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CO_2 Reduction to Formate in *Clostridium acidi-urici*

R. K. THAUER[1]

Department of Biochemistry, Case Western Reserve University, Cleveland, Ohio 44106

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Cell-free lysates of *Clostridium acidi-urici* catalyze the reduction of CO_2 to formate in the presence of reduced ferredoxin and nicotinamide adenine dinucleotide, reduced form.

In the purine fermentation of *Clostridium acidi-urici*, about 9% of the acetate formed is totally synthesized from CO_2 (5). The reduction of CO_2 to formate with reduced ferredoxin (Fd_{red}) as found in *C. pasteurianum* (1) is believed to be the initial reaction in this process (3). However, all published attempts have failed to demonstrate a formation of formate from CO_2 by *C. acidi-urici* (2). In this note, the conditions for the CO_2-reduction to formate are reported. There will be no distinction made between CO_2, HCO_3^-, or $CO_3^=$.

Frozen cells of *C. acidi-urici* grown on purines (2) were a gift of R. D. Sagers. Ferredoxin was prepared from *C. pasteurianum* by the method of Mortenson (4). Hydrogenase was partially purified from *C. pasteurianum* by anaerobically acidifying lysates (50 mg of protein/ml) with 1 N acetic acid to pH 4.9 and subsequent heating to 60 C for 25 min. After cooling and centrifugation at 40,000 × *g* for 30 min, the supernatant fraction containing the hydrogenase (5 to 10 IU per mg of protein) was adjusted to pH 8.0 with 1 N tris(hydroxymethyl)aminomethane (Tris) base. Crude lysates of *C. acidi-urici* (20 mg of protein/ml) were obtained by incubating 2 g of cells in 6 ml of 100 mM Tris acetate, pH 8.5, for 30 min at 35 C under hydrogen in the presence of 100 IU of *C. pasteurianum* hydrogenase with 5 mg of lysozyme and 0.5 mg of deoxyribonuclease, followed by centrifugation at 40,000 × *g* for 30 min. Ferredoxin-free lysates, which are also free from all nucleotides and formate, were prepared by passing 5 ml of the crude lysate anaerobically through a column filled with 1.5 ml of diethylaminoethyl-cellulose, a mixture of 2 g of Dowex-2-acetate, and 200 mg of charcoal (1).

Reduction of CO_2 to formate was studied in 1-ml reaction mixtures composed of imidazol acetate buffer (pH 7.0, 100 mM), mercaptoethanol (25 mM), potassium ^{14}C-carbonate (30,000 dpm/μmol; 30 mM, or as indicated), ferredoxin-free lysate (4 mg of protein, or as indicated), and electron donors in the form of regenerating systems (RS). NADH (0.5 mM) was regenerated with galactose (50 mM) and galactose dehydrogenase (0.5 IU). Nicotinamide adenine dinucleotide phosphate, reduced form, (NADPH; 0.5 mM) was regenerated with glucose-6-phosphate (25 mM) and glucose-6-phosphate dehydrogenase (1 IU). Fd_{red} (0.1 mg of protein) was regenerated with hydrogen and hydrogenase (2.5 IU), and reduced methyl viologen (MV_{red}; 10 mM) was regenerated with hydrogen and hydrogenase (2.5 IU). All assays were made at 35 C in 22-ml Thunberg tubes after repeated evacuation and filling with hydrogen. The reaction was stopped, and formate was determined as described earlier (1).

The lysates of *C. acidi-urici* readily catalyzed the reduction of CO_2 to formate (Table 1). The reduction was dependent on both NADH and Fd_{red}, for which half-maximal velocities were obtained at concentrations of 0.01 mM and 0.03 mg/ml, respectively. Neither the NADH-RS nor the Fd_{red}-RS were effective when tested alone. Also, other combinations of electron donors were without effect. NADPH and nicotinamide adenine dinucleotide phosphate, oxidized form, ($NADP^+$) did not substitute for NADH, and oxidized ferredoxin did not substitute for Fd_{red}. A reduction of CO_2 to formate was observed, however, with the Fd_{red}-RS plus nicotinamide adenine dinucleotide, oxidized form (NAD^+; 0.5 mM). This finding can be explained by NADH-formation from NAD^+ due to ferredoxin-NAD-reductase activity present in the lysates (6). Methyl viologen was an artificial but very effective

[1] Permanent address: Abteilung für Biologie der Ruhr-Universität Bochum, 463 Bochum, Pastfach 2148, Germany.

reductant independent of NADH or Fd_{red}.

