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Energy conservation in fermentative glutarate degradation by the bacterial strain WoGl3

Carola Matthies [1] and Bernhard Schink

Lehrstuhl Mikrobiologie I, Universität Tübingen, Tübingen, FRG

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1. SUMMARY

Dicarboxylic acids with 2–5 carbon atoms can be degraded fermentatively by pure cultures of various strictly anaerobic bacteria. The small amount of free energy released in these decarboxylations (about 20–25 kJ mol^{-1}) is conserved as sole source of growth energy either through sodium-pumping decarboxylases or through electrogenic substrate/product transport devices. In the glutarate-fermenting bacterial strain WoGl3 a glutaconyl-CoA-decarboxylating enzyme activity was detected. This enzyme was inhibited by avidin and was stimulated by sodium ions. The enzyme activity was partially associated with the cytoplasmic membrane, indicating that energy conservation is accomplished through a sodium-ion-pumping glutaconyl-CoA decarboxylase enzyme.

Correspondence to: B. Schink. Present address: Fakultät für Biologie, Universität Konstanz, Postfach 5560, D-7750 Konstanz, FRG.

[1] *Present address:* BITÖK-Institut, Universität Bayreuth, D-8580 Bayreuth, FRG.

2. INTRODUCTION

Dicarboxylic acids (with this term, we refer usually to alkanedioic acids) are widespread in nature and represent an important source of organic substrate for animals and microorganisms. Their nutritive value in aerobic degradation increases with their chain length: oxalate oxidation releases only one pair of electrons, malonate four pairs etc., which are used for ATP synthesis, mainly in electron transport phosphorylation. Fermenting bacteria, on the other hand, have very little advantage from degradation of such substrates; oxidation in the absence of oxygen, nitrate, or sulfate by a fermentative type of metabolism releasing hydrogen would require cooperation with e.g. hydrogen-oxidizing methanogenic bacteria in a so-called syntrophic association [1]; such bacteria have never been isolated so far. Instead, it was observed that bacteria could be enriched and isolated from anoxic natural sources with short-chain dicarboxylic acids as sole source of energy, and that these bacteria covered their whole energy metabolism by decarboxylation of the substrate to the respective fatty acid.

In the present article, we provide evidence that conservation of decarboxylation energy in the recently isolated strictly anaerobic bacterial strain WoGl3 is accomplished through a sodium-pumping glutaconyl-CoA decarboxylase enzyme system.

3. MATERIALS AND METHODS

Strain WoGl3 [2] was grown in a bicarbonate-buffered freshwater mineral medium supplied with 1% (v/v) rumen fluid [2]. Cells were harvested by centrifugation in closed vessels handled inside an anaerobic glove box, and were washed and resuspended in 50 mM morpholinoethanesulfonic acid (pH 6.5) reduced with 2.5 mM dithioerythritol. Cell extracts were prepared under strict exclusion of oxygen [3] by French press treatment at 136 MPa pressure. Extracts were subfractionated by centrifugation at $200\,000 \times g$ in closed anoxic vessels (Beckman Modell L5-50 centrifuge) for 60 min.

Glutaconyl-CoA decarboxylase activity was measured in two different assay systems. In a discontinuous assay, release of CO_2 was measured by gas chromatography [4] in oxygen-free 25 ml butylrubber-sealed serum bottles containing 0.5 ml reaction mixture with 50 mM morpholinoethane sulfonic acid buffer (pH 6.5), 0.1 mM butyryl-CoA, 0.1 mM NAD^+ and 10 mM disodium glutarate. For continuous measurement of glutaconyl-CoA decarboxylase, a photometric coupled test system was applied [5].

Protein was quantified according to Zamenhoff [6]. Sodium ion concentrations were determined with an Orion Na^+ electrode (System Ross 84-11, Colora, Lorch, FRG).

4. RESULTS

Decarboxylation of glutarate by cell-free extracts of strain WoGl3 was possible if either acetyl-CoA or butyryl-CoA was added to the reaction mixture at catalytic amounts, indicating that decarboxylation occurred with a coenzyme A-activated derivative. The measured activity was

