STIMULATION OF ACETYL-CoA CARBOXYLASE BY (−)-HYDROXYCITRATE

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Received 28 July 1972

1. Introduction

In addition to the known inhibitory effect on citrate cleavage enzyme (EC 4.1.3.8) [1], (−)-hydroxycitrate was shown recently [2] to stimulate fatty acid synthesis from acetate. This effect was observed both in the isolated perfused rat liver and in homogenates of this organ and was assumed to be due to an activation of acetyl-CoA carboxylase (EC 6.4.1.2) [2], in analogy to other tricarboxylates [3]. As (−)-hydroxycitrate is used as an experimental tool in studies on the relationship of carbohydrate and lipid metabolism [2,4–6], unequivocal proof of this assumption seemed to be warranted.

In this communication experiments are reported which show that (−)-hydroxycitrate does stimulate acetyl-CoA carboxylase. The reaction kinetics revealed (−)-hydroxycitrate as an effector of the V_{max} type.

2. Materials and methods

Phosphoenolpyruvate, ATP, NADH, pyruvate kinase (EC 2.7.1.40) and lactate dehydrogenase (EC 1.1.1.27) were products of Boehringer Mannheim GmbH. Albumin reinst was purchased from Behringwerke AG, Frankfurt/Main, acetyl-CoA (determination according to [7]) and glutathione from Serva, Heidelberg. (−)-Hydroxycitric acid lactone was prepared according to [8] from dried fruit rinds of *garcinia cambogia*, which were kindly supplied by Professor Dr. Deutschmann, Institut für Angewandte Botanik, Hamburg, W.Germany. The lactone was hydrolyzed according to [4] before use.

Rat liver acetyl-CoA carboxylase was purified 170-fold as described by Hashimoto and Numa [9] including DEAE-cellulose chromatography. After this

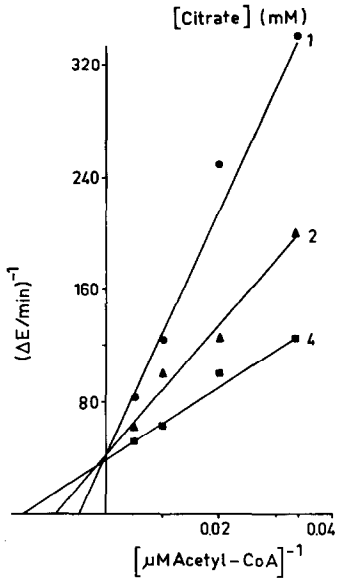

Fig. 1. Lineweaver–Burk plots of acetyl-CoA carboxylase activity with varying acetyl-CoA and citrate concentrations. Purified acetyl-CoA carboxylase (3.7 mg protein/ml) was diluted 3-fold with a solution containing 50 mM Tris-HCl pH 7.5, 3.75 mM glutathione, 0.75 mg/ml albumin and 10 mM citrate. After incubation at 37° for 30 min, 0.1 ml was added to the reaction mixture containing in a final volume of 1.0 ml: 50 μmoles Tris-HCl buffer pH 7.5; 10 μmoles $MgCl_2$; 3.75 μmoles glutathione; 0.75 mg albumin; 0.1 μmole NADH; 1–4 μmoles citrate; 1 μmole ATP; 0.1 μmole phosphoenolpyruvate; 10 μmoles $KHCO_3$; ~ 3 U pyruvate kinase and ~ 3 U lactate dehydrogenase (both enzymes dialyzed before use against 50 mM Tris·HCl buffer pH 7.5). Start with acetyl-CoA. Initial velocities were determined spectrophotometrically at 334 nm (temp. = 37°). Apparent K_m values of 70, 112 and 234 μM for acetyl-CoA in the presence of 1, 2 and 4 mM citrate, respectively, were obtained. Replotting the same data yielded apparent K_m values of 0.97, 1.9, 6.4 and 4.7 mM for citrate in the presence of 200, 100, 50 and 30 mM acetyl-CoA, respectively.

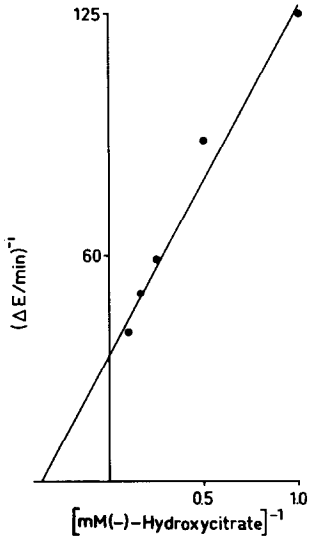

Fig. 2. Lineweaver–Burk plot of acetyl-CoA carboxylase activity preincubated with varying (−)-hydroxycitrate concentrations. Experimental conditions as in fig. 1, except that acetyl-CoA carboxylase was preincubated in all cases for 30 min with the (−)-hydroxycitrate concentrations indicated on the abscissa. The reaction was carried out with the same concentrations. Acetyl-CoA was 110 μM in all assays.

step a specific activity of 75.7 mU/mg protein was measured in the presence of 10 mM citrate as specified in fig. 1. Protein was determined with the biuret method [10]. The purified enzyme was meassured spectrophotometrically by coupling with pyruvate kinase and lactate dehydrogenaxe [9]; this assay was checked by the $H^{14}CO_3^-$-fixation method [9]. Both assays gave identical results. The reaction followed zero order kinetics with up to 8.7 mU of acetyl-CoA carboxylase. Preincubation of the enzyme with 2 U avidin/ml inhibited the reaction completely. Lineweaver–Burk plots were evaluated by the least squares fit.

