Reductive dechlorination of 3-chlorobenzoate is coupled to ATP production and growth in an anaerobic bacterium, strain DCB-1

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Abstract. Thermodynamic data that the reductive dechlorination of 3-chlorobenzoate is exergonic have led to the hypothesis that this reaction yields biologically useful energy. This hypothesis was tested with strain DCB-1, a dehalogenating bacterium. The organism was grown under strictly anaerobic conditions in vitamin-amended mineral medium with formate plus acetate as electron donor and 3-chlorobenzoate as electron acceptor. The cell yield increased stoichiometrically to the amount of 3-chlorobenzoate dechlorinated. No growth was observed in the absence of 3-chlorobenzoate, or when 3-chlorobenzoate was replaced by benzoate. To obtain further evidence on that energy is derived from dechlorination, 3-chlorobenzoate was added to starved cells. This amendment resulted in an increase in the ATP level of the cells at 10 nmol per mg protein versus 3 nmol per mg protein in non-amended controls. These data indicate that the reductive dehalogenation of chlorinated aromatic compounds can be coupled to a novel type of chemotrophy.

Key words: Reductive dehalogenation — Chemotrophy — Energy conservation — Anaerobic respiration

The first step in the microbially mediated anaerobic degradation of chlorinated aromatic compounds like (chloro)benzoates, -phenols, and -benzenes is a reductive dechlorination (Suflita et al. 1982; Tiedje et al. 1986; Quensen et al. 1988; Mikesell and Boyd 1986). Reductive dehalogenation generally makes these compounds less toxic and more amendable for further biodegradation (Bedard et al. 1987; Bosma et al. 1988). 3-Chlorobenzoate was the first chlorinated aromatic compound shown to be anaerobically degraded via a reductive dehalogenation step (Suflita et al. 1982), and its biodegradation has been studied extensively (Horowitz et al. 1985; Suflita et al. 1983; Tiedje and Stevens 1988; Shilton and Tiedje 1984). Recently thermodynamic data have been presented which show that the reductive dechlorination of 3-chlorobenzoate to benzoate is an exergonic reaction (Dolfing and Tiedje 1987). This has led to the hypothesis that reductive elimination of chlorine from 3-chlorobenzoate yields biologically useful energy (Dolfing and Tiedje 1987). Experimental results obtained with a defined 3-chlorobenzoate degrading methanogenic microbial consortium (Dolfing and Tiedje 1986) were indeed consistent with this hypothesis (Dolfing and Tiedje 1987), but since these results had been obtained with a mixed culture they did not provide unequivocal proof. To prove the hypothesis that bacteria can exploit reductive dechlorination as an energy generating reaction, it is necessary to grow them with a chlorinated aromatic compound as electron acceptor in pure culture under defined conditions. The only anaerobic bacterium available in pure culture that is able to reductively dechlorinate aromatic compounds is strain DCB-1 (Shelton and Tiedje 1984). This organism has been isolated from a 3-chlorobenzoate degrading methanogenic enrichment culture, with pyruvate as its energy source. In fact even growth with pyruvate in a defined basal salts medium has long been elusive (Stevens et al. 1988). Recently, however, it was reported that the addition of three vitamins, viz. nicotinamide, 1,4-naphthoquinone and thiamine, greatly stimulated growth of strain DCB-1 on pyruvate [DeWeerd KA and Suflita JM (1988) Nutritional requirements of a dehalogenating anaerobic bacterium, strain DCB-1. Abstr ASM Meeting I-108]. This finding has opened up the possibility of testing the hypothesis that strain DCB-1 is able to obtain energy for growth from the reductive dechlorination of 3-chlorobenzoate. The results presented here indicate that strain DCB-1 indeed conserves energy for growth from this reaction.

Materials and methods

Organism and growth conditions

Strain DCB-1 was a gift from J. M. Tiedje. The organism was grown in the anaerobic basal salts medium described earlier (Shelton and Tiedje 1984), supplemented with nicotinamide (500 µg/l), 1,4-naphthoquinone (200 µg/l) and thiamine (50 µg/l). These amendments were made from a 1000 times concentrated stock solution to which 10 ml/l acetic acid was added to facilitate dissolution of the vitamins. The basal salts medium was further supplemented with 5 mM sodium acetate and 6 mM sodium formate as...
Fig. 1. Growth yield of strain DCB-1 on 3-chlorobenzoate. Data points are from 12 replicative vials, 3 for each individual concentration.

