PYRUVATE SYNTHESIS FROM ACETYL COENZYME A AND CARBON DIOXIDE WITH $NADH_2$ OR $NADPH_2$ AS ELECTRON DONORS

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1. Introduction

In *Clostridium kluyveri* reduced ferredoxin is acting as electron donor in two reactions: the evolution of hydrogen gas during the ethanol–acetate fermentation [1] and the synthesis of pyruvate from acetyl-CoA CoA and CO_2 [2,3]. The first reaction is important because it enables *C. kluyveri* to gain ATP by substrate phosphorylation [4]. The second reaction provides the cell with pyruvate needed for the biosynthesis of cellular constituents [5]. The physiological reaction, however, by which Fd^+ is reduced and regenerated for the above processes is not known. In this paper we report that a cell-free system of *C. kluyveri* supplemented with $NADH_2$ or $NADPH_2$ and acetyl-CoA catalyzes the reduced Fd dependent synthesis of pyruvate in a nitrogen atmosphere. With a cell-free system of *C. pasteurianum* a similar reaction was not observed.

2. Materials and methods

C. kluyveri was grown and cell-free extracts were prepared as described previously [6]. *C. pasteurianum* was cultivated in the medium of ref. [7] and Fd was purified according to ref. [8]. The enzymic reactions were carried out in Warburg vessels containing Sephadex G-25 treated cell-free extract in 50 mM potassium phosphate buffer, pH 7.0, and 25 mM 2-mercaptoethanol in sidearm 1, $KH^{14}CO_3$ solution in sidearm 2 and the other reactants in the main compartment. The Warburg vessels were flushed with purified nitrogen gas for 10 min. Following a preincubation period of 20 min, the reactions were run for 20 min at 30°. After stopping with perchloric acid and removal of the $^{14}CO_2$ the remaining non-volatile radioactivity was measured in a Packard Tri-Carb using Bray's [9] scintillation fluid. Paper chromatography was carried out according to ref. [10]. LDH (360 U/mg) was purchased from Boehringer, Mannheim.

* Abbreviations used: ferredoxin = Fd; lactate dehydrogenase = LDH.

3. Results

As is well known [2,3] cell-free extracts of *C. kluyveri* catalyzed the synthesis of pyruvate from acetyl-CoA and CO_2 in a hydrogen atmosphere (table 1). The rate of pyruvate formation increased when LDH and $NADH_2$ were added as trapping reagents. An incorporation of $^{14}CO_2$ into non-volatile compounds was also observed when the reaction was carried out under nitrogen in the presence of relatively high concentrations of $NADH_2$ or $NADPH_2$. Pyruvate trapping reagents such as LDH or semicarbazide increased the rate of $^{14}CO_2$ fixation. Similar experiments were performed with cell-free extracts of *C. pasteurianum*. Although the pyruvate synthase system was active in these extracts no $^{14}CO_2$ was incorporated with $NADH_2$ or $NADPH_2$ as terminal electron donors.

Reactions 5 and 7 of table 1 were run with $^{14}CO_2$ of a higher specific radioactivity and the products formed were identified. Chromatography and cochro-

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Table 1
Reductive carboxylation of acetyl-CoA by cell-free extracts of *C. kluyveri* and of *C. pasteurianum* with hydrogen or $NAD(P)H_2$ as terminal electron donors.

Components added or omitted	Atmosphere	$^{14}CO_2$ incorporated			
		C. kluyveri		*C. pasteurianum*	
		(cpm/assay)	(U/g of protein)	(cpm/assay)	(U/g of protein)
1. –	H	24,460	1.42	–	–
2. + $NADH_2$; + LDH	H	144,130	8.38	110,200	9.75
3. –	N	90	–	116	–
4. + $NADH_2$	N	3,650	0.21	166	–
5. + $NADH_2$; + LDH	N	57,170	3.32	180	–
6. + $NADH_2$; + LDH; – acetyl phosphate	N	140	–	120	–
7. + $NADPH_2$	N	5,790	0.34	284	–
8. + $NADPH_2$; + semicarbazide	N	9,550	0.55	196	–

