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Anaerobic degradation of 3-aminobenzoate by a newly isolated sulfate reducer and a methanogenic enrichment culture

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Abstract. A new rod-shaped, gram-negative, non-sporing sulfate reducer, strain mAB1, was enriched and isolated from marine sediment samples with 3-aminobenzoate as sole electron and carbon source. Strain mAB1 degraded 3-aminobenzoate completely to CO_2 and NH_3 with stoichiometric reduction of sulfate to sulfide. Cells contained carbon monoxide dehydrogenase, cytochromes, and sulfite reductase P582. Strain mAB1 degraded also benzoate, 4-aminobenzoate, hydroxybenzoates, and some aliphatic compounds. Besides sulfates, also sulfite was reduced with 3-aminobenzoate as electron donor, but not thiosulfate, sulfur, nitrate, or fumarate. The strain grew in sulfide-reduced mineral medium supplemented with 7 vitamins. Strain mAB1 was tentatively affiliated with the genus *Desulfobacterium*. Experiments with dense cell supsensions showed benzoate accumulation during 3-aminobenzoate degradation under conditions of sulfate limitation or cyanide inhibition. 3-Aminobenzoate was activated to 3-aminobenzoyl-CoA by cell extracts in the presence of ATP, coenzyme A, and Mg^{2+}. Acitivity of 3-aminobenzoyl-CoA synthetase was 16 nmol per min and mg protein, with a K_M for 3-aminobenzoate lower than 50 µM. Cell extract of 3-aminobenzoate-grown cells activated also 3-hydroxybenzoate (31.7 nmol per min and mg protein) and benzoate (2.3 nmol per min and mg protein), but not 2-aminobenzoate or 4-aminobenzoate. In the presence of NADH of NADPH, 3-aminobenzoyl-CoA was further metabolized to a not yet identified reduced product.

Freshwater enrichments with 3-aminobenzoate in the absence of an external electron acceptor led to a stable methanogenic enrichment culture consisting of three types of bacteria. 3-Aminobenzoate was degraded completely to CO_2 and stoichiometric amounts of CH_4, with intermediary acetate accumulation.

Key words: 3-Aminobenzoate – Anaerobic degradation – Sulfate-reducing bacterium – Methanogenic enrichment culture – 3-Aminobenzoyl–CoA synthetase – 3-Aminobenzoyl-CoA reduction

While 2- and 4-aminobenzoate occur in nature, 3-aminobenzoate is to our knowledge a xenobiotic compound produced mainly for synthesis of azo dyes. A toxic effect of 3-aminobenzoate was reported for humans, dogs and rabbits, which metabolize 3-aminobenzoate partly to 3-ureidobenzoic acid and 3-aminohippuric acid (Neumüller and Otto-Albrecht 1979).

Aerobically, 3-aminobenzoate is degraded through hydroxylation to 5-aminosalicylic acid, which is further metabolized by a 1,2-dioxygenase to cis-4-aminocarboxy-2-oxohexan-2,5-dienic acid (Russ, Stolz and Knackmuss, Abstr. Ann. Meetg. VAAM, Freiburg, 1991, Poster P136).

Anaerobic degradation of 3-aminobenzoate has not yet been observed so far. Of primary interest is the influence of the amino-group in 3-position on the degradation pathway, in comparison to amino-groups in 2- and 4-position of benzoate. 2-Aminobenzoate is degraded through the CoA ester; an activation to 2-aminobenzoyl-CoA wa proven with nitrate-reducing (Ziegler et al. 1987; 1989), sulfate-reducing, and fermenting bacteria (Schnell 1991), but the further metabolism is unknown. In the pathway of anaerobic 4-aminobenzoate degradation, activation to 4-aminobenzoyl-CoA and reductive deamination to benzoyl-CoA was shown with *Desulfobacterium anilini* (Schnell and Schink 1991). Anaerobic degradation of 3-aminobenzoate is reported in the present study.

Material and methods

Source of organisms

The methanogenic enrichment culture DemAB was enriched from black anoxic sediment of a canal in Delft (Netherlands), and the nitrate-reducing culture was enriched from mud of a polluted ditch near Konstanz (FRG).

