GLYOXYLATE INHIBITION OF CLOSTRIDIAL PYRUVATE SYNTHASE*

Rudolf K. THAUER, Eckhard RUPPRECHT and Kurt JUNGERMANN
Biochemisches Institut, Albert Ludwigs Universität, 78 Freiburg, Germany

Received 2 July 1970

1. Introduction

Formate derived from CO_2 is the predominant precursor of tetrahydrofolate carried one-carbon units in *Clostridium kluyveri* [1,2]. The reduction of CO_2 to formate is mediated by two enzymes [3], pyruvate synthase and pyruvate formate lyase. Pyruvate synthase is assumed to catalyze pyruvate synthesis from acetyl-CoA and CO_2 with reduced ferredoxin as electron donor, pyruvate dehydrogenation to acetyl-CoA and CO_2, and a pyruvate CO_2 exchange. Pyruvate formate lyase catalyzes pyruvate cleavage to acetyl-CoA and formate and a pyruvate formate exchange.

For the study of formate formation from CO_2 in cell-free extracts it is important to specifically block one of the two enzymes involved. In this communication it will be shown that in *Cl. kluyveri* glyoxylate is a potent inhibitor of the three pyruvate synthase activities while the two pyruvate formate lyase activities are not affected. As pyruvate synthase of *Cl. pasteurianum* is also inhibited, glyoxylate may turn out to be a useful tool for the study of processes in which formate is likely to be the key intermediate and pyruvate synthase a key enzyme such as the catabolic CO_2 reduction to acetate or methane and the anabolic C_1-unit synthesis from CO_2 or pyruvate.

2. Materials and methods

Clostridium kluyveri (obtained from Boehringer Mannheim) was maintained and grown on ethanol-acetate-bicarbonate [4] and *Cl. pasteurianum* (ATCC 6013) on synthetic media with glucose as the sole carbon and energy source [5].

Cell-free lysates were prepared by incubating freshly harvested cells (0.25 g wet cells/ml) with lysozyme (2 mg/ml) and DNAase (0.1 mg/ml) under an atmosphere of hydrogen for 30 min at 37° and by centrifuging at 40,000 *g* for 30 min. Dowex-2-acetate treated lysates were obtained as described [3].

All assays (see legends to figures and tables) were carried out at 37° either in 22 ml Thunberg tubes or in 3 ml anaerobic cuvettes after repeated evacuation and refilling with the desired gas. Hydrogen was determined by gas-chromatography [6], radioactivity by liquid scintillation counting and reduced benzyl-viologen by photometry at 578 nm.

3. Results

In cell-free lysates of *Cl. kluyveri* all the reactions catalyzed by the ferredoxin dependent pyruvate synthase were specifically inhibited by glyoxylate. At 6.1×10^{-5} M glyoxylate the dehydrogenation of pyruvate and its synthesis from acetyl-CoA, CO_2 and H_2, and the pyruvate-CO_2 exchange were reduced to half maximum velocity (fig. 1). 2 mM concentrations of glycollate, oxalate, glycolaldehyde or glycine were without effect.

Pyruvate cleavage to acetyl-CoA and formate was not influenced by glyoxylate at concentrations up to 5 mM; pyruvate formate exchange was apparently increased due to the glyoxylate inhibition of pyruvate degradation to acetyl-CoA and CO_2 (table 1).

* This project was supported by grants from the Deutsche Forschungsgemeinschaft, Bad Godesberg, through the Sonderforschungsbereich "Molekuläre Grundlagen der Entwicklung", Freiburg.

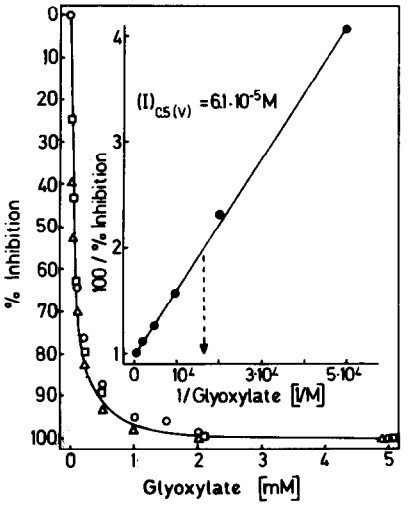

Fig. 1. Glyoxylate inhibition of pyruvate synthase of *Clostridium kluyveri.*
□: pyruvate synthesis from acetyl-CoA, CO_2 and H_2;
△: pyruvate dehydrogenation to acetyl-CoA, CO_2 and H_2;
○: pyruvate CO_2 exchange.

All assays were carried out in 22 ml Thunberg tubes; reaction time was 5 minutes.

