Effect of ascorbate on oxygen uptake and growth of *Escherichia coli* B

HOLLY E. RICHTER, JACEK SWITALA, and PETER C. LOEWEN[1]

Department of Microbiology, University of Manitoba, Winnipeg, MB., Canada R3T 2N2

Received November 17, 1988

Accepted January 26, 1988

RICHTER, H. E., SWITALA, J., and LOEWEN, P. C. 1988. Effect of ascorbate on oxygen uptake and growth of *Escherichia coli* B. Can. J. Microbiol. **34**: 822–825.

The addition of ascorbate to aerobically growing cultures of *Escherichia coli* B caused only a short pause in growth and no subsequent change in the rate or extent of growth. The effect of ascorbate on oxygen uptake varied from inhibition in minimal medium to stimulation in rich medium. Cyanide-resistant growth and oxygen uptake were stimulated by ascorbate. Both the rate and extent of anaerobic growth were stimulated in proportion to the amount of ascorbate added when fumarate was the terminal electron acceptor. Ascorbate had no effect on any aspect of anaerobic growth in the absence of a terminal electron acceptor or in the presence of nitrate.

RICHTER, H. E., SWITALA, J., et LOEWEN, P. C. 1988. Effect of ascorbate on oxygen uptake and growth of *Escherichia coli* B. Can. J. Microbiol. **34** : 822–825.

L'addition d'ascorbate à des cultures aérobiques d'*Escherichia coli* B s'est traduite par une courte pause de la croissance, dans en altérer subséquemment le taux et l'étendue. Les offets de l'ascorbate sur l'absorption de l'oxygène ont varié depuis une inhibition sur un milieu minimal à une stimulation sur un milieu enrichi. L'ascorbate a stimulé la résistance de la croissance en présence de cyanure ainsi que l'absorption de l'oxygène. Dans les cultures anaérobiques, le taux et l'étendue de la croissance ont été l'un et l'autre stimulés en proportion de la quantité d'ascorbate ajoutée, lorsque du fumarate était un accepteur terminal d'électrons. L'ascorbate n'a eu aucun effect sur la croissance anaérobique en l'absence de cet accepteur terminal d'électrons, pas plus d'ailleurs qu'en présence de nitrate.

[Traduit par la revue]

Ascorbate is a molecule with a very diverse chemistry and an even more diverse biochemistry. Its principal reaction pathway involves oxidation to dehydroascorbate via an intermediate ascorbate free radical resulting from the loss of one proton and one electron. It is involved in many enzymatic reactions, usually as an electron donor (Lewin 1976) and has been shown to cause DNA damage in viruses (Bissel et al. 1980) and bacteriophage (Murata and Kitagawa 1973; Richter and Loewen 1982*b*). Despite the prevalence of bacteria in the human gut and the current practice of ingesting large quantities of ascorbate, little attention has been given to the effect of ascorbate on bacteria. Ascorbate was found to induce a five- to seven-fold increase in catalase HPI levels in *Escherichia coli* involving hydrogen peroxide formed during ascorbate oxidation (Richter and Loewen 1981) and it reversibly inhibited glucose uptake by the glucose phosphotransferase system but not the mannose phosphotransferase system (Loewen and Richter 1983). Ascorbate was not taken up by the cell suggesting that it affected cellular metabolism by interacting with components of the cell membrane. This communication describes an extension of these studies to an investigation of the effect of ascorbate on bacterial respiration and anaerobic growth.

A prototrophic strain of *E. coli* B, B23, was used throughout this study and growth conditions were as described (Richter and Loewen 1981) in LB medium (Miller 1974) and in glycerol–salts or glucose–salts medium (Richter and Loewen 1982*a*) supplemented as indicated in the individual experiments. Oxygen uptake experiments were performed in a Gilson oxygraph fitted with a Clark electrode. Cultures and ascorbate solutions, prepared fresh before each experiment, were made anaerobic by flushing with nitrogen.

