Relationship between the Production of Spirosomes and Anaerobic Glycolysis Activity in *Escherichia coli* B

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The effects of culture conditions (aerobic or anaerobic) and glucose in the medium on the production of spiroosomes in *Escherichia coli* B were studied by SDS-PAGE and electron microscopy. The M_r of the spiroosome of *E. coli* B was estimated to be 97 000. Electron microscopy revealed that the amount of spiroosomes derived from anaerobic cultures was about eightfold larger than that from aerobic cultures. In SDS-PAGE, the bands of spiroosome protein derived from anaerobic cultures were more intense than those derived from aerobic cultures, either in peptone water or in Davis–Mingioli's minimal medium. With increased glucose concentration under aerobic conditions, the intensity of the band of spiroosome protein was similar to that observed under anaerobic conditions in basal media. These results suggest that spiroosome production by *E. coli* B is related to its anaerobic glycolysis activity.

INTRODUCTION

Holt & Canale-Parola (1968) observed an array of tightly packed longitudinally arranged helices below the outer cell sheath and axial filament in *Spirochaeta stenostrepta*. They also found that ballistic disintegration loosened the helices so that they separated from the cells. Similar helical structures have since been observed in various bacteria – Gram-positive and Gram-negative, cocci and bacilli, facultative and obligate anaerobes, including *Acholeplasma* (Ueda & Takagi, 1972; Kawata *et al.*, 1975, 1976, 1979; Kawata, 1984; Kessel *et al.*, 1981; Nieves *et al.*, 1981; Ueki *et al.*, 1982; Matayoshi & Oda, 1985); these helices were termed spiroosomes by Kawata *et al.* (1975). Studies on the fine structure, chemical components and physicochemical properties of the spiroosomes have shown that the basic helix of spiroosomes is left-handed and consists of a single protein with an apparent M_r of around 95 000 (Kawata *et al.*, 1975, 1976, 1979; Kawata, 1984; Kessel *et al.*, 1981; Ueki *et al.*, 1982; Matayoshi & Oda, 1985). Although there has been some speculation on the physiological function of spiroosomes, including suggestions that their function is related to flexibility and motility in *Spirochaeta* (Holt & Canale-Parola, 1968), to a cytoskeletal role in *Acholeplasma* (Kessel *et al.*, 1981) and to a contractile protein (Kawata *et al.*, 1982), their true function is currently uncertain. We considered that the function of spiroosomes might be related to anaerobic metabolism because no obligately aerobic bacteria have been reported to possess spiroosomes.

In this report we describe the relationship between spiroosomes and anaerobic glycolysis activity in *Escherichia coli* B.

METHODS

Organism, media and growth conditions. *E. coli* B was grown aerobically for 16 h in each basal liquid medium. Samples of the cell suspensions were stored at -80°C and used as inocula for all experiments. Peptone water, pH 7.2, and Davis–Mingioli's minimal medium (DM broth), pH 7.3 (7 g K_2HPO_4 , 2 g KH_2PO_4 , 0.1 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1 g $(\text{NH}_4)_2\text{SO}_4$, 0.5 g trisodium citrate $\cdot 2\text{H}_2\text{O}$, and 2 g glucose per litre of distilled water) were prepared as the basal media. For aerobic cultivation a reciprocal shaker was used at 160 r.p.m., and anaerobic cultivation was carried out in a jar under an O_2 -free gas mixture of 90% N_2 and 10% CO_2 . The cells were cultivated for 16 h or 40 h at 37°C .