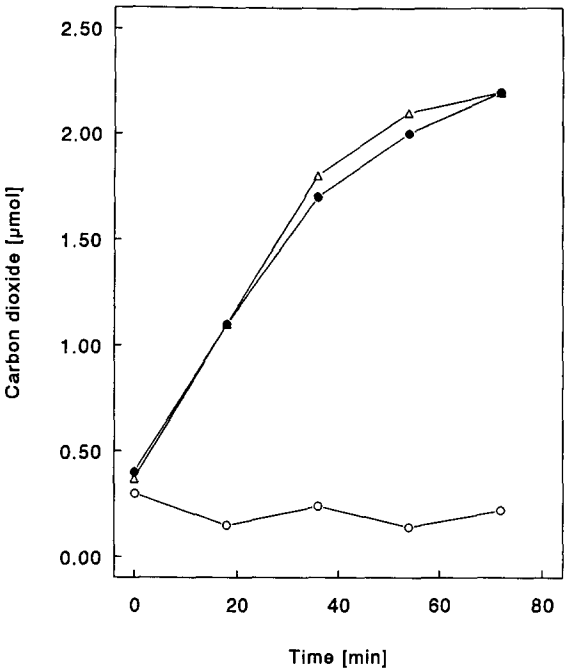

Fig. 1. Inhibition of glutaconyl-CoA decarboxylase activity (photometric assay) by avidin in cell-free extracts of strain WoGl3. △, no addition; ○, extract incubated with avidin (0.27 mg (mg protein)$^{-1}$; ●, extract in the presence of the same amount of avidin pre-incubated with excess biotin.

in the range of 40–50 nmol min^{-1} (mg protein)$^{-1}$ which corresponds to about 5% of the physiological activity in growing cells [2]. Decarboxylation was inhibited completely by addition of avidin (Fig. 1). Control experiments with avidin preincubated with excess biotin revealed that this inhibition was due to a specific binding of free avidin to enzyme-bound biotin.

If the cell-free extract was dialysed or ultrafiltrated prior to the enzyme assay, the measurable decarboxylation activity was decreased to less than 10% of the original activity (Fig. 2). Addition of various electron acceptors at catalytic amounts increased the activity considerably; full recovery of the original activity was obtained with NAD^+ as acceptor (Fig. 2). These findings indicate that decarboxylation occurs with an oxidized derivative of the coenzyme A-activated glutarate. Control experiments with a coupled enzyme assay system for glutaconyl-CoA decarboxylase [5] re-

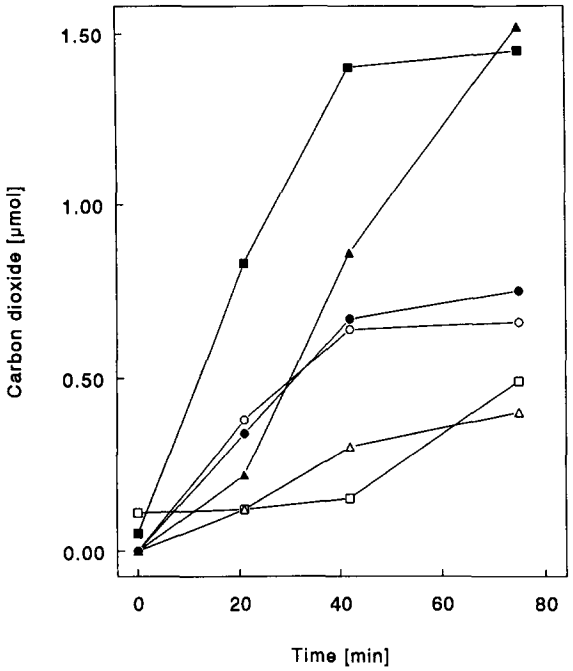

Fig. 2. CO_2 formation from glutarate by cell-free extracts before and after ultrafiltration (Amicon membrane, exclusion limit $M_r = 3000$) in the presence and absence of oxidized electron carriers. ■, crude extract, no addition. All others ultrafiltrated extract; △, without further additions; or plus addition of 0.1 mM NAD^+ (▲); phenazine methosulfate (○); methylene blue (●); or dichlorophenol indophenol (□).

vealed that actually glutaconyl-CoA was decarboxylated by cell extracts of strain WoGl3 at the same rate as was glutarate in the discontinuous enzyme assay.

The glutaconyl CoA decarboxylase activity of strain WoGl3 was stimulated significantly by addition of sodium chloride (Fig. 3). Potassium chloride had no stimulating effect. Half-maximal stimulation was observed with 4.9 mM NaCl.

Membrane preparations obtained after high-speed centrifugation of crude extracts exhibited very little glutaconyl-CoA decarboxylase activity (< 5% of crude extract). The cytoplasmic fraction thus prepared contained about 20% of the original activity. Combination of both fractions recovered nearly the full activity (about 80%) of the original extract.

5. DISCUSSION

5.1. Biochemistry of glutarate decarboxylation by strain WoGl3

In the present study, activity of the decarboxylating enzyme involved in glutarate fermentation by strain WoGl3 is demonstrated for the first time. Measurement of this activity requires an acyl-CoA derivative and, in dialysed extracts, an oxidizing agent such as NAD. Glutaryl-CoA: acetate CoA transferase and glutaryl-CoA : butyrate CoA transferase, as well as butyryl-CoA dehydrogenase and glutaryl-CoA dehydrogenase, both reacting with $K_3Fe(CN)_6$, were detected earlier in cell-free extracts [2]. These findings prove that glutarate is first activated to glutaryl-CoA, oxidized to glutaconyl-CoA, decarboxylated to crotonyl-CoA, and the latter reduced again to butyryl-CoA [2]. Our present data show that glutaryl-CoA oxidation and crotonyl-CoA reduction