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The sulfate reducer strain mAB1 was isolated from marine enrichment cultures inoculated with black reducing sediment from Etang de Prevost, a salt marsh near Marseille (France). Other sulfate-reducing enrichment cultures were started with organic-rich anoxic sediment from Rio de la Pergola, a canal in Venice (Italy).

Media and growth conditions

Anaerobic defined mineral media of various salinities were prepared as described in detail by Widdel and Pfennig (1984), using trace element solution SL10a (Widdel 1986), 2 ml per 1 medium. 10 Vitamins were added as a modified 7-vitamin solution described by Widdel and Pfennig (1981) containing in addition 3 mg folic acid and 1 mg DL-α-lipoate in 100 ml H_2O. Vitamin B_{12} (5 mg in 100 ml H_2O) and riboflavin solutions (5 mg in 100 ml 20 mM acetic acid) were added separately (1 ml per 1 autoclaved medium). Dithionite (final concentration about 0.3 mM) was added to the sulfate-reducing cultures as anoxically prepared solution, or as dry crystals. Stock solutions of 3-aminobenzoate and the other aromatic substrates were prepared anoxically, filter sterilized, and kept under nitrogen.

All procedures of isolation, cultivation, determination of growth parameters, and characterization were basically as published earlier (Widdel and Pfennig 1984; Schnell et al. 1989; 1991).

Ultra-thin sections and electron microscopy of strain mAB1 were performed by Prof. Dr. F. Mayer, Göttingen, FRG.

Determination of stoichiometries

Stoichiometries of substrate degradation by sulfate-reducing cultures were determined in 100-ml screw-cap bottles. Samples for measurement of optical density (Hitachi photometer 100-40, Tokyo, Japan), substrate and product concentrations were taken, and the bottles were filled up with sterile glass rods as non-diluting spacers. For fermenting bacteria, serum bottles with a headspace were used and samples were taken with syringes. Cell dry mass was determined in 1-l batch cultures (Widdel and Pfennig 1981).

Pigment analysis and DNA base composition

Cytochromes were identified in cell extracts by recording reduced-minus-oxidized difference spectra with an Uvicon 860 spectrophotometer (Kontron, Zürich, Switzerland). Desulfoviridin was identified as described by Postgate (1956). Sulfite reductase P582 was identified through CO-difference spectra of cell extract (Trudinger 1970).

The DNA base composition was measured by direct determination of the guanine-plus-cytosine content by high performance liquid chromatography (Tamaoka and Komagata 1984; Mesbah et al. 1989). The DNA was isolated according to Cashin et al. (1977).

Experiments with dense cell suspensions

Cells were grown in 1000-ml bottles and harvested in the late exponential growth phase by centrifugation (20000 × *g*, 20 min). Centrifuge tubes were preincubated and filled in an anoxic chamber where also the cell suspensions were prepared after centrifugation. Cells were washed once and resuspended to a final density of 1 mg protein · ml^{-1} in anoxic medium.

Assays were performed at room temperature in 2-ml vials closed with butyl rubber stoppers and gassed with N_2/CO_2 (90/100). The reaction mixture contained in 1 ml of medium 300 µg cell protein, 0.5–1.0 mM substrates, 0.25 mM dithionite, and in some cases 0.2–1.2 mM cyanide as inhibitor of carbon monoxide dehydrogenase. All additions and samples were handled with gas-tight Unimetrics microliter syringes to prevent access of air. Samples (100 µl) were taken at regular intervals, injected into 400 µl of 0.1 M H_3PO_4, and kept on ice to be analyzed within 1 h.

Experiments with cell extracts

Cells were harvested anoxically as described above, washed and suspended in N_2-sparged 50 mM sodium phosphate buffer, pH 7.0, with 20 mM $MgCl \cdot 6\,H_2O$ and 2.5 mM 1,4-dithiotreitol. Cells were disrupted in a chilled French pressure cell gassed with N_2. The crude extract was centrifuged again in N_2-gassed vials at 5000 × *g* at 4 °C to remove cell debris.

