INDUCTION BY SODIUM OF THE CITRATE FERMENTATION ENZYMES IN *KLEBSIELLA AEROGENES*

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1. Introduction

The anaerobic metabolism of citrate by *Aerobacter indologenes* and *Klebsiella aerogenes* proceeds via a cleavage of citrate to oxalacetate (OAA), catalysed by citrate lyase, followed by decarboxylation of the OAA to pyruvate, catalysed by OAA decarboxylase [1–3]. This is termed the fermentation pathway. The latter enzyme in *K. aerogenes* NCTC418 is dependent on Na^+ for activity [4] and Na^+ is also essential for anaerobic growth on citrate [3]. Aerobic growth of strain NCTC418 on citrate is independent of Na^+ [5] and the metabolism of citrate is effected via the citric acid cycle [5,6].

Under aerated conditions, (defined as those in which no oxygen tension can be detected in the culture even though it is aerated), the presence of Na^+ has a pronounced effect on the growth and metabolism of strain NCTC418. Growth on Na^+-citrate medium is faster but less efficient than growth on K^+-citrate medium. Metabolism of citrate occurs via both the citric acid cycle and the fermentation pathway in the Na^+-citrate cultures whereas metabolism is solely via the citric acid cycle in the K^+-citrate cultures [5].

The above results seem to indicate that Na^+ is essential for the induction of the fermentation enzymes under aerated conditions and it is the purpose of this communication to present evidence to support this hypothesis.

2. Experimental

2.1. *Growth of the organism*

Klebsiella aerogenes NCDO 711 (NCTC418) was grown at 35°C on K^+-citrate medium [7] in a growth vessel fitted with a Clark-type oxygen electrode [8]. Aerated conditions were maintained continuously throughout the whole of the growth period by adjusting the rate of the air flow to maintain a zero oxygen tension [5]. Growth was monitored by measuring the absorbance of the culture at 680 nm and samples of the culture were collected, clarified by centrifugation and were stored at −20°C until analysed for citrate. Samples (50 ml) of the culture were removed at intervals after the addition of NaCl (final concentration, 37 mM) to the growing culture and the cells were harvested by centrifugation at 16 000 *g* for 15 min at 5°C and were stored at −20°C for 16 hr.

2.2. *Preparation of cell extracts and enzyme assays*

The frozen cells were thawed in 2 ml of 0.05 M Tris–HCl buffer, pH 7.0, and extracts were prepared as previously described [7]. Citrate lyase was assayed spectrophotometrically by determining the rate of formation of OAA and pyruvate from citrate by coupling the reaction to the endogenous OAA decarboxylase, and added lactate and malate dehydrogenases. The incubation system contained (in μmoles in a final volume of 1 ml): Tris–HCl buffer, pH 7.0, 100; $MgCl_2$, 10; sodium citrate, 5; NADH, 0.02; lactate and malate dehydrogenases 1 unit of each and cell extract. The enzyme was also assayed by a discontinuous assay procedure [3] as a check on the spectrophotometric assay. OAA decarboxylase activity was measured by determining the rate of formation of pyruvate from OAA in the presence of 20 mM Na^+ by the discontinuous assay procedure of Stern [4]. Substrates, coractors and enzymes used in the assay

procedures were obtained from Sigma Chemical Corp., St. Louis, Mo., USA.

2.3. *Other assay procedures*

Citrate in the spent culture liquour was assayed by the acetic anhydride–pyridine method [9] and protein by the biuret method [10].

3. Results

The growth of *K. aerogenes* on K^+-citrate under aerated conditions is shown in fig.1. Under these conditions growth was logarithmic with a mean generation time of 138 min and a molar growth yield of 39.8 g dry weight of cells per mol of citrate utilised. After 4 hr of growth, NaCl (final concentration 37 mM) was added to the culture. This concentration of Na^+ gives almost maximum stimulation of citrate uptake in strain NCTC418 [11]. One hour after the addition of the Na^+ the logarithmic growth rate increased to