Partial purification of spiroosomes. The purification procedure was as described by Matayoshi & Oda (1985). The organism was grown anaerobically at 37 °C in 200 ml peptone water containing 1% (w/v) glucose for 5 h, by which time exponential growth had commenced. The cells were washed twice with phosphate-buffered saline (PBS, pH 7.4) by centrifugation (1500 g, 20 min) and the pellets were resuspended in 60 ml PBS. The suspension was sonicated at 35 W for 3 s with a Branson model B-12 sonifier and then centrifuged at 1500 g for 30 min to remove the bulk of the cells. The supernatant was further centrifuged at 40000 g for 1 h to remove the remaining cells and membrane fragments. The resultant supernatant was centrifuged at 160000 g for 2 h to obtain a spiroosome-rich fraction. For the determination of the M_r , the spiroosome preparation was solubilized in 0.05 M-Tris buffer (pH 7.0), 2% (w/v) SDS, 5% (v/v) 2-mercaptoethanol, 0.005% (w/v) bromophenol blue, and 6% (w/v) sucrose at 100 °C for 5 min. The dissociated polypeptides were analysed by discontinuous SDS-PAGE according to the method of Spear and Roizman (1972), which is based on that described by Laemmli (1970). After electrophoresis, the gel was fixed and stained with 0.04% (w/v) Coomassie brilliant blue R250 in 25% (v/v) 2-propanol with 10% (v/v) acetic acid, and destained in 10% (v/v) 2-propanol with 10% (v/v) acetic acid. The M_r of the spiroosome was determined as described (Matayoshi & Oda, 1985).

Quantification of spiroosomes by electron microscopy. Cell suspensions derived from aerobic or anaerobic cultivation, with or without glucose, were prepared at a fixed volume and cell concentration (usually 5×10^8 cells ml^{-1}) and sonicated at 35 W for 3 s. These samples were negatively stained with 1% (w/v) uranyl acetate and photographed in a Hitachi H-300 electron microscope at an accelerating voltage of 75 kV. Eight $1 \times 1 \mu\text{m}$ areas of the specimens were chosen at random and the total length of the spiroosomes was determined. These observations were carried out in three separate experiments.

Quantification of spiroosome production by SDS-PAGE. Cells were cultivated under aerobic and anaerobic conditions with various concentrations of glucose. A 50 ml volume of each basal medium was inoculated with a loopful of subcultured cell suspension and incubated under aerobic (shaking) or anaerobic conditions for 16 or 48 h at 37 °C. The cells were harvested and washed twice with PBS as above, and then washed once with distilled water. The pellets were suspended in distilled water (4–6 ml) and sonicated three times at 60 W for 9 min at 4 °C with a Branson model B-12 sonifier. The cells were disrupted completely by this sonication and the suspensions were lyophilized immediately. Each sample was solubilized in SDS-PAGE sample buffer at a concentration of 25 mg ml^{-1} at 100 °C for 5 min and 4 μl of each sample solution was subjected to electrophoresis as described above. These experiments were carried out in duplicate.

RESULTS AND DISCUSSION

Spiroosomes of E. coli B

An electron micrograph of negatively stained spiroosomes of *E. coli B* is shown in Fig. 1 (a). The length of the spiroosomes ranged from 39 to 235 nm (mean $\pm$ SD, 123 ± 45 nm; $n = 50$). The width of the helices ranged from 13.1 to 18.3 nm (mean 15.4 ± 1.2 nm; $n = 50$). The spiroosomes of *E. coli B* were slightly larger than spiroosomes of other anaerobes (Matayoshi & Oda, 1985). In the partially purified fraction, aggregated and loosened spiroosomes were observed (Fig. 1 b). The spiroosomes of *E. coli B* were much easier to loosen during the purification procedure than were those of the obligately anaerobic bacteria which we studied previously (Matayoshi & Oda, 1985). The electrophoretic pattern of the spiroosome fraction in SDS-PAGE showed one major polypeptide band and a few minor bands. The M_r of the spiroosome protein was estimated to be about 97000 (Fig. 2). This M_r is slightly larger than those reported for other species, i.e. *Peptostreptococcus productus* and *Eubacterium aerofaciens* (94000; Matayoshi & Oda, 1985), *Lactobacillus brevis* (95000; Ueki *et al.*, 1982) and *Acholeplasma laidlawii* (95000; Kawata *et al.*, 1986; or 100000; Kessel *et al.*, 1981).