Formate formation from CO_2 in the presence of the NADH- plus the Fd_{red}-RS was linear with protein up to 6 mg and with time up to 10 min (Fig. 1). A broad pH optimum with a maximum at 7 was observed in imidazol acetate and Tris acetate buffers. The half-maximal velocity was obtained with 8 mM CO_2 (Fig. 2).

There are two possible explanations of these results: (i) CO_2 is reduced to formate with Fd_{red}, and this reduction is dependent on NADH as an obligate positive effector; or (ii) NADH is the reductant, and Fd_{red} is either an obligate positive effector, or it is needed to keep the enzyme in a reduced active state. Due to the presence of the ferredoxin-NAD-reductase in the cell-free lysates of *C. acidi-urici*, these possibilities could not be delineated.

TABLE 1. *Reduction of CO_2 to formate in ferredoxin- and nucleotide-free lysates of C. acidi-urici*

Electron donor[a]	Formate formed (nmol/10 min/ 4 mg of protein)
—	<10
NADH-RS	17
Fd_{red}-RS	22
NADPH-RS	<10
NADH-RS + Fd_{red}-RS	480
NADPH-RS + Fd_{red}-RS	<10
$NADP^+$ [b] + Fd_{red}-RS	<10
NAD^+ [b] + F_{red}-RS	200
NADH-RS + Fd_{ox}-RS[c]	34
MV_{red}-RS	2,500

[a] RS, Regenerating system.
[b] 0.5 mM.
[c] Oxidized ferredoxin (0.1 mg protein), hydrogenase (2.5 IU), gas phase: argon.

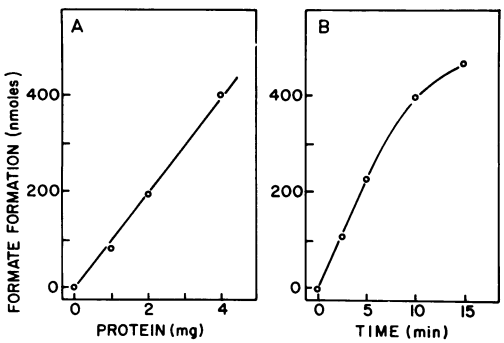

FIG. 1. *Reduction of CO_2 to formate in the presence of reduced ferredoxin and NADH using regenerating systems. A, Protein dependence (Time, 10 min). B, Time dependence (ferredoxin-free lysate, 4 mg of protein).*

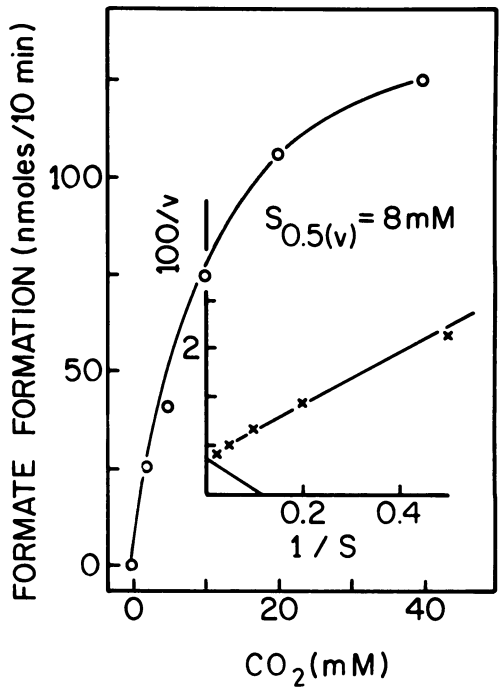

FIG. 2. *Effect of the concentration of CO_2 in the reduction to formate in the presence of reduced ferredoxin and NADH using regenerating systems. The reaction mixture contained 2 mg of protein of the ferredoxin-free lysate. $[S]_{0.5(v)}$ is the CO_2 concentration corresponding to half-maximal velocity.*

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