3. Results

Measurements of acetyl-CoA carboxylase activity after citrate activation in the presence of different acetyl-CoA concentrations (fig. 1) confirmed the conclusions of Hashimoto and Numa [9] that citrate stimulation is of the K_m type for acetyl-CoA under these conditions.

The stimulation of acetyl-CoA carboxylase activity by (−)-hydroxycitrate was concentration dependent (fig. 2) resulting in an apparent K_m value for (−)-hydroxycitrate of 2.8 mM. Enzyme activity was maximal with 10 mM (−)-hydroxycitrate; higher concentrations (20 mM) resulted in decreasing activities. This activation was time dependent (fig. 3), maximal activation being attained after 30 min.

Experiments on the kinetics of activation (fig. 4) indicated that the stimulation of enzyme activity was due to an increase of the V_{max} value, while the apparent K_m value for acetyl-CoA remained unchanged (fig. 4A). The 1/v *vs.* 1/ (−)-hydroxycitrate plot gave a similar kinetic pattern, i.e. change of maximal velocity with only minor changes of the apparent K_m value for (−)-hydroxycitrate (fig. 4B).

4. Discussion

The stimulation of acetyl-CoA carboxylase activity by (−)-hydroxycitrate as revealed in this study substantiates the findings reported recently [2] of an increased fatty acid synthesis from acetate caused by this substance.

It can be concluded that (−)-hydroxycitrate is a positive effector of acetyl-CoA carboxylase. The similarity of (−)-hydroxycitrate and citrate activation of enzyme activity is apparent in the time for maximal stimulation of enzyme activity (30 min for both) and in the concentrations for half-maximal (2.8 mM and 1.9 mM, respectively) and maximal stimulation (about 10 mM for both).

More extensive kinetic analyses, on the other hand, demonstrated a difference of both effectors. Numa reported recently [9] that citrate leads to an activation of rat liver acetyl-CoA carboxylase of the K_m type, if the enzyme was preincubated in the presence of high concentrations of this effector (10 mM). This finding was confirmed in this study (fig. 1). In contrast to citrate, however, (−)-hydroxycitrate proved to be an activator of the V_{max} type, if the same preincubation procedure was chosen (fig. 4).

From these and previous [2] data, it can be concluded that (−)-hydroxycitrate acts as an inhibitor of lipogenesis only, if cytoplasmic acetyl-CoA is produced by the citrate cleavage enzyme reaction. But it will activate fatty acid synthesis whenever an

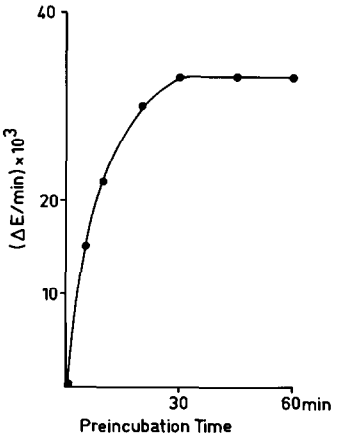

Fig. 3. Effect of preincubation time on acetyl-CoA carboxylase activity. Experimental conditions as in fig. 2, except that acetyl-CoA carboxylase was preincubated for indicated times with 10 mM (−)-hydroxycitrate and the reaction was carried out with the same concentration.

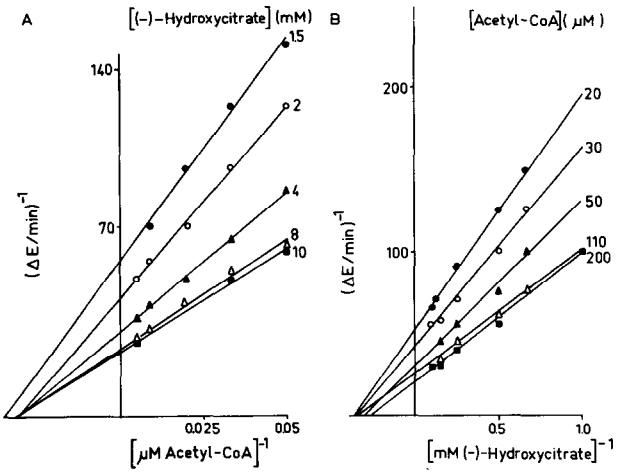

Fig. 4. Lineweaver–Burk plots of acetyl-CoA carboxylase activity with varying acetyl-CoA and (−)-hydroxycitrate concentrations. All conditions as in fig. 1, except that (−)-hydroxycitrate was employed instead of citrate. Mean values of two identical sets of experiments are given. A and B are different plots of the same data.

alternate source of cytoplasmic acetyl-CoA, e.g. acetate, is available.

Quantitative conclusions as to the importance of the citrate cleavage enzyme reaction derived from measurements of lipogenesis in the presence of (−)-hydroxycitrate must take into account this activating effect.

Acknowledgements

The skillful technical assistance of Mrs. J. Nowack and Mr. H. Henninger is gratefully acknowledged. This project was supported by grants from Deutsche Forschungsgemeinschaft, Bad Godesberg, W.Germany.

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