Assays for acyl-CoA synthetase were performed in 2-ml vials closed with butyl ruber stoppers and gassed with N_2. The reaction mixture contained in 1 ml of N_2-gassed 50 mM sodium phosphate buffer, pH 7.0, 0.5–1.0 mM aromatic acid, 0.5–1.0 mM CoASH, 1–2 mM ATP, 20 mM $MgCl_2$, 2.5 mM dithiotreitol, and about 450 µg cell protein. Samples (100 µl) were taken with gas-tight syringes, and the reaction was stopped by injection into 400 µl of 0.1 M H_3PO_4.

Experiments on 3-aminobenzoyl-CoA reduction were performed in a modified acyl-CoA synthetase assay: Various reductants were added after approximately 1-mM 3-aminobenzoyl-CoA was produced by cell extract, and samples were analyzed by high pressure liquid chromatography.

Carbon monoxide dehydrogenase activity was determined photometrically (Diekert and Thauer 1978), 2-oxoglutarate-ferredoxin oxidoreductase activity after Brandis-Heep et al. (1983). Methyl viologen and dithionite served as electron acceptor and reducing agent, respectively.

Protein was determined after Bradford (1976) with bovine serum albumin as standard.

Synthesis and hydrolysis of 3-aminobenzoyl-CoA

3-Aminobenzoyl-CoA was synthesized chemically after Merkel et al. (1989), from 5 µmol CoASH and 10 µmol 3-aminobenzoate. The reaction was followed by high pressure liquid chromatography. For quantification of 3-aminobenzoyl-CoA, an aliquot was hydrolyzed in 50 mM NaOH, and the correlation between the disappearing peak area and the amount of produced 3 aminobenzoate and CoASH was determined.

Analyses of substrates and products

Nonvolatile organic compounds were analysed with a Beckman (Munich, FRG) System Gold high pressure liquid chromatograph equipped with a Merck (Darmstadt, FRG) LiChrospher 60 RP-select B column (5 mm × 12.5 cm). The mobile phase contained 100 mM ammonium phosphate buffer, pH 2.6, and methanol. Various gradients were used between 10 and 70% methanol to separate ATP, AMP, CoASH, aromatic acids, and acyl-CoA esters, at 1 ml/min flow rate. Samples of 20 µl were injected with a Spark Promis I autosampler. A Beckman 167 scanning variable wavelength detector was used at the respective appropriate wavelength for each compound. Data were analysed by a computer program and quantified by comparison with external and internal standards of known composition. Peaks were identified by comparison of retention times and UV-spectra recorded by on-line spectral scans with standards samples.

Sulfide was analysed photometrically by the methylene blue method (Cline 1969), and ammonia according to Chaney and Marbach (1962) after precipitation of sulfide with zinc acetate.

Methane, acetate, and other fatty acids were measured by gas chromatography (Platen and Schink 1987).

Results

Enrichment of 3-aminobenzoate-degrading bacteria

Enrichment cultures with 3-aminobenzoate and sulfate inoculated with marine reducing sediment samples showed sulfide production after 4 months. In the enrichment

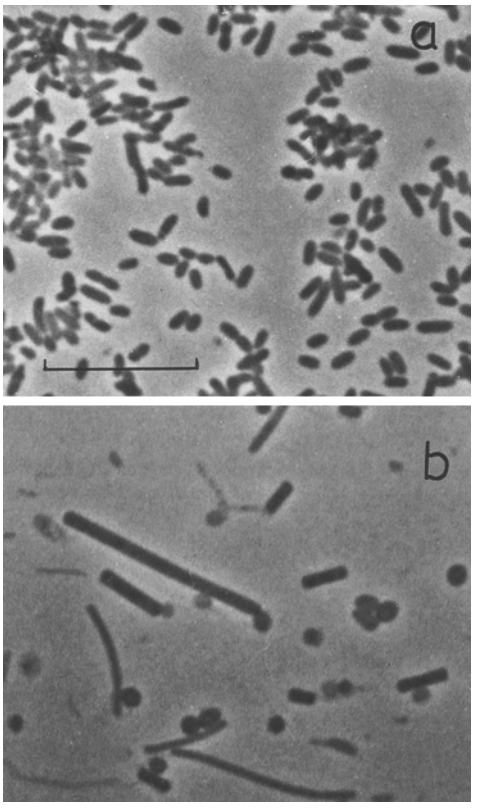

Fig. 1. Phase-contrast photomicrographs of the sulfate-reducing bacterium strain mAB1 **(a)** and the methanogenic coculture DemAB on 3-aminobenzoate **(b)**. *Bar* equals 10 µm

culture inoculated with sediment from Venice, bacteria grew in flocs consisting mainly of *Desulfosarcina*-like cells. Repeated homogenisation and subcultivation did not prevent flox formation. The enrichment culture with sediment samples from Marseille contained mainly short rods. Strain mAB1 (Fig. 1a) was isolated from this culture.