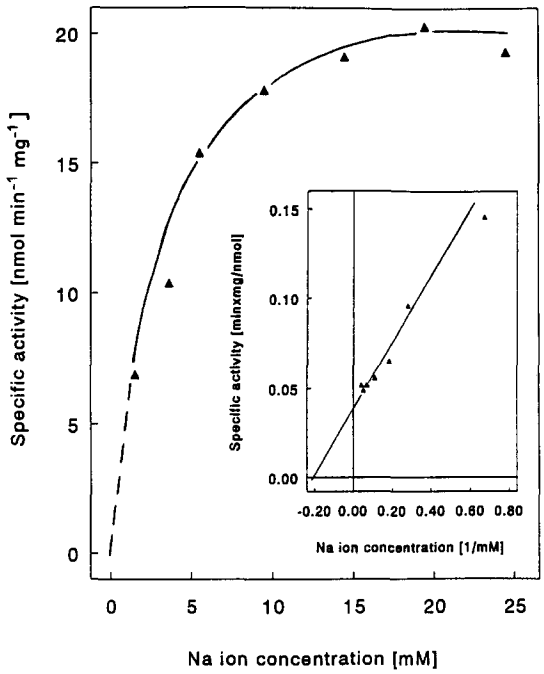

Fig. 3. Effect of sodium ion concentration on glutaconyl-CoA decarboxylase activity (photometric assay) in extracts of strain WoGl3. Background Na^+ concentration was 1.5 mM. Maximum acitvity: 26 nmol min^{-1} (mg protein)$^{-1}$. Inset: Lineweaver-Burk plot.

both operate best with NAD^+, which is probably the physiological electron carrier in both cases. The low redox potential of the NAD^+/NADH couple (−320 mV) as compared to those of the acyl-CoA couples (−125 mV for crotonyl-CoA/butyryl-CoA; [7]) keeps the acyl-CoA derivatives largely in the reduced state, thus preventing spontaneous decarboxylation of glutaconyl-CoA.

Our results prove further that glutaconyl-CoA decarboxylase in our strain is a biotin enzyme and is activated by sodium ions, similar to the glutaconyl-CoA decarboxylases described earlier [8,9]. These enzymes were shown to operate as sodium ion pumps to establish a sodium ion gradient across the cytoplasmic membrane, which can contribute significantly to the energy budget of glutamate-fermenting bacteria [10]. In our case, this sodium ion gradient has to be the only source of growth energy for strain WoGl3 because no other ATP-yielding reactions are involved in glutarate fermentation to butyrate and isobutyrate [2]. Unfortunately, the glutaconyl-CoA decarboxylase of this strain was not firmly bound to the cytoplasmic membrane; results of the subfractionation experiments mentioned above indicate that both membrane-bound and soluble or artificially solubilized components are required for full enzyme activity.

5.2. Energy conservation in strain WoGl3

All the data presently at hand indicate that energy conservation in glutarate fermentation by strain WoGl3 is accomplished through a membrane-associated, sodium-pumping glutaconyl-CoA decarboxylase, similar to the corresponding enzymes in glutamate-fermenting bacteria [10]. This way of energy conservation resembles the one operating in succinate fermentation by *Propionigenium modestum* [11]: methylmalonyl-CoA is decarboxylated by a membrane-bound decarboxylase which acts as a primary sodium ion pump [12]. The sodium ion gradient thus established drives ATP synthesis by a membrane-bound, sodium-pumping ATPase [13], the only reliably proven sodium ATPase existing so far. In this case, the free energy of decarboxylation is converted directly into an ion gradient. Thus, energy conservation will be rather independent of substrate/product concentration relations, provided that both are at similar concentrations.

An entirely different mechanism of energy conservation in fermentative dicarboxylate degradation was described for oxalate fermentation by *Oxalobacter formigenes*: oxalyl-CoA is decarboxylated inside the cytoplasm by a soluble enzyme, and the free energy of this decarboxylation reaction is conserved in a proton gradient across the cytoplasmic membrane by means of an electrogenic oxalate : formate antiporter [14]. Thus, not the decarboxylation reaction itself energizes the membrane, but the inwardly oriented substrate gradient together with the outwardly oriented product gradient maintain a proton gradient across the membrane which drives ATP synthesis.

The question remains how such a system can work at substrate concentrations in the micromolar range as they are typical of natural anoxic environments, especially sediments. Maintenance of a protonmotive force in the range of 150–200 mV across the cytoplasmic membrane by a single substrate or product gradient would require a concentration ratio of about 1 : 3000; two such gradients could add up to this value by an antiporter mechanism if they were at least 1 : 50 each. If such a system should operate at low substrate concentrations, either the CoA transferase must have an extremely high affinity for the substrate, or the product has to accumulate inside the cell up to 3000 times the outside concentration to allow establishment of a proton gradient. The ecological significance of such an energy conservation system still needs to be established.

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