REGULATION OF CITRATE SYNTHASE ACTIVITY IN STRICT AND FACULTATIVELY AUTOTROPHIC THIOBACILLI

B. F. Taylor

Rosenstiel School of Marine and Atmospheric Sciences
University of Miami, Miami, Florida 33149

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Summary

The citrate synthases of Thiobacillus denitrificans and T. neapolitanus are inhibited by ∝-ketoglutarate but not by NADH. These characteristics are in accord with a purely biosynthetic role for the tricarboxylic acid (TCA) cycle in strict autotrophs. In contrast, the citrate synthases from two facultatively autotrophic thiobacilli, T. novellus and Thiobacillus A2, are not inhibited by ∝-ketoglutarate. The enzyme from Thiobacillus A2 resembles those of other Gram-negative bacteria in being inhibited by NADH whereas that from T. novellus is not. This may account, to some degree, for the better heterotrophic potential of Thiobacillus A2 compared with T. novellus.

Introduction

Citrate synthase is a key enzyme of the tricarboxylic acid (TCA) cycle and is subject to a variety of metabolic controls in different organisms. The citrate synthases of Gram-negative bacteria are inhibited by NADH (Weitzman and Jones, 1968) whereas those of Gram-positive bacteria are inhibited by ATP (Jangaard et al., 1968). ATP and NADH are end-products of the TCA cycle which regulate the rate of oxidation of acetate. The citrate synthases from facultatively anaerobic Gram-negative bacteria are also inhibited by ∝-ketoglutarate (Weitzman and Dunmore, 1969, a). Escherichia coli growing fermentatively lacks ∝-ketoglutarate dehydrogenase (Amarasingham and Davis, 1965). Under these conditions the TCA cycle fulfills a biosynthetic function only and consequently ∝-ketoglutarate, not NADH, is the metabolic regulator of citrate synthase (Weitzman and Dunmore, 1969, a). Several strictly autotrophic thiobacilli lack ∝-ketoglutarate dehydrogenase: T. neapolitanus (Kelly, 1967), T. thioparus and a strain of

T. thio-oxidans (Smith et al., 1967), and T. denitrificans (Taylor et al , 1969). The TCA cycle presumably plays a purely biosynthetic role in these organisms and consequently T. denitrificans and T. neapolitanus were examined for the effect of α-ketoglutarate and NADH on the activity of their citrate synthases. The citrate synthases of two facultatively autotrophic thiobacilli were also examined for comparative purposes. T. novellus (Taylor, unpublished data) and Thiobacillus A2 (Taylor et al., 1969) both possess a complete TCA cycle even when grown autotrophically.

Methods

The organisms were grown autotrophically on thiosulfate; Thiobacillus A2 and T. novellus on the medium of Taylor and Hoare (1969); T. neapolitanus on the medium of Vishniac and Santer (1957); T. denitrificans on the medium of Baalsrud and Baalsrud (1954). Cells were harvested by centrifugation, washed twice with cold 0.05M potassium phosphate (pH 7.5) and then broken by one passage through a French Press at 15,000 p. s. i. A crude extract was obtained by centrifugation at 30,000 x g for 30 min. at 5° C. The citrate synthases of the strict autotrophs were studied without further treatment. With crude extracts of the facultative autotrophs malate dehydrogenase activity interfered with a study of the effect of NADH on the activity of citrate synthase. Malate dehydrogenase was separated from citrate synthase by chromatography on a column of DEAE-sephadex (5 x 1 cm.) using 1 - 2 ml. of crude extract (about 20 mg. of protein). The column was equilibrated with 0.05M potassium phosphate (pH 7.5) containing 0.05M NaCl. Malate dehydrogenase activity was eluted with this buffer. After washing with 0.1M NaCl in 0.05M potassium phosphate (pH 7.5), citrate synthase was eluted by increasing the NaCl concentration to 0.15M. The partially purified enzyme was dialyzed overnight (14 - 16 hrs.) against at least 100 volume of 0.05 potassium phosphate (pH 7.5) at 5°C.