Freshwater enrichments with or without sulfate added did not produce sulfide, but showed methane production after 5–6 months. The culture DemAB enriched with river sediment from Delft consisted of three different bacterial types (Fig. 1b): short rods, *Methanospirillum*-like bacteria, and long thick rods similar to *Methanothrix* sp.

Isolation and characterization of strain mAB1

Strain mAB1 was isolated in subsequent agar dilution series with 1 mM 3-aminobenzoate and 20 mM sulfate. The short rods were non-motile, 0.5 µm in width, and 1–2 µm long (Fig. 1a). By Gram-staining, no reliable attribution to the gram-negative or gram-positive type was possible; in electron microscopic examination of ultra-thin sections, a gram-negative cell wall structure appeared (not shown).

Strain mAB1 grew best in saltwater medium. In brackish water medium or in saltwater medium supplemented with additional 60 mM NaCl and 10 mM $MgCl_2$, growth was slower, and no growth occurred in freshwater medium. No growth was found in complex medium under aerobic or microaerobic conditions. Addition of 0.3 mM dithionite shortened the lag-phase of growth.

The pH-range of growth was pH 6.8 to 8.0, with an optimum at pH 7.0 to 7.5. No growth occurred below 20 °C or above 40 °C. The shortest doubling time was measured at 30 °C (260 h with 3-aminobenzoate as substrate). With benzoate or 3-hydroxybenzoate, strain mAB1 grew approximately three times faster. 3-Aminobenzoate concentrations higher than 1 mM increased the lag-phase of growth significantly.

Strain mAB1 grew in the presence of sulfate with 3-aminobenzoate, 3- and 4-hydroxybenzoate, 4-aminobenzoate, and benzoate; no further aromatic substrate was utilized. It grew also with H_2/CO_2, fatty acids of 1–6 carbon atoms, with succinate, fumarate, ma-

Table 1. Substrates tested as electron donors and carbon sources for strain mAB1, with sulfate as electron acceptor. Added substrate concentration are given in mM in parentheses

Compounds utilized:
Formate (10), acetate (10), propionate (10), butyrate (5), *n*-valerate (5), *n*-caproate (4), succinate (5), fumarate (5), malate (5), glutarate (5), pyruvate (20), benzoate (2), 3-hydroxybenzoate (1), 4-hydroxybenzoate (2), 3-aminobenzoate (1), 4-aminobenzoate (1)

Compounds not utilitzed:
2-Methylbutyrate (5), 3-methylbutyrate (3), malonate (5), adipate (2), pimelate (1), lactate (20), methanol (10), ethanol (10), butanol (5), cyclohexanol (1); cyclohexanecarboxylate (1), phenol (1), catechol (1), hydroquinone (1), resorcinol (1), pyrogallol (1), phloroglucinol (1), *o*-cresol (1), *m*-cresol (1), *p*-cresol (1), aniline (1), 2 aminobenzoate (1), 2-hydroxybenzoate (1), phenylacetate (1), phenylpropionate (1), 2,3-dihydroxybenzoate (1), 2,4-dihydroxybenzoate (1), 2,5-dihydroxybenzoate (1), 2,6-dihydroxybenzoate (1), 3,4-dihydroxybenzoate (1), 3,5-dihydroxybenzoate (1), *o*-phthalate (1), tyrosine (1), tryptophane (1), phenylalanine (1)

