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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 repertory of the enzyme in chiral synthesis.



## 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 [1,6,7]. 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 enzymes. Our synthetic program was initiated by making substitutions of the enzyme at the putative binding site of the γ-carboxyl group of the substrate so that the enzyme would accept substrate analogs lacking the charged γ-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 *E. 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 γ-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 (Fig. 1). The products and enantiomeric excess (ee) were confirmed by circular dichroism (CD), $^1$H nuclear magnetic resonance (NMR), and chiral gas chromatography (GC). This

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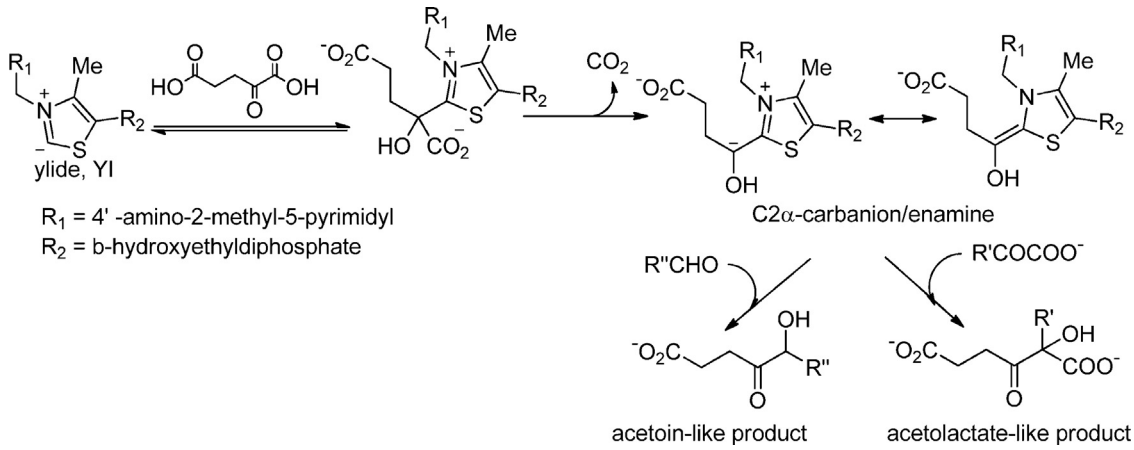


**Scheme 1.** E1o catalyzed reaction mechanism of carboligase product.

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

### 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

#### 2.2.1. CD spectroscopy

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

#### 2.2.2. 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.

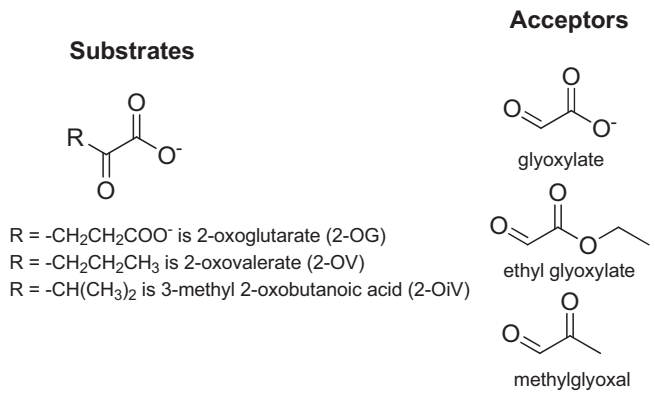


**Fig. 1.** Substrates and acceptors for carboligase reaction by E1o.

#### 2.2.3. 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.

#### 2.2.4. 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).

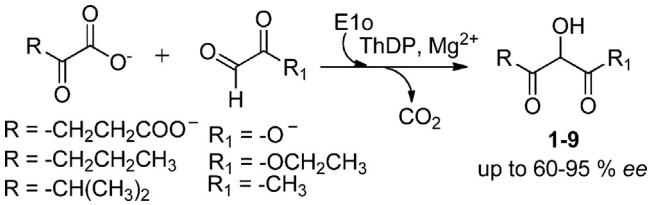


**Fig. 2.** E1o catalyzed formation of acetoin-like chiral product using different substrates and acceptors.