Citrate synthase activity was assayed by the method Srere et al. (1963). Assay mixtures contained 20mM Tris-HCl (pH 8.0), 1mM EDTA-Na_2, 0.05mM oxalo-

TABLE 1 Citrate synthases of strictly autotrophic thiobacilli.

Addition to assay	Concentration in assay (mM)	*T. denitrificans* Specific activity (nmoles coenzyme A produced/min./mg. protein)	*T. denitrificans* % of control	*T. neapolitanus* Specific activity (nmoles coenzyme A produced/min./mg. protein)	*T. neapolitanus* % of control
None	-	8.60	100	74.4	100
NADH	1	9.40	109	71.8	97
NADPH	1	9.77	114	64.2	86
ATP	1	10.2	118	33.3	45
α-keto-glutarate	1	1.95	23	33.3	45
" "	5	0.10	9	1.3	2

Assay mixtures contained in μmoles: Tris-HCl (pH 8.0, 20; EDTA-Na_2, 1; sodium oxaloacetate, 0.05; acetyl coenzyme A, 0.16; DTNB, 0.1; and crude extract. Total volume, 1.0 ml.

acetate, 0.16mM acetyl CoA and 0.1mM 5, 5'-dithiobis-(2-nitro-benzoic acid) (DTNB). Enzymic activity was measured by following the increase in extinction at 412nm using a split-beam spectrophotometer. The blank cuvette contained all the assay components except oxaloacetate. Acetyl coenzyme A was prepared by the method of Stadtman (1951). Protein in crude extracts was measured by the method of Lowry et al. (1951). The method of Kalcker (1947) was employed to estimate the protein concentration of column eluates.

Results and Discussion

The citrate synthases of T. denitrificans and T. neapolitanus, unlike those of other Gram-negative bacteria (Weitzman and Jones, 1968), were not inhibited by NADH (1mM) (Table 1). Weitzman and Jones (1968) using similar assay conditions, found that 0.3mM NADH was sufficient to completely inhibit citrate synthase activity in crude extracts of Escherichia coli and Azotobacter vinelandii. The citrate synthases of strictly autotrophic thiobacilli, in contrast to the enzymes from other strictly aerobic Gram-negative bacteria (Weitzman and Dunmore, 1969, a), were inhibited by α-ketoglutarate (1mM). This inhibition was relieved by an increase in ionic strength (Figure 1). This "desensitization" of the enzyme to α-ketoglutarate is indicative of an allosteric regulatory mechanism (Atkinson, 1966) and has been observed with citrate synthases from facultatively anaerobic Gram-negative bacteria (Weitzman and Dunmore, 1969, a). ATP (1mM) did not affect the activity of the citrate synthase of T. denitrificans but caused a 50% inhibition of that from T. neapolitanus. ATP normally has no effect on citrate synthases of Gram-negative bacteria (Weitzman, 1966) and this inhibition of the activity of the enzyme from T. neapolitanus requires further investigation. It is possible that during periods of active growth on thiosulfate, or sulfur, large amounts of ATP are produced and, in order to maintain the correct ratio of reduced pyridine nucleotide and ATP required for biosynthesis, high levels of ATP generally inhibit key biosynthetic enzymes, such as citrate synthase, and ATP utilization is directed towards the generation of reduced pyridine nucleo-

