Inhibition of the α -Ketoglutarate Dehydrogenase Complex from Bakers' Yeast by Acetaldehyde and Glyoxylate

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A condensation reaction between α -ketoglutarate and acetaldehyde to form 5-hydroxy-4-ketohexanoic acid was originally detected in studies on alcohol metabolism *in vitro* in systems from mammals¹⁾ and yeast.^{2,3)} This reaction is analogous to the formation of acetoin from pyruvate plus acetaldehyde, or to the formations of other acyloins from α -keto acids plus aldehydes.

α -Ketoglutarate also reacts with glyoxylate. A synergistic decarboxylation of α -ketoglutarate and glyoxylate catalyzed by enzyme from bacteria or mammalian tissues has been reported by many workers.⁴⁻¹²⁾ This reaction was first demonstrated by Crawhall and Watts⁵ and the enzyme which catalyzes it was purified about 100-fold by Koch and Stokstad⁸ and by Stewart and Quayle¹¹⁾ from rat and

TABLE I. CARBOLIGASE ACTIVITY OF THE α -KETOGLUTARATE DEHYDROGENASE COMPLEX

Substrate	Specific activity	
	KG-Glyoxylate carboligase (units/mg protein)	KG-Acetaldehyde carboligase (units/mg protein)
Glyoxylate-U- ¹⁴ C	0.6	—
Glyoxylate-U- ¹⁴ C plus α -ketoglutarate	21.5	—
Acetaldehyde	—	0
Acetaldehyde plus α -ketoglutarate	—	12.2

α -Ketoglutarate: glyoxylate carboligase activity was assayed essentially as described by Koch *et al.*⁸⁾ Incubations were performed for 30 min at 30°C in Warburg vessels. The main compartment contained: enzyme, 100 μ moles of potassium phosphate buffer (pH 7.0), 5 μ moles of MgCl₂, 0.2 μ mole of TPP and 2.5 μ moles of sodium α -ketoglutarate. The reaction was started by adding 2 μ moles of U-¹⁴C-glyoxylate (100,000 cpm) from a side arm. The final volume was 2.5 ml. The reaction was stopped by adding a mixture [of 0.1 ml of 25% trichloroacetic acid and 0.1 ml of 0.2 M 4-amino-antipyrine from the other side arm. ¹⁴CO₂ was absorbed by alkali on filter paper in the center well and counted in a scintillation counter (Beckman LS-100). One unit of enzyme was defined as the amount producing 1 μ mole of [¹⁴C] CO₂/hr. Protein was determined by the method of Lowry *et al.*¹⁷⁾

α -Ketoglutarate: acetaldehyde carboligase activity was assayed as described by Schlossberg *et al.*¹³⁾ The incubation medium contained: 10 μ moles of sodium α -ketoglutarate, 20 μ moles of acetaldehyde, 100 μ moles of potassium phosphate buffer (pH 7.0), 0.4 μ mole of TPP, 10 μ moles of MgCl₂, 20 mg of albumin and enzyme. The final volume was 3 ml. After incubation for 30 min at 30°C, the proteins were precipitated by injecting 0.3 ml of 70% perchloric acid and the mixture was centrifuged. The supernatant was neutralized with potassium hydroxide and centrifuged to remove potassium perchlorate. 5-Hydroxy-4-ketohexanoic acid was measured as described by Bloom *et al.*¹⁾ One unit of enzyme was defined as the amount producing 1 μ mole of 5-hydroxy-4-ketohexanoic acid per hr.

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pig liver mitochondria, respectively.

Recently, Schlossberg *et al.*¹³⁾ and Saito *et al.*¹⁴⁾ reported that α -ketoglutarate dehydrogenase complexes prepared from beef heart and pig heart, respectively, also catalyzed the carboligase reaction between α -ketoglutarate and glyoxylate or acetaldehyde. The present paper reports that an α -ketoglutarate dehydrogenase complex from bakers' yeast catalyzes the carboligase reaction between α -ketoglutarate and glyoxylate or acetaldehyde and is inhibited by acetaldehyde or glyoxylate.

The α -ketoglutarate dehydrogenase complex was prepared from bakers' yeast (Oriental Yeast Co., Ltd.) by the method of Hirabayashi *et al.*¹⁵⁾ It was essentially homogenous

upon ultracentrifugation and had a sedimentation coefficient ($s_{20,w}$) of 20.9 S.

As shown in Table I, $^{14}\text{CO}_2$ formation from glyoxylate- $\text{U-}^{14}\text{C}$ was strongly enhanced by addition of α -ketoglutarate. Furthermore, the formation of 5-hydroxy-4-ketohexanoic acid from acetaldehyde and α -ketoglutarate was catalyzed by the α -ketoglutarate dehydrogenase complex. The mechanism of the synergistic decarboxylation of α -ketoglutarate and glyoxylate or acetaldehyde seemed to be very similar to that resulting in acetoin formation from pyruvate and acetaldehyde catalyzed by the pyruvate dehydrogenase complex.¹⁶⁾

Glyoxylate and acetaldehyde markedly inhibited the reduction of NAD^+ catalyzed by the α -ketoglutarate dehydrogenase complex. Glyoxylate and acetaldehyde caused mixed type inhibition of the overall oxidation activity of the α -ketoglutarate dehydrogenase complex with α -ketoglutarate (Fig. 1). The K_i value of glyoxylate was 3.3 mM and that of acetaldehyde was 7.2 mM. The present results on the inhibition of the α -ketoglutarate dehydrogenase complex indicate that the functions of this complex in yeast may be regulated by the metabolism of ethanol through the influence of the latter on the level of acetaldehyde.

FIG. 1. Effects of Glyoxylate and Acetaldehyde on the α -Ketoglutarate Dehydrogenase Complex from Bakers' Yeast.

Additions: A, none; B, 2.5 mM glyoxylate; C, 5.0 mM acetaldehyde.

The reaction mixture contained 150 μ moles of potassium phosphate buffer (pH 7.2) 0.5 μ mole of MgCl_2 , 0.08 μ mole of CoA, 5 μ moles of L-cysteine, 0.3 μ mole of NAD, 0.5 μ mole of TPP, sodium α -ketoglutarate and glyoxylate or acetaldehyde in a final volume of 2.0 ml. The reaction was initiated at 25°C by the addition of enzyme and the rate of formation of NADH was followed at 340 m μ using a Hitachi, model 124, spectrophotometer in conjunction with a Hitachi, model QPD 34, recorder. One unit of α -ketoglutarate dehydrogenase complex is defined as the amount catalyzing the oxidation of one μ mole of α -ketoglutarate per min.

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