REGULATION OF CITRATE SYNTHASE ACTIVITY BY α-KETOGLUTARATE. METABOLIC AND TAXONOMIC SIGNIFICANCE

P.D.J.WEITZMAN and Patricia DUNMORE
Department of Biochemistry, University of Leicester, Leicester, England

Received 25 April 1969

1. Introduction

Citrate synthase – a key enzyme of the tricarboxylic acid cycle – has been shown to vary in regulatory behaviour according to its organism of origin [1, 2]. In a study of the effects of the metabolic regulators NADH and AMP it was found that citrate synthases from diverse organisms fall into three distinct categories which correlate with established taxonomic divisions. We have also shown that these differences in regulatory behaviour are accompanied by gross differences in the molecular sizes of the enzymes [3].

Wright, Maeba and Sanwal [4] have shown that α-ketoglutarate inhibits the citrate synthase of *E. coli*. Since there are variations in the response of different citrate synthases to NADH and AMP we have investigated the incidence of inhibition by α-ketoglutarate in a number of citrate synthases. We here report our results which indicate a significant pattern in the regulation of the enzyme by this metabolite.

2. Experimental

The organisms used in the study are listed in table 1. Bacteria were grown aerobically at 30° or 37° in salts-acetate medium or nutrient broth. The cells were harvested, washed, suspended in buffer of composition 20 mM Tris, 1 mM EDTA, pH 8.0 and disrupted by ultrasonication. Citrate synthases from baker's yeast and wheat germ were obtained by passage of thick suspensions of these materials twice through a French press. In all cases cell debris was removed by centrifugation and the supernatants were studied without further purification. Purified enzyme from *E. coli* was prepared as described elsewhere [5]. Pig heart enzyme was obtained commercially (Boehringer).

Enzyme activity was measured spectrophotometrically at 412 mμ [6] in the presence of the chromogen 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB). Assay mixtures contained 20 mM Tris, pH 8.0, 1 mM EDTA, 0.05 mM oxaloacetate, 0.16 mM acetyl coenzyme A and 0.1 mM DTNB. The effect of α-ketoglutarate was examined at a concentration of 1 mM. In some cases assays were also carried out polarographically [7]; the results confirmed those obtained spectrophotometrically.

3. Results and discussion

Experiments with both crude and purified *E. coli* citrate synthase confirmed the published report [4] that α-ketoglutarate is an inhibitor of this enzyme. The effect of α-ketoglutarate on the activities of the other citrate synthases is summarised in table 1.

The results are interesting from a taxonomic standpoint. The inhibition by α-ketoglutarate appears to be restricted to one family of Gram negative organisms, namely the Enterobacteriaceae. The other bacteria, both Gram negative and Gram positive, and the higher organisms all showed no such inhibition of their citrate synthases. In all cases the enzymes which respond to α-ketoglutarate may be "desensitised" to this inhibitor

Table 1
Regulation of citrate synthases by α-ketoglutarate. Strains of bacteria are those quoted by Weitzman and Jones [2]. See text for experimental details.

Inhibition by α-ketoglutarate	No inhibition by α-ketoglutarate		
	Gram negative bacteria	Gram positive bacteria	Eucaryotes
Aerobacter aerogenes	*Acinetobacter lwoffi*	*Bacillus megaterium*	Baker's yeast
Arizona arizonae	*Azotobacter vinlandii*	*Kurthia zopfii*	Wheat germ
Escherichia coli	*Moraxella calcoacetica*	*Streptomyces somaliensis*	Pig heart
Proteus vulgaris	*Pseudomonas aeruginosa*		
Salmonella anatum	*Pseudomonas fluorescens*		
	Pseudomonas ovalis		

by raising the ionic strength (presence of 0.2 M KCl), suggesting an allosteric regulatory mechanism. This correlation between citrate synthase regulation and microbial taxonomy augments such relationships established in our previous studies [2].