The addition of ascorbate to aerobic cultures of *E. coli* caused a short pause in growth but no subsequent change in the rate or extent of growth (Fig. 1). The strong reducing properties of ascorbate suggested that if ascorbate was interacting with membrane components, it might interfere with normal electron flow and thereby affect the growth rate immediately after addition. This was investigated by studying the effect of ascorbate on oxygen uptake. As shown in Table 1, the rate of oxygen uptake by cells grown in glucose–salts medium was inhibited by ascorbate, but the presence of amino acids reduced the inhibitory effect to an extent that in rich LB medium there was a slight enhancement of oxygen uptake. These ascorbate effects, whether inhibition or enhancement, were increased by adding ascorbate to the growth medium prior to isolating the cells for oxygen uptake determinations. In all of these experiments, it was necessary to have EDTA present in the uptake medium to chelate metal

[1]Author to whom all correspondence should be addressed.

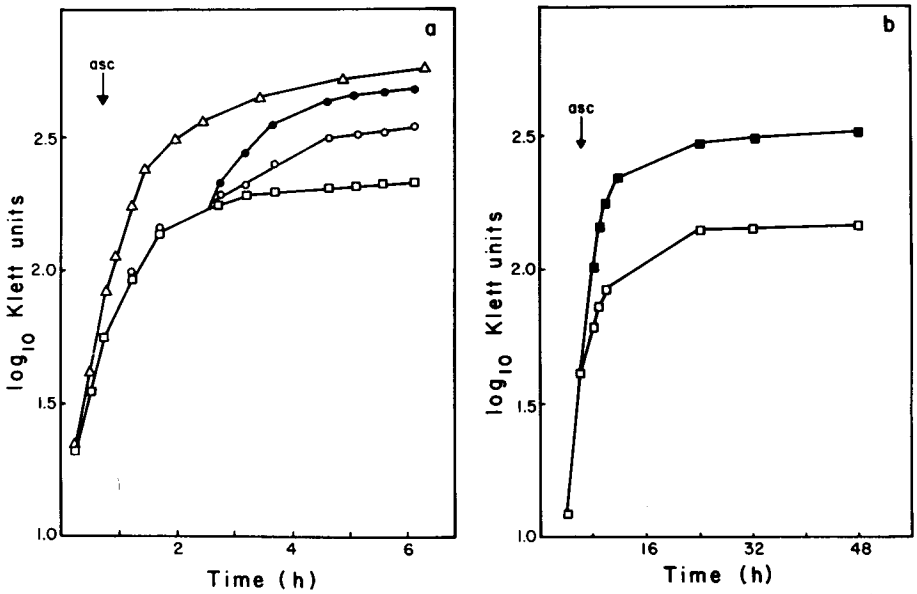

FIG. 3. Effect of ascorbate on anaerobic growth of *E. coli* B in (*a*) LB medium and (*b*) glycerol-salts-fumarate medium. (*a*) Cultures were grown aerobically (△) and anaerobically (□) with no supplements. Anaerobic cultures were also supplemented with 2.8 (○) and 5.7 mM (●) ascorbate at the time indicated by the arrow. (*b*) Anaerobic cultures were grown in glycerol-salts-fumarate medium containing 20 mM glycerol and 20 mM fumarate and supplemented with 0 (□) or 2.8 mM (■) ascorbate at the time indicated by the arrow. Fumarate could be replaced with 20 mM aspartate without significantly changing the growth curves.

Because bacteria in the human gut live in a largely anaerobic environment, the effect of ascorbate on anaerobic growth of *E. coli* was investigated. In anaerobic LB medium, ascorbate had no significant effect on the rate of growth until the primary carbon source, presumably glucose, was depleted. Then there was a second burst of growth, the rate and extent of which was proportional to the amount of ascorbate added (Fig. 3*a*). LB medium is rich in amino acids including aspartate which can be nonoxidatively deaminated to fumarate for use as a terminal electron acceptor by the anaerobically induced fumarate reductase. To confirm that ascorbate could donate electrons to the fumarate reductase system, ascorbate was added to an anaerobic culture growing in glycerol-salts medium supplemented with fumarate resulting in an enhancement of both the rate and extent of growth (Fig. 3*b*). A mixture of fumarate and nitrate or aspartate alone as terminal electron acceptor supported a similar response to ascorbate, but nitrate alone did not support ascorbate enhancement of growth. No catalase was induced in cells growing anaerobically in LB medium or glycerol-salts-fumarate medium confirming that the culture was anaerobic and that hydroperoxides capable of inducing HPI were not being produced.