The assay system contained in a total volume of 2.0 ml: potassium phosphate buffer, pH 7.0, 50 mM; 2-mercaptoethanol, 25 mM; potassium lithium acetyl phosphate, 25 mM; $KH^{14}CO_3$ (279,000 cpm/μmole), 10 mM; coenzyme A, 0.5 mM; and 0.4 ml of Sephadex G-25 treated extract of *C. kluyveri* (7.7 mg of protein/ml) and of *C. pasteurianum* (5 mg of protein/ml), respectively. $NADH_2$, 2.5 mM; $NADPH_2$, 5 mM; LDH, 100 μg; and semicarbazide, 10 mM were added as indicated. The experimental conditions were described in Methods. U = μmoles/min.

Table 2
Effect of ferredoxin on the reductive carboxylation of acetyl-CoA with $NADH_2$ as terminal electron donor.

Ferredoxin added (μg/assay)	$^{14}CO_2$ incorporated	
	(cpm/assay)	(U/g of protein)
0	80	–
10	140	–
25	4,230	0.52
50	9,970	1.23

The assay system contained the components described in table 1. $NADH_2$, 5 mM; LDH, 100 μg; and cell-free extract (3.6 mg protein/ml), 0.4 ml were added. To remove ferredoxin the Sephadex G-25 treated extract was passed through a DEAE-cellulose column (3.0 × 0.8 cm) equilibrated against 50 mM potassium phosphate buffer, pH 7.0.

matography with authentic samples in two solvent systems revealed the formation of pyruvate (74%) and alanine (26%) in reaction 7. Reaction 5 yielded lactate as the only radioactive product. It was identified by chromatography in 1-propanol: ammonia and isolated by chromatography of the reaction mixture on Dowex-1-formate. Degradation of the ^{14}C-lactate by manganese dioxide [11] revealed that the radioactivity was present exclusively in the carboxyl group.

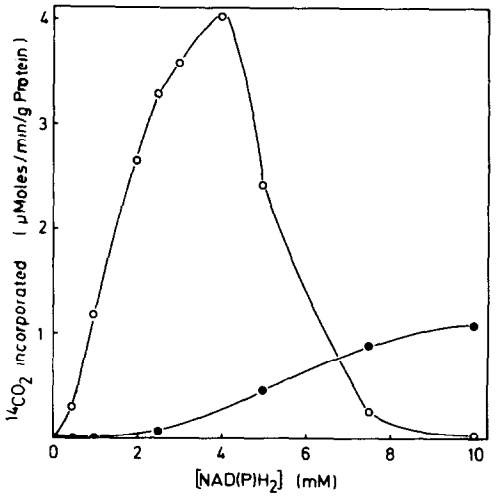

Fig. 1. Dependence of the reductive carboxylation of acetyl-CoA on the concentration of $NADH_2$ and $NADPH_2$. The assay system contained the components described in table 1. LDH was added to the reaction mixtures containing $NADH_2$ and semicarbazide to the mixtures containing $NADPH_2$. The Sephadex G-25 treated extract of *C. kluyveri* contained 7.0 mg of protein/ml. –○—○– $NADH_2$. –●—●– $NADPH_2$.

It is apparent from table 2 that the reductive carboxylation of acetyl-CoA with $NADH_2$ as electron donor was dependent on the presence of Fd. Extracts

passed through a DEAE-cellulose column catalyzed the reaction only after the addition of Fd.

The dependence of the amount of $^{14}CO_2$ fixed on the concentration of $NADH_2$ and $NADPH_2$ is shown in fig. 1. It can be seen that high concentrations of $NADH_2$ strongly inhibit the reaction. At concentrations up to 5 mM, $NADH_2$ is more effective as an electron donor than $NADPH_2$.

4. Discussion

Cell-free extracts of *C. kluyveri* catalyze the synthesis of pyruvate from acetyl-CoA, CO_2 and $NADH_2$ or $NADPH_2$. The reaction is not catalyzed by extracts of *C. pasteurianum* and *Chromatium* as was shown by Buchanan et al. [12]. The enzyme activity responsible for the reduction of Fd is therefore not necessarily present in all anaerobes containing NAD or NADP reductase.

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