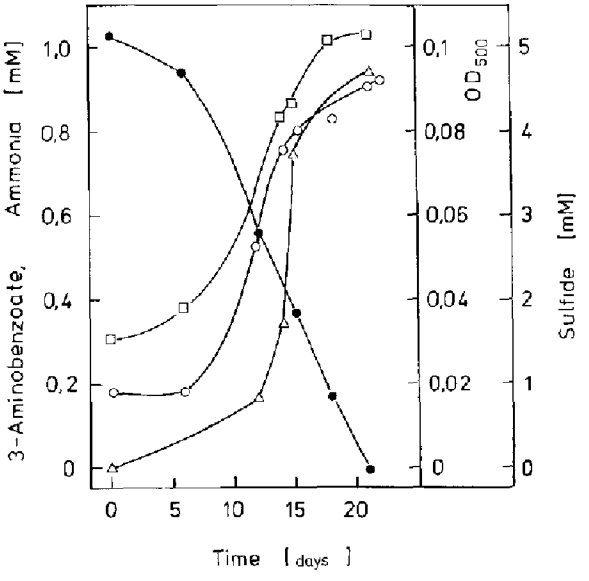

Fig. 2. Growth curve of the sulfate-reducing bacterium strain mAB1; 3-aminobenzoate (●), ammonia (△), sulfide (□), optical density (○). 3-Aminobenzoate was added at the start and again after 30 days

late, glutarate, and pyruvate (Table 1). Besides sulfate, also sulfite, but not thiosulfate, elemental sulfur, nitrate, or fumarate was reduced.

Redox difference spectra of dithionite-reduced minus air-oxidized cell extract showed absorption maxima at 553, 530, and 420 nm indicating the presence of c-type cytochromes.

CO-difference spectra demonstrated the presence of sulfite reductase P582 (absorption maxima at 420, 553 and 579 nm, and absorption minima at 440, 536, 565 and 593 nm).

The G + C content of the DNA was determined to be 52.6 +/− 0.2 mol% by direct base ratio determination by high pressure liquid chromatography.

Stoichiometry of 3-aminobenzoate degration

A time course of 3-aminobenzoate oxidation by strain mAB1 is presented in Fig. 2; the electron balance of this growth experiment came up to 105% (Table 2), according to the following equation for complete 3-aminobenzoate oxidation (The ΔG^{f0} value for aminobenzoates (−200 kJ per mol) was calculated by comparison with values for carboxylation, hydroxylation and amination of aromatic compounds published in Thauer et al. 1977; Karapet'yants and Karapet'yants 1970):

$$2\,C_7H_6NO_2^- + 7\,SO_4^{2-} + 11\,H^+ \rightarrow 14\,CO_2 + 2\,NH_4^+ + 7\,HS^- + 4\,H_2O \quad (1)$$

$\Delta G'_0 = -186$ kJ per mol 3-aminobenzoate

Table 2. Stoichiometry of 3-aminobenzoate oxidation by strain mAB1

3-Aminobenzoate degraded [mmol]	1.03
Sulfide produced [mmol]	3.59
Ammonia produced [mmol]	0.94
Cell dry mass produced [mg]	12.7
Groth yield [g · mol^{-1}]	13.0
3-Aminobenzoate assimilated[a] [mmol]	0.055
3-Aminobenzoate dissimilated [mmol]	0.975
Molar ratio of produced sulfide over Dissimilated 3-aminobenzoate	3.68
Electron balance [%]	105.3

[a] Substrate consumed for cell mass synthesis was calculated after the following equation:

$$17\,C_7H_7O_2N + 11\,NH_4^+ + 43\,H_2O \rightarrow 28\,[C_4H_8O_2N] + 7\,HCO_3^- + 18\,H^+$$

5.95 mmol 3-aminobenzoate is assimilated into 1 g cell dry mass

Table 3. Stoichiomety of 3-aminobenzoate degradation by the methanogenic coculture DemAB

3-Aminobenzoate degraded [μmol]	143
Methane produced [μmol]	539
Methane expected [μmol][a]	501
Electron balance [%]	107.6

[a] The amount of methane expected for complete oxidation of 3-aminobenzoate was calculated after the following equation:

$$2\,C_7H_6O_2N^- + 10\,H_2O + 4\,H^+ \rightarrow 7\,CO_2 + 7\,CH_4 + 2\,NH_4^+$$

From the doubling time of 260 h ($\mu = 0.0026\ h^{-1}$) and a growth yield of 13 g per mol 3-aminobenzoate, an in vivo degradation activity of 21 nmol per min and mg protein was calculated.