The citrate synthases of Gram negative bacteria are considerably larger molecules than those of Gram positive bacteria or of higher, eucaryotic, organisms and, furthermore, it is only the "large" citrate synthases which exhibit allosteric inhibition by NADH [3]. We proposed that it is only these "large" enzymes – presumably polymeric molecules – which are capable of undergoing the conformational rearrangements which constitute the response to NADH. It is therefore interesting to find that those citrate synthases which show inhibition by α-ketoglutarate are also all of the "large" type. Interaction of these enzymes with α-ketoglutarate may well lead to a rearrangement of sub-units similar to that observed by electron microscopy in the case of NADH [8].

Examination of table 1 shows that within the Gram negative bacteria the strict aerobes contain citrate synthases which are insensitive to α-ketoglutarate, whereas inhibition by α-ketoglutarate is shown only by the facultative anaerobes. In these organisms, which can grow anaerobically and generate energy by fermentation rather than through the tricarboxylic acid cycle, citrate synthase is still required to fulfil a biosynthetic role, leading to α-ketoglutarate. Studies with *E. coli* have suggested [9] that under such conditions the cycle is modified to a branched non-cyclic pathway as schematised in fig. 1. Such a scheme emphasises the end-product nature of α-ketoglutarate; its inhibitory effect on citrate synthase is then a typical case of end-product inhibition of the initial enzyme of a pathway. There is evidence that even during the aerobic growth of *E. coli* on glucose the tricarboxylic acid cycle functions primarily for biosynthesis [10], and the apparent absence of α-ketoglutarate dehydrogenase under such conditions [9] suggests that even in certain stages of aerobic growth *E. coli* may possess the modified tricarboxylic acid cycle as in fig. 1. Our observations of the regulation of citrate synthase by

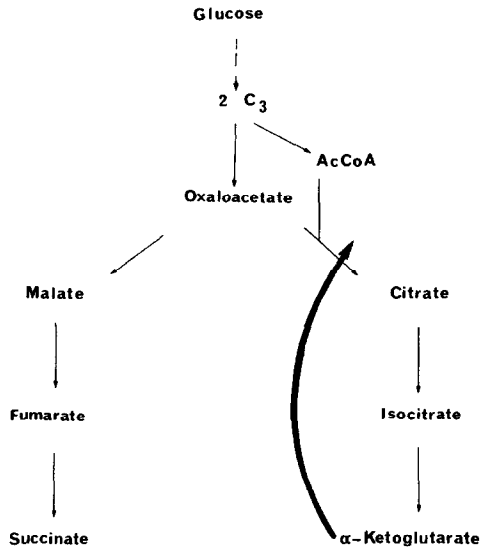

Fig. 1. Modified tricarboxylic acid cycle in *E. coli* [9] showing end-product inhibition of citrate synthase. See text for explanation.

α-ketoglutarate only in those organisms which are related to *E. coli* and which can grow anaerobically are consistent with the operation of this metabolic pathway.

Acknowledgements

We thank Professor H.L.Kornberg for helpful discussions and Dr. Dorothy Jones for supplying some of the organisms used in this work.

References

[1] P.D.J.Weitzman, Biochim. Biophys. Acta 128 (1966) 213.
[2] P.D.J.Weitzman and D.Jones, Nature 219 (1968) 270.
[3] P.D.J.Weitzman and P.Dunmore, Biochim. Biophys. Acta 171 (1969) 198.
[4] J.A.Wright, P.Maeba and B.D.Sanwal, Biochem. Biophys. Res. Commun. 29 (1967) 34.
[5] P.D.J.Weitzman, in: Methods in Enzymology, vol. 13, ed. J.M.Löwenstein (Academic Press, New York, 1969) p. 22.
[6] P.A.Srere, H.Brazil and L.Gonen, Acta Chem. Scand. 17 (1963) S129.
[7] P.D.J.Weitzman, in: Methods in Enzymology, vol. 13, ed. J.M. Löwenstein (Academic Press, New York, 1969) p. 365.
[8] A.J.Rowe and P.D.J.Weitzman, J. Mol. Biol., in press.
[9] C.R.Amarasingham and B.D.Davis, J. Biol. Chem. 240 (1965) 3664.
[10] R.B.Roberts, D.B.Cowie, R.J.Britten, E.T.Bolton and P.H.Abelson, Proc. Natl. Acad. Sci. U.S. 39 (1953) 1013.