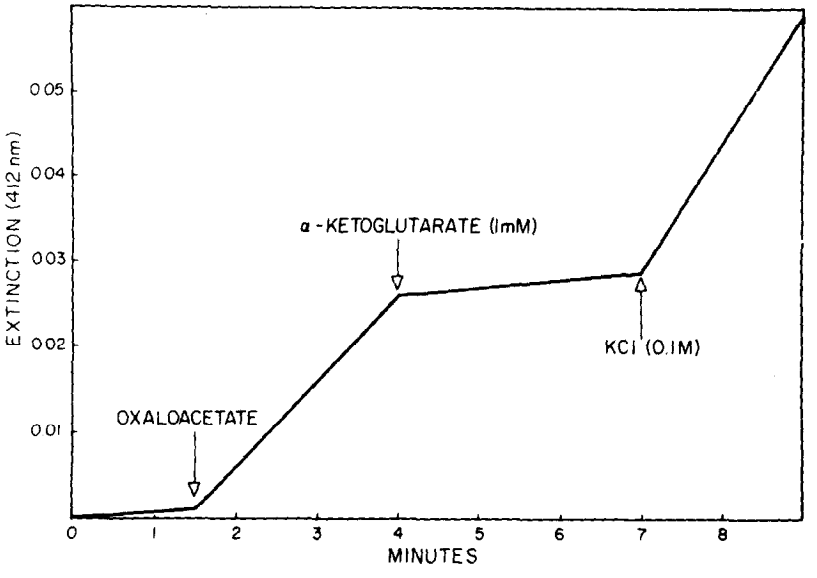

Figure 1. Effect of ionic strength on the inhibition of citrate synthase of T. denitrificans by α-ketoglutarate. Reaction mixture contained in μmoles: Tris-HCl (pH 8.0), 20; EDTA-Na_2, 1; sodium oxaloacetate, 0.05; acetyl coenzyme A, 0.16; DTNB, 0.1; crude extract, 0.60 mg. of protein. Assay performed at 30° in a total volume of 1.06 ml.

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tides by reversed electron transport (Aleem et al., 1963).

The citrate synthase from T. novellus was not inhibited by NADH, ATP or α-ketoglutarate (Table 2). α-ketoglutarate (10mM) actually stimulated the activity of citrate synthase in the facultative autotrophs. The enzyme from Thiobacillus A2, unlike those of the other thiobacilli examined, was extremely sensitive to inhibition by NADH. This inhibition was relieved by an increase of ionic strength (0.2M KCl) indicative of an allosteric type of inhibitory mechanism (Weitzman and Dunmore, 1969, b). In these respects, the citrate synthase of Thiobacillus A2 resembled that of a typical Gram-negative bacterium (Weitzman and Jones, 1968).

NADPH (1mM) had no effect on any of the enzymes examined and also the enzymes from T. denitrificans and Thiobacillus A2 were not affected by AMP (1mM), ADP (1mM), NAD (1mM), NADP (1mM), and sodium pyruvate (10mM).

The thiobacilli are strictly aerobic Gram-negative bacteria and as such, according to the work of Weitzman and Jones (1968), the activity of their

TABLE 2 Citrate synthases of facultatively autotrophic thiobacilli.

Addition to assay	Concentration in assay (mM)	T. novellus		Thiobacillus A2	
		Specific activity (nmoles coenzyme A produced/min./mg. protein)	% of control	Specific activity (nmoles coenzyme A produced/min./mg. protein)	% of control
None	-	13.8	100	58.2	100
NADH	0.1	-	-	9.7	17
	1	15.7	107	4.9	8
NADPH	1	17.7	121	56.2	97
ATP	1	17.2	117	48.6	83
α-keto-glutarate	1	17.2	117	-	-
" "	10	27.5	187	70.8	126

Assay mixtures contained in μmoles: Tris-HCl (pH 8.0), 20; EDTA-Na_2, 1; sodium oxaloacetate, 0.05; acetyl coenzyme A, 0.16; DTNB, 0.1; and enzyme from DEAE-sephadex column. Total volume, 1.0 ml.

citrate synthases should be regulated by NADH. In the strictly autotrophic thiobacilli, the TCA cycle has been modified by the loss of α-ketoglutarate dehydrogenase to fulfill a purely biosynthetic role. A citrate synthase sensitive to inhibition by α-ketoglutarate is advantageous for the control of cellular metabolism under these conditions. In agreement with the lack of an oxidative TCA cycle the citrate synthases of the strictly autotrophic thiobacilli are not inhibited by NADH. The facultatively autotrophic thiobacilli possess α-ketoglutarate dehydrogenase even when grown autotrophically. Their citrate synthases are therefore not inhibited by α-ketoglutarate. The citrate synthase from Thiobacillus A2 was inhibited by NADH whereas that of T. novellus was not. This may be a contributory factor to the observation that Thiobacillus A2 has a much better heterotrophic potential than T. novellus (Taylor and Hoare, 1969).

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