional group have a very useful spectral property: all exhibit this same CD band, a band that is also very useful for kinetic measurements [14]. It was earlier shown at Rutgers that the column used for GC analysis interacts more favorably with (*S*) enantiomers [compared to the (*R*) enantiomer], increasing their retention time. This observation was used to identify the enantiomers and to calculate their *ee* value (Figure 3). Before carrying out *ee* measurements of the new compounds, racemic benzoin and (*R*)-benzoin were used as standards for the chiral GC column, further confirming the (*R*) or (*S*) enantioselectivity, while also providing an approximate retention time expected of the new products on the chiral column.

This study reports use of an ester and a 2-oxoaldehyde as acceptors for the first time in a ThDP enzyme catalyzed carboligase reaction. This is important since some of the carboligase reactions produce α -ketoacids, which are prone to thermal decarboxylation.

4. Conclusions

The E1o component of OGDHc catalyzes the formation of acetoin-like products for all of the reactions tested in good enantiomeric excess. In addition, the products produced by E1o have different enantioselectivities, depending on both substrate and acceptor being used. For example, E1o yields the (*R*)-enantiomer with 2-OG as the substrate and glyoxylate acceptor. On the other hand, the (*S*)-enantiomer is produced with 2-OV as the substrate and glyoxylate as acceptor. Two other issues impact on the significance of the work: (1) The E1o plasmid from *E. coli* is available to all from the source mentioned in the Experimental Section and can provide active enzyme in good yield, and (2) The work is readily extended to the synthesis of longer chain 2-oxoacids, since preliminary results suggest that 2-oxoadipic acid and glyoxylate form the corresponding carboligase product (*S*)-2-hydroxy-3-oxoheptanedioic acid. The results suggest that E1o from *E. coli* offers a good starting point for protein engineering and optimization to synthesize stable chiral intermediates for fine chemical synthesis. Compared to a recent publication using E1o for chiral synthesis [13], this contribution expands the scope of both the 2-oxo acid donor and the aldehyde acceptor significantly for their use in carboligase condensation reactions by this enzyme.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Müller M, Gocke D, Pohl M. FEBS J. 2009; 276:2894–2904. [PubMed: 19490096]
2. Lindqvist Y, Schneider G, Ermler U, Sundström M. EMBO J. 1992; 11:2373–2379. [PubMed: 1628611]
3. Kaplun A, Binshtein E, Vyazmensky M, Steinmetz A, Barak Z, Chipman DM, Tittmann K, Shaanan B. Nat Chem Biol. 2008; 4:113–118. [PubMed: 18176558]
4. Brammer LA, Meyers CF. Org Lett. 2009; 11:4748–4751. [PubMed: 19778006]
5. Mosbacher TG, Müller M, Schulz GE. FEBS J. 2005; 272:6067–6076. [PubMed: 16302970]
6. Kokova M, Zavrel M, Tittmann K, Spiess AC, Pohl M. J Molec Cat B: Enzymatic. 2009; 61:73–79.
7. Shaanan B, Chipman DM. FEBS J. 2009; 276:2447–2453. [PubMed: 19476486]
8. Shim DJ, Nemeria NS, Balakrishnan A, Patel H, Song J, Wang J, Jordan F, Farinas FET. Biochemistry. 2011; 50:7705–7709. [PubMed: 21809826]
9. Baykal A, Chakraborty S, Dadoo A, Jordan F. Bioorg Chem. 2006; 34:380–393. [PubMed: 17083961]
10. Sergienko EA, Jordan F. Biochemistry. 2001; 40:7382–7403. [PubMed: 11412092]
11. Nemeria N, Tittmann K, Joseph E, Zhou L, Vazquez-Coll MB, Arjunan P, Hübner G, Furey W, Jordan F. J Biol Chem. 2005; 280:21473–21482. [PubMed: 15802265]
12. Schlossberg MA, Bloom RJ, Richert DA, Westerfeld WW. Biochemistry. 1970; 9:1148–1153. [PubMed: 5418712]
13. Beigi M, Waltzer S, Fries A, Eggeling L, Sprenger GA, Müller M. Org Lett. 2013; 15:452–455. [PubMed: 23317369]
14. Patel H, Nemeria NS, Brammer LA, Freel Meyers CL, Jordan F. J Am Chem Soc. 2012; 134:18374–79. [PubMed: 23072514]

Substrates

R = $-\text{CH}_2\text{CH}_2\text{COO}^-$ is 2-oxoglutarate (2-OG)

R = $-\text{CH}_2\text{CH}_2\text{CH}_3$ is 2-oxovalerate (2-OV)

R = $-\text{CH}(\text{CH}_3)_2$ is 3-methyl 2-oxobutanoic acid (2-OiV)

Acceptors

Figure 1.
Substrates and acceptors for carbolygase reaction by E1o.

Figure 2.

E1o catalyzed formation of acetoin-like chiral product using different substrates and acceptors.

Figure 3.
Structure and nomenclature of the chiral products produced from the E1o catalyzed reaction by using variety of acceptors and substrates.

Scheme 1.
E1o catalyzed reaction mechanism of carbolligase product.

Table 1

Enantiomeric excess of acetoin-like product formation by E1o using chiral GC.

Substrates	Acceptors	Product	ee% ^a
2-OG	Glyoxylate	1	90 % (<i>R</i>)
2-OV		2	96 % (<i>S</i>)
2-OiV		3	67 % (<i>S</i>)
2-OG	Ethyl-glyoxylate	4	81 % (<i>R</i>)
2-OV		5	52 % (<i>S</i>)
2-OiV		6	81 % (<i>S</i>)
2-OG	Methyl-glyoxal	7	83 % (<i>S</i>)
2-OV		8	81 % (<i>R</i>)
2-OiV		9	>90 % (<i>S</i>)

^a A yield of ~ 95–100% for all the acetoin products was determined by ¹H NMR as no starting material (substrate) was detected at the end of overnight incubation.