The stoichiometry of 3-aminobenzoate degradation by the methanogenic enrichment culture was examined in batch culture. The growth curve presented in figure 3 and the fermentation balance (Table 3) indicate complete substrate degradation according to the equation:

$$2\,C_7H_6O_2N^- + 10\,H_2O + 4\,H^+ \rightarrow 7\,CO_2 + 7\,CH_4 + 2\,NH_4^+ \quad (2)$$

$\Delta G'_0 = -144$ kJ per mol 3-aminobenzoate

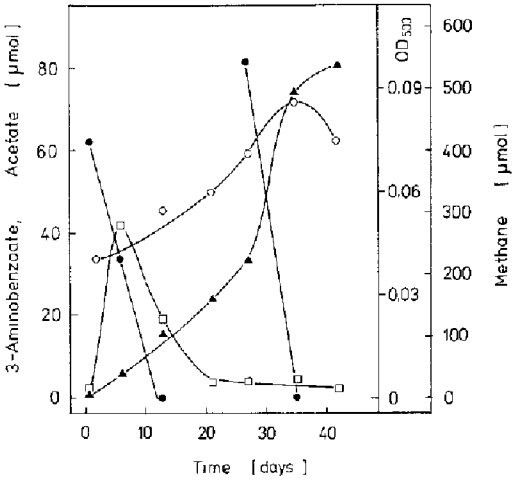

Fig. 3. Growth curve of the methanogenic enrichment culture DemAB; 3-aminobenzoate (●), methane (▲), acetate (□), optical density (○)

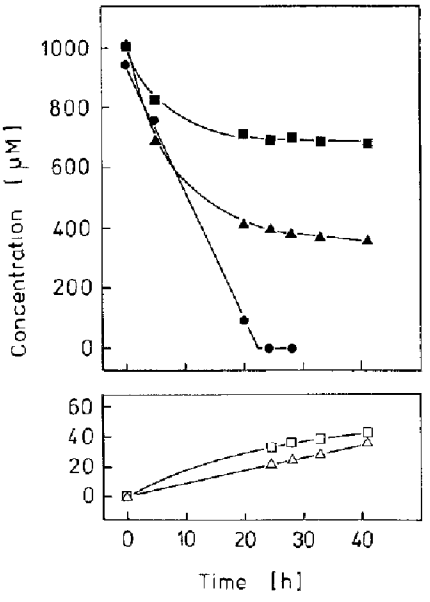

Fig. 4. Degradation of 3-aminobenzoate in dense cell suspensions of strain mAB1 under sulfate limitation. Degradation of 3-aminobenzoate with 10 mM sulfate (●), with 1 mM sulfate (▲), and without added sulfate (■). Benzoate accumulation in the assays without sulfate (□) and with 1 mM sulfate (△)

Experiments with dense cell suspensions of strain mAB1

Degradation of 3-aminobenzoate in dense cell suspensions was significantly slower in sulfate-limited assays than in control assays with 10 mM sulfate (Fig. 4). Addition of 1 mM sulfate caused fast degradation at the beginning, but after 20 h the substrate consumption was as slow as in the sulfate-free assay. Under sulfate limitation, benzoate accumulated during 3-aminobenzoate degradation; without additional sulfate, 42 µM benzoate accumulated within 41 h, and with 1 mM sulfate at the beginning, 36 µM benzoate was found. Cyanide, an inhibitor of carbon monoxide dehydrogenase, in the range of 0.2 to 0.8 mM decreased the degradation rate in dense cell suspensions significantly, and about 25 µM benzoate appeared in the supernatant (data not shown).

Experiments with cell-free extracts of strain mAB1

Cell-free extracts catalyzed a stoichiometric turnover of 3-aminobenzoate to 3-aminobenzoyl-CoA in the presence of ATP, coenzyme A, and Mg^{2+} (Fig. 5).

The measurable specific activity of 3-aminobenzoyl-CoA synthetase was identical with 50 µM to 1 mM 3-aminobenzoate added (12 nmol per min and mg protein) indicating that the K_m-value for 3-aminobenzoate was considerably lower than 50 µm. 3-Hydroxybenzoate was activated by extracts of 3-aminobenzoate-grown cells at higher activity (31.7 nmol per min and mg protein) than 3-aminobenzoate. Low activity of acyl-CoA synthetase was measured with benzoate (2.3 nmol per min and mg protein), and no activity with 2-aminobenzoate and 4-aminobenzoate.