In summary, ascorbate could influence bacterial metabolism despite not being transported into the cell. Electrons from the ascorbate were transferred directly to membrane components and either enhanced or inhibited oxygen uptake depending on the growth medium. This effect was not sufficiently strong to visibly affect the rapid aerobic growth of cells in rich medium except for a brief pause after which the cells had adapted to the presence of ascorbate. However, when growth was significantly slower, as when cyanide was present or under anaerobic conditions, the growth rate was visibly enhanced. The influence of ascorbate on anaerobic growth was quite specific in requiring an active fumarate reductase system which could not be replaced by the nitrate reductase complex. Because bacteria grow predominantly in an anaerobic environment in a relatively rich medium in the human gut, it is very likely that large quantities of ascorbate could enhance the growth of bacteria in their natural environment.

Acknowledgements

This work was supported by grant A9600 and a postgraduate scholarship to H. E. R. from the Natural Sciences and Engineering Research Council.

BISSEL, M., HATIE, C., FARSON, D., SCHWARZ, R., and SOO, W. J. 1980. Ascorbic acid inhibits replication and infectivity of avian RNA tumor virus. Proc. Natl. Acad. Sci. U.S.A. **77**: 2711–2715.

INGLEDEW, W. J., and POOLE, R. K. 1984. The respiratory chains of *Escherichia coli*. Microbiol. Rev. **48**: 222–271.

LEWIN, S. 1976. Vitamin C: its molecular biology and medical potential. Academic Press, London.

LOEWEN, P. C., and RICHTER, H. E. 1983. Inhibition of sugar uptake by ascorbic acid in *Escherichia coli*. Arch. Biochem. Biophys. **226**: 657–665.

MILLER, J. H. 1974. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

MURATA, A., and KITAGAWA, K. 1973. Mechanism of inactivation of bacteriophage J1 by ascorbic acid. Agric. Biol. Chem. **37**: 1145–1151.

RICHTER, H. E., and LOEWEN, P. C. 1981. Induction of catalase in *Escherichia coli* by ascorbic acid involves hydrogen peroxide. Biochem. Biophys. Res. Commun. **100**: 1039–1046.

RICHTER, H. E., and LOEWEN, P. C. 1982*a*. Catalase synthesis in *Escherichia coli* is not controlled by catabolite repression. Arch. Biochem. Biophys. **215**: 72–77.

———1982*b*. Rapid inactivation of bacteriophage T7 by ascorbic acid is repairable. Biochem. Biophys. Acta, **697**: 25–30.

TABLE 1. Effect of ascorbate on oxygen uptake by *Escherichia coli* B23 grown in various media

Growth medium[a]	Oxygen uptake (nmol/min per mg dry cell weight) ±EDTA[b]	EDTA + ascorbate	NaCN	NaCN + ascorbate
Glucose–salts	207	158	12.5	41.3
Glucose–salts–ascorbate	100	5.4	20.4	50.4
Glucose - salts - amino acids	210	194	7.5	31.7
Glucose - salts - ascorbate - amino acids	166	137	6.3	31.7
LB	997	1260	2.1	91.1
LB–ascorbate	1010	1760	3.2	71.2

[a]Cells were incubated with 2.8 mM ascorbate for 15 min in growth medium where specified, collected by centrifugation, washed two times in fresh medium, and resuspended in the same medium at the same cell density (approximately 70 Klett units) for the assay of oxygen uptake. Casamino acids were present, where indicated, at a concentration of 0.1% (w/v).
[b]EDTA was 1 mM.