Fig. 2. Effect of the addition of 3-chlorobenzoate on the ATP concentration in energy-starved cells. 3-Chlorobenzoate was added at t = 0 to replicate vials (●, ■), a third vial received an equal amount of benzoate (○).

carbon and electron donors. Incubations were static at 35°C in the dark.

**ATP experiments**

Strain DCB-1 was grown on 3-chlorobenzoate (3.2 mM) plus formate (6 mM) and acetate (5 mM) in replicate cultures. After growth had ceased, samples were taken for protein and (3-chloro)benzoate analysis. Once it had been established that all 3-chlorobenzoate had been dechlorinated to benzoate, the ATP measurements were started. ATP was extracted from the cells by rapidly adding 0.3 ml samples to 5 ml boiling Tris buffer (20 mM; pH = 7.8). After 5 min of boiling, the samples were frozen and stored until analysis. At the time indicated in Fig. 2, 3-chlorobenzoate was added to the cultures to a final concentration of 2 mM and samples were taken for ATP analysis at appropriate intervals. ATP was measured by the luciferin-luciferase method (Kimmich et al. 1975) in an LKB photometer.

**Chemical analyses**

For protein determinations cells were harvested by centrifugation, washed in 4 mM phosphate buffer pH = 7, and analyzed for protein by the method of Lowry as described by Hanson and Phillips (1981).

Benzoate and 3-chlorobenzoate were analyzed by HPLC.

**Results and discussion**

**Growth yield experiments**

In preliminary experiments, strain DCB-1 was able to dechlorinate 3-chlorobenzoate to benzoate when growing in basal salts medium on pyruvate in the presence of the mentioned vitamins (data not shown). Subsequent subcultures of strain DCB-1 in vitamin-amended basal salts medium grew with 3-chlorobenzoate as electron acceptor. In this medium with formate plus acetate as electron donors, 3-chlorobenzoate was stoichiometrically dechlorinated to benzoate: between 85% and 100% of the 3-chlorobenzoate added was recovered as benzoate. The growth yield increased stoichiometrically with the amount of 3-chlorobenzoate dechlorinated. The molar growth yield was 6.6 g protein per mol of 3-chlorobenzoate added (Fig. 1). This growth yield was not affected when formate was omitted from the medium or was replaced by hydrogen (data not shown). No growth was observed when 3-chlorobenzoate was replaced by benzoate. These observations indicate that growth of strain DCB-1 was indeed coupled to the reductive dechlorination of 3-chlorobenzoate. The growth yield reported here is more than twice as high as the apparent growth yield of strain DCB-1 in the defined consortium (Dolfing and Tiedje 1987), which suggests that the cross-feeding with growth factors in the consortium is suboptimal for strain DCB-1. The doubling time of strain DCB-1 when grown in pure culture on the reductive dechlorination of 3-chlorobenzoate (8 to 10 days) was significantly lower than its doubling time in the consortium which was 3 days (Dolfing and Tiedje 1986). Thus the growth conditions for the pure culture seem to be amendable for improvement, too.

Mohn and Tiedje [(1989) Growth yield increase linked to reductive dechlorination by strain DCB-1. Abstracts of the annual meeting of the American Society for Microbiology K-66] recently obtained sluggish growth of strain DCB-1 on dechlorination. These authors showed that in the absence of acetate formate served as electron donor for reductive dechlorination of 3-chlorobenzoate. In the experiments reported here it is not clear whether acetate or formate served as the source of reducing equivalents. The presence of acetate and other differences in the composition of the media used in our independent studies may have caused the higher growth rate and growth yield observed in the present study.

**ATP experiments**

To obtain further evidence that strain DCB-1 derives energy from the reductive dechlorination of 3-chlorobenzoate, the concentration of ATP was followed after the addition of 3-chlorobenzoate to energy-starved cells. The concentration of ATP in benzoate-amended starved cells remained low and stable over the 2 h of the experiment, while the addition of 3-chlorobenzoate resulted in a rapid increase in the ATP level in the cells from 3 nmol per mg protein to 9 nmol per mg protein (Fig. 2).

**Conclusions**

Together these results confirm the hypothesis that strain DCB-1 is able to obtain energy for growth from the reductive dechlorination of 3-chlorobenzoate, and extend the known