After approximately 1 mM 3-aminobenzoyl-CoA was produced by cell-free extracts of 3-aminobenzoate grown cells, various electron donors (NADPH, NADH, titanium citrate, titanium nitrilotriacetate, cobalt sepulchrate, dithionite-reduced methyl viologen, benzyl viologen, anthraquinone-2,6-disulfonate and safranin T) were added.

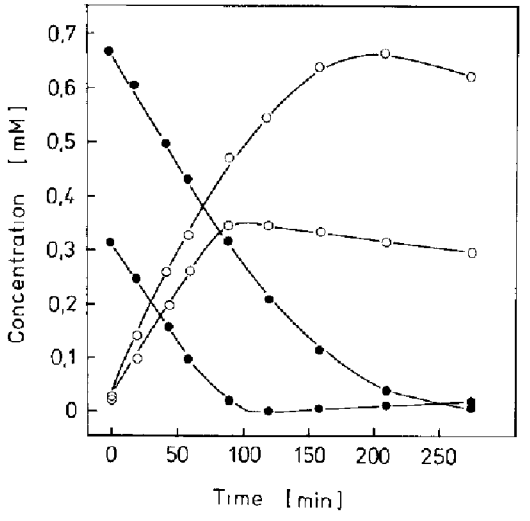

Fig. 5. Activation of 3-aminobenzoate (●) to 3-aminobenzoyl-CoA (○) by cell-free extract of strain mAB1. Two sets of data with two different 3-aminobenzoate concentrations are presented

Within 2 h of incubation, a so far unidentified product was formed with all electron donors applied, but not in 3-aminobenzoate-free control assays. The highest amounts of this product were observed with NADPH or NADH, and 75 µM resp. 37 µM 3-aminobenzoate CoA was consumed at the same time, respectively. The retention time of the product was similar (t_R = 8.24 min) to but not identical with benzoyl-CoA (t_R = 7.81 min); co-elution of commercial benzoyl-CoA and the unidentified product clearly showed two different peaks. The UV-absorption spectrum of the unidentified compound showed a peak at 260 nm and a high extinction below 220 nm, not much different from benzoyl-CoA or other acyl-CoA esters.

The pathway of acetate oxidation was studied by enzyme measurements in cell-free extracts of strain mAB1. Benzoate-grown cells showed CO-dehydrogenase activity of 10.3 µmol per min and mg protein. Also a low 2-oxoglutarate methylviologen oxidoreductase activity of 0.43 µmol per min and mg protein was measured. The same activity was determined with pyruvate as substrate.

Discussion

Anaerobic degradation of 3-aminobenzoate

The present study documents for the first time that 3-aminobenzoate, although not known to be a natural product, can be degraded in the absence of molecular oxygen. Three different types of bacteria were found to decompose 3-aminobenzoate, a sulfate-reducing bacterium in pure culture, a methanogenic and a nitrate-reducing enrichment culture.

The sulfate reducer, strain mAB1, oxidized 3-aminobenzoate completely to CO_2 with concomitant reduction of sulfate to sulfide, and release of ammonium. High activity of CO-dehydrogenase indicated that acetyl-CoA is oxidized via the CO-dehydrogenase pathway (Schauder et al. 1986) although low 2-oxoglutarate synthase activity was found as well. However, a similar activity was found with pyruvate as substrate, and perhaps both synthase activities can be attributed to the same enzyme that is needed in the assimilatory metabolism. CO-dehydrogenase and pyruvate synthase are probably key enzymes as well during autotrophic growth with hydrogen and sulfate of this bacterium.