Pyruvate synthesis: Tris acetate, pH 7.5, 100 mM; glutathione red., 2.5 mM; acetylphosphate K, Li, 25 mM; coenzyme A, 0.5 mM; $K_2{}^{14}CO_3$, 10 mM (200,000 dpm/μmole) phosphotransacetylase, 0.5 U; monosodium glutamate, 20 mM; glutamate pyruvate transaminase, 5 U; 8 mg crude lysate protein (avidin pretreated [3]; water to 1 ml; gas phase: hydrogen.

Pyruvate CO_2 exchange: Tris acetate, pH 7.5, 100 mM; glutathione red., 2.5 mM, $K_2{}^{14}CO_3$ 10 mM (200,000 dpm/μmole); sodium pyruvate, 10 mM; 8 mg crude lysate protein (avidin pretreated [3]); water to 1 ml; gas phase: hydrogen. Assay mixtures were acidified by injection of 0.5 ml 5% TCA and at 0° repeatedly evacuated and filled with $^{12}CO_2$. Aliquots were then counted.

Pyruvate degradation: Tris acetate, pH 7.5, 100 mM; glutathione red., 2.5 mM; coenzyme A, 0.5 mM; sodium arsenate, 5 mM; 8 mg crude lysate protein: water to 1 ml; gas phase: argon. Hydrogen was determined by gas-chromatography as has been described [6].

In cell-free lysates of *Cl. pasteurianum* pyruvate synthesis from acetyl-CoA and CO_2 was also inhibited by low concentrations of glyoxylate ($I_{0.5(V)} = 5.7 \times 10^{-5}$ M). Pyruvate dehydrogenation and pyruvate CO_2 exchange, however, were less sensitive to glyoxylate than in *Cl. kluyveri*. 100% inhibition was obtained only with concentrations above 5 mM. This may be correlated with the finding that the K_m for pyruvate

Table 1
Effect of glyoxylate on pyruvate formate lyase activities in cell-free extracts of *Clostridium kluyveri.*

	A ^{14}C-Formate formed (nmoles/5 min)	B ^{14}C-Formate exchanged (nmoles/5 min)
Complete	115	80
plus glyoxylate 2 mM	100	---
plus glyoxylate 2 mM	105	135

Complete: (A) Pyruvate cleavage to acetyl-CoA and formate: tris acetate, pH 7.5, 100 mM; glutathione red., 2.5 mM; coenzyme A, 0.5 mM; sodium arsenate, 5 mM; 1-^{14}C-pyruvate, 5 mM (200,000 dpm/μmole): phosphotransacetylase, 1U; 8 mg crude lysate protein; water to 1 ml; gas phase: hydrogen. Additions: sodium glyoxylate as indicated.

(B) Pyruvate formate exchange: tris acetate, pH 7.5, 100 mM; glutathione red., 2.5 mM; coenzyme A, 0.5 mM: sodium pyruvate. 5 mM: ^{14}C-formate, 5 mM (200,000 dpm/μmole): 8 mg crude lysate protein; water to 1 ml; gas phase: hydrogen. Additions: sodium glyoxylate as indicated. Assay mixture were heat stopped (100°/30 sec); pyruvate was converted to alanine by addition of 0.1 ml 1 M glutamate and 0.4 ml of an aqueous solution of 1 U of glutamate pyruvate transaminase and by further incubation at 37° for 30 min; this mixture was then acidified with 0.5 ml 5% TCA, supplemented with 0.5 ml acetone and centrifuged; 1.25 ml of the supernatant was chromatographed on 1.5 g Dowex-2-formate columns (0.5 cm ϕ) with 0.4 N HCOOH, as has been described [7]. This method allows the rapid separation of ^{14}C-pyruvate (as ^{14}C-alanine) from ^{14}C-formate.

in pyruvate dehydrogenation** is 200 times larger for *Cl. pasteurianum* ($K_m = 1.8 \times 10^{-2}$ M) than for *Cl. kluyveri* ($K_m = 9.8 \times 10^{-5}$ M).

Information on the mechanism of the glyoxylate inhibition was obtained by following pyruvate dehydrogenation photometrically with benzylviologen as electron acceptor. Initial velocity was independent of glyoxylate concentration. However, the rate dropped to zero in a process dependent on inhibitor concentration. Complete inhibition could not be reversed by high concentrations of pyruvate (fig. 2). This indicated that either an inhibitor was formed from glyoxylate or that glyoxylate inactivated the enzyme. Available evidence points to an inactivation as all possible compounds related to glyoxylate were without effect.