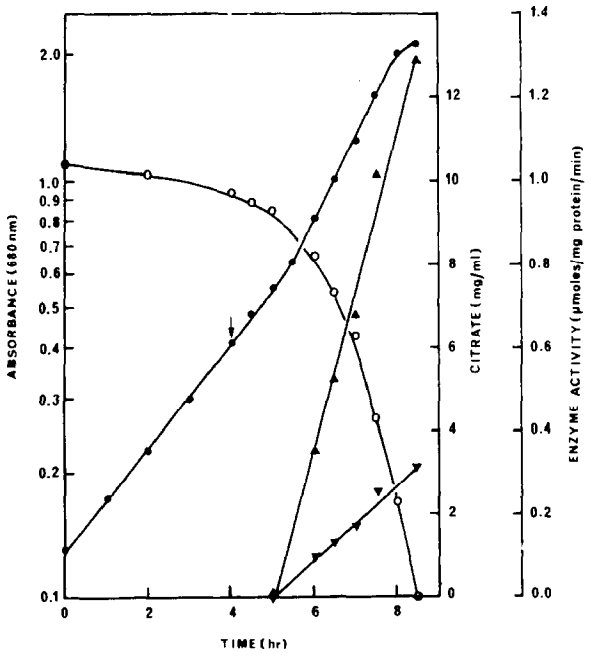

Fig.1. Growth rate (●), citrate utilisation (○), and the induction of citrate lyase (▲), and OAA decarboxylase (▼) in *K. aerogenes* grown under aerated conditions on K^+-citrate, before and after the addition of Na^+. NaCl (final concentration 37 mM) was added at the time indicated by the arrow.

give a new mean generation time of 96 min, but the molar growth yield fell to 16.5 g dry weight of cells per mol of citrate utilised. Growth continued at this rate until the medium was depleted of citrate.

An examination of the extracts prepared from cells harvested at intervals after the addition of Na^+ showed that synthesis of both of the fermentation enzymes, citrate lyase and the Na^+-dependent OAA decarboxylase, commenced between one and two hours after the addition of the Na^+. This time interval is of the same order as the mean generation of 96 min observed after the addition of Na^+ and indicates that the enzymes were induced in the first generation of cells following the addition of Na^+. The spec. act. of both enzymes increased linearly throughout the second phase of growth with a constant ratio of activity of citrate lyase to OAA decarboxylase of approximately 4:1.

The rate of citrate utilisation was low in the aerated K^+-citrate medium but increased rapidly about 1 hr after the addition of the Na^+. Similar results on growth, enzyme induction and citrate utilisation were observed when NaCl was replaced by Na_2SO_4 indicating that the observed effects were due to Na^+ and not to the increased concentration of the anion.

4. Discussion

The results clearly indicate that the addition of Na^+ to a culture of *K. aerogenes* growing on K^+-citrate under aerated conditions caused an increase in both the growth rate and the utilisation of citrate but a decrease in the molar growth yield. Thus, the faster but less efficient, growth observed after the addition of Na^+ duplicates the previous results obtained when *K. aerogenes* was grown on Na^+-citrate under aerated conditions [5].

The observed changes in growth can be attributed to the induction by Na^+ of the citrate fermentation enzymes which apparently occurred at the same time as both the increased growth rate and the increased citrate utilisation. Prior to the addition of Na^+, metabolism of citrate presumably occurred via the citric acid cycle, as previously observed [5]. However, after the addition of Na^+, metabolism was able to proceed via both the citric acid cycle and the fermentation pathway thus accounting for the faster growth rate and the more rapid utilisation of citrate.

Since the latter pathway is a fermentative one, a lower growth yield would be expected after the addition of Na^+ and in fact was observed. The need for Na^+ for the induction of the fermentation enzymes provides a second explanation for the Na^+ requirement for anaerobic growth of *K. aerogenes* on citrate [3], the first being the activation of the OAA decarboxylase [4]. It is interesting to note that the induction of the fermentation enzymes was not observed when the organism was grown on Na^+-citrate under aerobic conditions [5].

Transport of citrate by *K. aerogenes* NCTC418, under anaerobic conditions, is Na^+-dependent [12] and Wilkerson and Eagon [11] have shown that uptake of citrate under conditions which may approximate aerated is stimulated by Na^+. Thus, Na^+ appears to be involved in three functions in *K. aerogenes* grown on citrate under anaerobic or aerated conditions, namely, 1) the transport of citrate, 2) the induction of the fermentation enzymes and 3) the activation of the OAA decarboxylase. It is conceivable that two transport systems, one Na^+-independent and one Na^+-dependent, are operative in cells grown on Na^+-citrate under aerated conditions. This is currently being investigated.

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