3-Aminobenzoate degradation is an exergonic process: Complete oxidation with sulfate according to Eq. (1) yields −186 kJ per mol. Also fermentative degradation appears to be possible:

$$C_7H_6O_2N^- + 6\,H_2O \rightarrow 3\,C_2H_3O_2^- + NH_4^+ + CO_2 + H^+ + 2\,H_2$$

$$\Delta G_0' = -30\ \text{kJ per mol 3-aminobenzoate}$$

Release of this small amount of free energy could allow growth of a fermenting bacterium in pure culture, but such a bacterium could not be isolated. Rather, we observed fermentative degradation of 3-aminobenzoate in a methanogenic mixed culture consisting of three

different types of bacteria: a 3-aminobenzoate-fermenting bacterium (short rod), a H_2-degrading *Methanospirillum*-like bacterium, and an acetate-degrading *Methanothrix*-like bacterium. The energy yield of 3-aminobenzoate degradation to CO_2, CH_4 and NH_4^+ (Eq. (2)) is -144 kJ per mol 3-aminobenzoate.

Initial reactions in 3-aminobenzoate degradation by strain mAB1

The first step in 3-aminobenzoate degradation by strain mAB1 was found to be an activation to 3-aminobenzoyl-CoA. Further reduction of 3-aminobenzoyl-CoA did not yield benzoyl-CoA but a different product which is tentatively described as a reduced CoA-ester.

Activation of 3-aminobenzoate to 3-aminobenzoyl-CoA by cell extracts depended on the presence of CoASH, ATP, and Mg^{2+}, and was detected at an activity equivalent to 54% of the calculated in vivo activity. 3-Aminobenzoate-grown cells activated also 3-hydroxybenzoate at high activity, benzoate at low activity, but not 2-aminobenzoate or 4-aminobenzoate. Acyl-CoA synthetase reactions were identified as initial step in degradation of benzoate by *Rhodopseudomonas palustris* (Harwood and Gibson 1986), of 2-aminobenzoate by a denitrifying *Pseudomonas* sp. (Ziegler et al. 1987) and by sulfate-reducing and fermenting bacteria (Schnell 1991), of 4-hydroxybenzoate by denitrifying bacteria (Merkel et al. 1989; Glöckler et al. 1988) and of 4-aminobenzoate by *Desulfobacterium anilini* (Schnell und Schink 1991).

The further metabolism of 3-aminobenzoyl-CoA is still enigmatic. Excretion of benzoate during 3-aminobenzoate degradation by dense cell suspension of strain mAB1 in the presence of cyanide or in the absence of sulfate indicates that a 3-aminobenzoyl derivative is reductively converted to a benzoyl compound. However, reduction of 3-aminobenzoyl-CoA with NADH or NADPH by cell-free extracts of strain mAB1 led to accumulation of a not yet identified reduced product, which was definitively different from benzoyl-CoA. Whether this compound or benzoyl-CoA is the physiological intermediate of 3-aminobenzoate degration in non-inhibited intact cells has still to be elucidated. Other substituted benzoates such as 4-hydroxybenzoate or 4-aminobenzoate are reduced in the CoA-activated form to benzoyl-CoA (Glöckler et al. 1989; Schnell and Schink 1991). With 3-hydroxybenzoate and 2-aminobenzoate, an activation to the CoA-derivative was shown (Heising et al. 1991; Ziegler et al. 1987 and 1989), but no reductive elimination of the substituents was observed so far in cell-free extracts.

Taxonomy of strain mAB1

Strain mAB1 is the only known strictly anaerobic bacterium in pure culture growing with 3-aminobenzoate as sole carbon and energy source. 3-Aminobenzoate is oxidized completely to CO_2. Acetyl-CoA residues are probably oxidized through the carbon monoxide dehydrogenase pathway (Schauder et al. 1986). Strain mAB1 is able to grow autotrophically with H_2, CO_2, and SO_4^{2-}, or heterotrophically with some aliphatic and aromatic substrates; sulfate or sulfite serves as electron acceptor. Cells are rod-shaped (0.5 by 1–2 μm), Gram negative, non-sporing and non-motile. They contain cytochromes and sulfite reductase P582. The G + C content of the DNA is 52.6 mol%.

These properties allow the affiliation of strain mAB1 with the genus *Desulfobacterium*. Some of the described *Desulfobacterium* species are able to degrade aromatic compounds, but none of them can grow with 3-aminobenzoate. Strain mAB1 differs also from all described *Desulfobacterium* species in its growth physiology. Therefore, an appropriate classification of strain mAB1 would require the establishment of a new species.

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