Comparison of spiroosome production in aerobic and anaerobic cultures by electron microscopy

Cell suspensions derived from aerobic and anaerobic cultures were gently sonicated (see Methods) and examined by electron microscopy. There was greater spiroosome production in the samples obtained from anaerobic cultures than in those of the aerobic cultures, in both peptone water and DM broth containing 1% (w/v) glucose (Table 1). The amount of spiroosomes was smallest in the sample derived from aerobic culture in peptone water. These results were confirmed in the following SDS-PAGE experiments.

Fig. 1. Electron micrographs of (a) negatively stained spiroosomes of *E. coli* B before purification, and (b) a partially purified spiroosome fraction of *E. coli* B obtained by differential centrifugation. Bars, 100 nm.

Table 1. *Effect of culture conditions on the production of spiroosomes assayed by electron microscopy*

Medium	Culture conditions	Glucose (%)	Total length of spiroosomes (nm μm^{-2})*
Peptone water	Aerobic	0	77 $\pm$ 67
Peptone water	Aerobic	1	377 $\pm$ 205
Peptone water	Anaerobic	1	822 $\pm$ 148
DM broth	Aerobic	1	90 $\pm$ 78
DM broth	Anaerobic	1	702 $\pm$ 227

* Data are the means of eight replicates $\pm$ SD.

Comparison of the production of spiroosomes in aerobic and anaerobic cultures by SDS-PAGE

Fig. 3 shows the SDS-PAGE profiles of *E. coli* B whole-cell lysates derived from aerobic and anaerobic cultures in peptone water and DM broth, both containing 1% glucose. The M_r 97 000 (97K) spiroosome bands of the cells from anaerobic cultures were clearly more intense than those of aerobically cultured cells. When the glucose concentration was reduced from 1% to 0.2% in the aerobic culture medium, the intensity of the band decreased further (data not shown). The 97K band of *E. coli* B from a standing culture was also more intense than that of the aerobic culture (data not shown).

Some other monosaccharides (arabinose, mannose, mannitol and galactose) were used as a carbon and energy source in place of glucose. In all cases, the intensity of the 97K spiroosome bands was higher in samples from the anaerobic culture than in those from the aerobic culture. If glucose is present at high concentration, facultative anaerobes initially carry out fermentation under aerobic conditions (Hempfling, 1970). To confirm the relationship between fermentation and the amount of 97K spiroosome protein, the glucose concentration was increased to 4% in DM broth, the cultures were incubated aerobically for 16 h at 37 °C, and the amount of spiroosome protein was compared with that obtained in normal DM broth (glucose 0.2%). The 97K band was more intense in the samples from cells cultured in DM broth containing the higher concentration of glucose (Fig. 4).

Fig. 2. SDS-PAGE of a spiroosome preparation of *E. coli* B. Lane 1, partially purified spiroosome fraction; lane 2, whole-cell preparation completely disrupted by sonication in distilled water. The M_r of the spiroosomes was determined as 97000 (arrow) by using two electrophoresis calibration kits (M_r 14000–94000 and 45000–200000; Pharmacia).

Fig. 3. Effect of culture conditions on the production of spiroosome protein determined by SDS-PAGE. Completely disrupted whole-cell preparations were lyophilized and samples of the same concentration and volume were subjected to SDS-PAGE. Lane 1, cultured aerobically in peptone water. Lane 2, cultured anaerobically in peptone water. Lane 3, cultured aerobically in DM broth. Lane 4, cultured anaerobically in DM broth. Both media contained 1% (w/v) glucose. The spiroosome bands are indicated by arrows.

Fig. 4. Effect of glucose concentration on the production of spiroosome protein determined by SDS-PAGE. *E. coli* B was grown aerobically for 16 h in DM broth containing 4% (lane 1) or 0.2% (lane 2) glucose. The arrow indicates the spiroosome bands.

All these observations indicate that the production of spiroosomes by *E. coli* B is related to anaerobic glycolysis. Therefore we are now investigating the possibility that the spiroosome protein is an inducible enzyme related to glycolysis.

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