** measured with benzylviologen (see fig. 2).

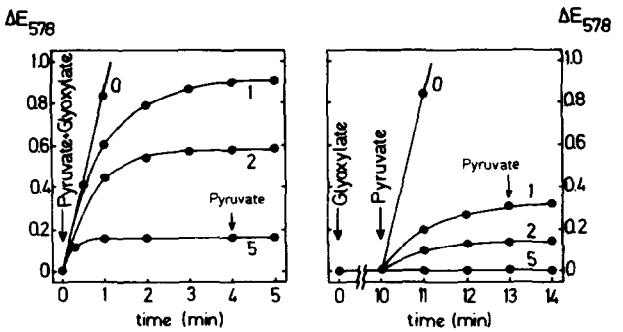

Fig. 2. Inactivation of pyruvate dehydrogenation by glyoxylate in cell-free extracts of *Clostridium kluyveri*. Assays were carried out anaerobically: 3 ml cuvettes, lightpath 1 cm, were filled with the assay mixture, closed with a soft rubber stopper, evacuated and filled with argon. After lifting the stopper the lysate was added; the cuvettes were immediately closed, evacuated and filled with argon. After incubation at 37° for 10 min, which was necessary to reduce residual oxygen with NADH, the reaction was started by injection of evacuated substrate and inhibitor solutions as indicated. Complete: Tris acetate, pH 7.5, 100 mM; glutathione red., 2.5 mM; coenzyme A, 0.5 mM; sodium arsenate, 5 mM; phosphotransacetylase, 1U: benzylviologen, 10 mM: NADH, 0.1 mM; 0.5 mg crude lysate protein: water to 2 ml; start as shown with sodium pyruvate, 2 mM, and/or sodium glyoxylate (0-5 mM) as indicated in the graph; restart where indicated with sodium pyruvate, 20 mM.

Inactivation by preincubation of the enzyme with glyoxylate in the absence of pyruvate could be achieved only with relatively high concentrations (5 mM) of the inhibitor (fig. 2b). At lower concentrations inactivation required the presence of pyruvate.

Inactivation was reversible as cell-free extracts incubated for 20 min with 20 mM glyoxylate or with 20 mM glyoxylate plus 20 mM pyruvate, sufficient to inactivate the enzyme, were active after removal of glyoxylate with Dowex-2-acetate.

4. Discussion

Pyruvate synthase has so far not been purified probably due to its extreme lability and sensitivity to oxygen. Therefore no definite information on the mechanism of the reactions catalyzed by the enzyme is available. Thus speculations on the mechanism of glyoxylate inactivation are inappropriate.

Clostridial pyruvate synthases [8–10] seem to be TPP dependent enzymes. As both pyruvate decarboxylase [11] and heart pyruvate dehydrogenase [12], which are TPP enzymes, are also inactivated by glyoxylate there may be similarities in the mechanism of inactivation.

The specific inhibition of pyruvate synthase by glyoxylate is a useful tool for the study of processes where it is desirable to block the enzyme such as anabolic [1–3] or catabolic [13–16] CO_2 reduction to formate. It should be pointed out that varying glyoxylate concentrations might be necessary in different Clostridia as indicated by the comparison of the enzymes from *Cl. kluyveri* and *Cl. pasteurianum*.

References

[1] K. Decker, K. Jungermann, R.K. Thauer and S.V. Hunt, Biochim. Biophys. Acta 141 (1967) 202.
[2] K. Jungermann, R.K. Thauer and K. Decker, European J. Biochem. 3 (1968) 351.
[3] R.K. Thauer, E. Rupprecht and K. Jungermann, FEBS Letters (1970) in press.
[4] R.K. Thauer, K. Jungermann, H. Henniger, J. Wenning and K. Decker, European J. Biochem. 4 (1968) 173.
[5] W. Lovenberg, B.B. Buchanan and J.C. Rabinowitz, J. Biol. Chem. 238 (1963) 3899.
[6] K. Jungermann, R.K. Thauer, E. Rupprecht, Chr. Ohrloff and K. Decker, FEBS Letters 3 (1969) 144.
[7] K. Jungermann, E. Rupprecht and R.K. Thauer, Anal. Biochem. 8 (1970) 304.
[8] I.G. Andrew and J.G. Morris, Biochim. Biophys. Acta 97 (1965) 176.
[9] R. Bachofen, B.B. Buchanan and D.I. Arnon, Proc. Natl. Acad. Sci. U.S. 51 (1964) 690.
[10] S. Raeburn and J.C. Rabinowitz, Biochem. Biophys. Res. Commun. 18 (1965) 303.
[11] A. Schellenberger, Wiss, Z. Univ. Halle XVIII 69 M, 319.
[12] G. Kohlhaw and H. Holzer, Mosbacher Colloquium 14 (Springer, Berlin, 1964) S. 245.
[13] A.Y. Sun, L. Ljungdahl and H.G. Wood, J. Bacteriol. 98 (1969) 842.
[14] L.G. Ljungdahl and H.G. Wood, Ann. Rev. Microbiol. 23 (1969) 515.
[15] B.A. Blaylock and T.C. Stadtman, Arch. Biochem. Biophys. 116 (1966) 138.
[16] T.C. Stadtman, Ann. Rev. Microbiol. 21 (1967) 139.