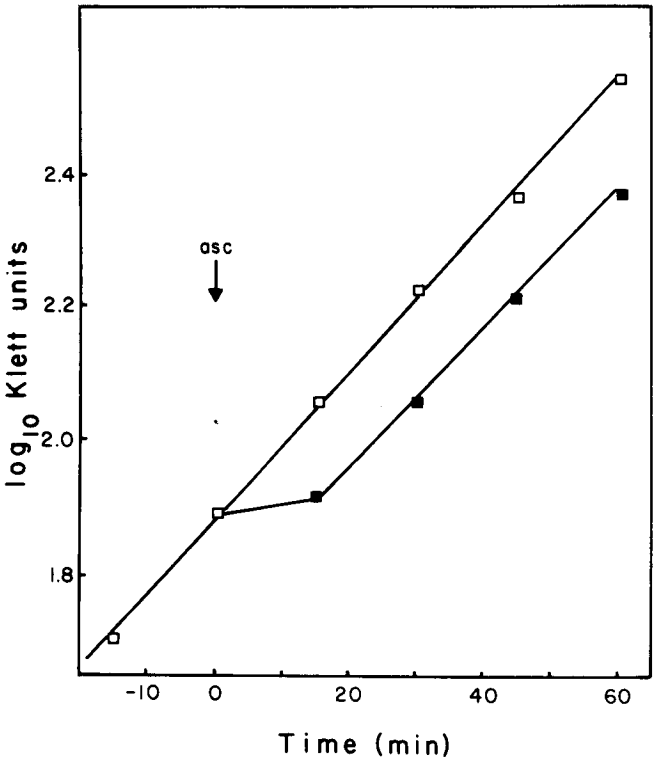

FIG. 1. Effect of ascorbate (asc) on the growth of *E.coli* B in LB medium. Ascorbate was added to a concentration of 5.7 mM at time 0 (arrow) and growth (■) was monitored; growth was also monitored for a culture to which buffer with no ascorbate was added (□).

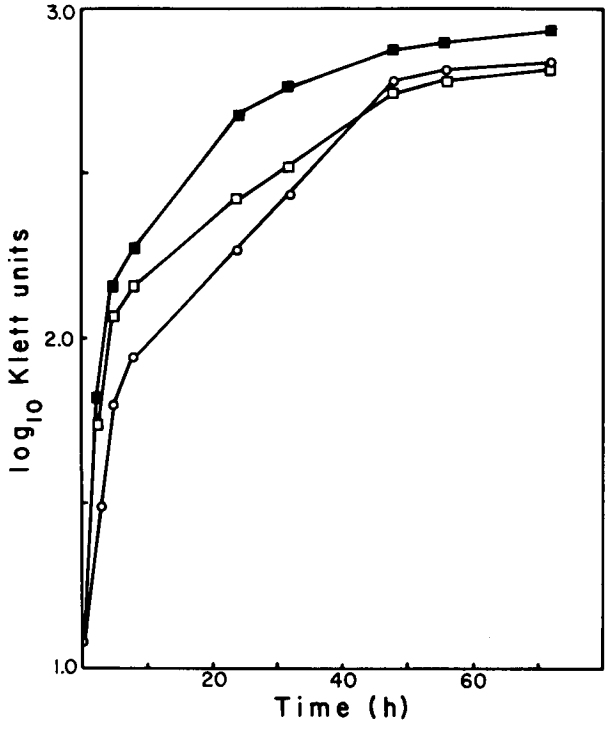

FIG. 2. Effect of ascorbate on cyanide-resistant growth of *E. coli* B in LB medium. Cultures containing 1.25 mM NaCN were supplemented with 0 (○), 2 (□), and 4mM (■) ascorbate and growth was monitored.

ions that catalyze the rapid oxidation of ascorbate giving the appearance of oxygen uptake by the cells. EDTA alone did not affect oxygen uptake, but ascorbate with EDTA did affect oxygen uptake by cells (Table 1) indicating that electron flow was being affected by the ascorbate. Other possible explanations for the pause in growth (Fig.1) include the transient stress of coming in contact with the ascorbate–H_2O_2 solution requiring adaptation through catalase synthesis (Richter and Loewen 1981) and the transient inhibition of glucose uptake (Loewen and Richter 1983). However, it was clear that despite these several mechanisms for ascorbate affecting cellular metabolism, ascorbate did not have a significant effect on rapid aerobic growth once the culture had adapted to its presence.

Escherichia coli has two main pathways for aerobic electron transport distinguishable by the terminal cytochrome oxidases, cytochrome *o* and cytochrome *d*, of which cytochrome *d* has a higher K_i for cyanide ion (Ingeldew and Poole 1984). When ascorbate was added to cells respiring in the presence of 1.25 mM NaCN, oxygen uptake was increased even in glucose–salts medium (Table 1). This suggested that electrons transferred into the cytochrome *d* system could stimulate oxygen uptake, but that the effect was not strong enough to be observed above the normal rate of oxygen uptake in the absence of NaCN. Only when cyanide was present in LB medium reducing the rate of growth did ascorbate significantly increase the rate of growth (Fig. 2) but not the extent of growth. It should also be noted here that the cyanide ion was as effective as EDTA in preventing the rapid oxidation of ascorbate in the growth medium.