STUDIES OF CITRATE TRANSPORT IN AEROBACTER AEROGENES: BINDING OF CITRATE BY A MEMBRANE BOUND OXALACETATE DECARBOXYLASE

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Summary: Citrate is a competitive inhibitor of the Na^+-activated oxalacetate (OAA) decarboxylase, a membrane-bound enzyme participating in citrate catabolism by Aerobacter aerogenes. It is proposed that this OAA decarboxylase also functions as a carrier protein in the transport of citrate by a novel mechanism.

The specific Na^+ requirement for anaerobic growth of Aerobacter aerogenes on citrate as sole carbon source (1) can be explained by the specific Na^+ requirement of the oxalacetate (OAA) decarboxylase step of the citrate fermentation pathway (2). This membrane bound decarboxylase (3), a soluble citritase and the citrate transport system were induced by growth on citrate and repressed by growth on glucose (2, 4). Citrate uptake by washed suspensions of citrate grown cells also specifically required Na^+ (5). Uptake occurred without detectable internal accumulation of citrate or OAA and was accompanied by extrusion of pyruvate, acetate and bicarbonate from the cell. These and other observations led us to postulate that the membrane-bound OAA decarboxylase might be involved in citrate transport as well as its catabolism.

This paper demonstrates that citrate is a competitive inhibitor of OAA decarboxylase. Thus the decarboxylase can bind citrate and meets the

criteria of a membrane-bound transport protein (6). A mechanism of citrate transport involving the decarboxylase is presented.

METHODS

A. aerogenes was grown anaerobically as previously described (1). The cells were subjected to sonic disruption and then centrifuged at 27,000 g. OAA decarboxylase was extracted from the particulate fraction with 1% Triton X-100 (3). This extract catalyzed the decarboxylation of 1.0 μmole of OAA per min per mg protein at 28°. OAA decarboxylase activity was measured spectrophotometrically at 290 nm (d = 0.5 cm; volume 1.50 ml) using a Gilford Model 2000 recorder with expanded absorbance scale. The reaction was carried out in 100 mM potassium phosphate buffer pH 7.0 at 25° with disodium OAA and potassium citrate added as indicated. The reaction was started by addition of enzyme (0.58 mg).

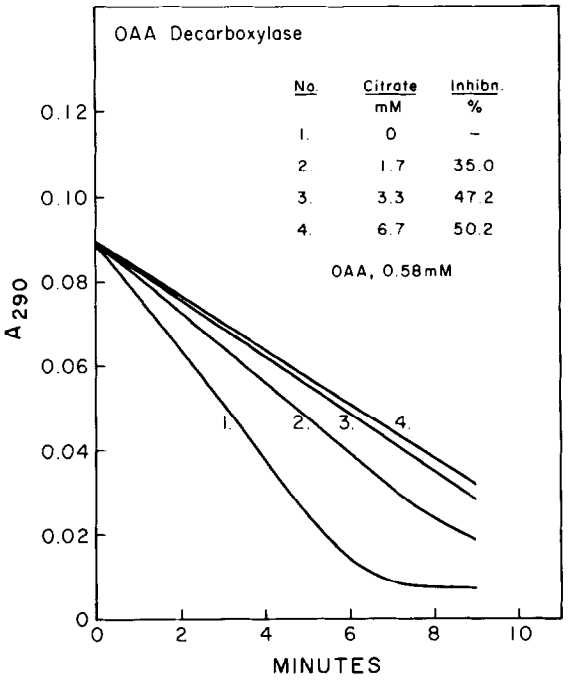

Figure 1. Inhibition of OAA Decarboxylase by Citrate.

RESULTS AND DISCUSSION

As illustrated in Fig. 1, citrate inhibited OAA decarboxylase, and the degree of inhibition increased with increasing concentrations of citrate. Kinetic analysis of the inhibition by citrate demonstrated that this inhibition was competitive in nature (Fig. 2). The K_m for OAA was 1.42×10^{-4}M while the K_i for citrate was 6.32×10^{-4}M (at 1.67 mM citrate). The latter value was in the range of the K_m values we have determined for citrate uptake by washed cell suspensions of A. aerogenes, namely 1.43×10^{-4}M for cells grown anaerobically on citrate and 3.20×10^{-4}M for cells grown aerobically on citrate.

The following observations had led us to postulate that OAA decarboxylase, besides participating in citrate catabolism, also functions as a carrier protein ("permease") for citric acid in A. aerogenes and S. typhimurium.

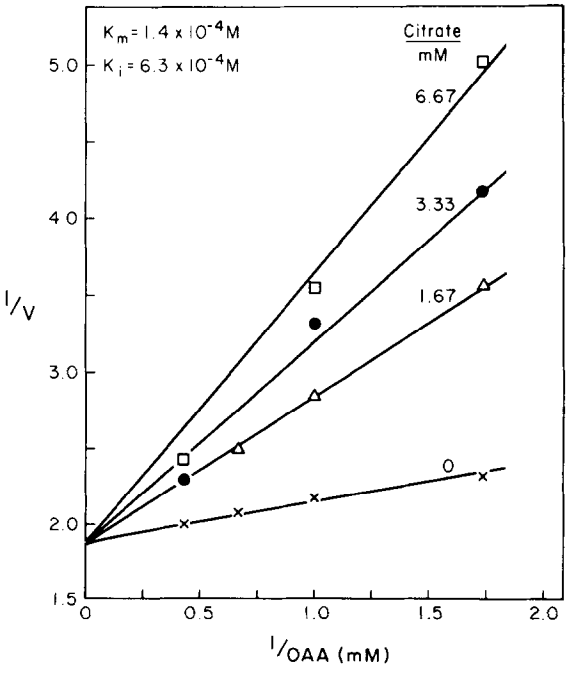

Figure 2. Citrate Inhibition as a Function of OAA Concentration. The data are plotted according to Lineweaver and Burk.

(a) The decarboxylase was located on the cytoplasmic membrane while citritase was present in the cytoplasm (3).

(b) Both OAA decarboxylase and citritase were induced by growth on citrate and were absent when A. aerogenes was grown on glucose or glycerol (2).

(c) Both growth on citrate and citrate uptake by the cell required Na^+ specifically as did the OAA decarboxylase (5).

(d) A. aerogenes cells grown on glucose were unable to transport or catabolize citrate with or without added Na^+ and they lacked the Na^+ dependent (or other) OAA decarboxylase.

(e) In S. typhimurium Na^+ was required for growth on citrate even though citrate grown cells possessed all the enzymes of the citric acid cycle, none of which require Na^+. Citrate grown cells also possessed citritase, the Na^+-dependent, avidin sensitive membrane-bound OAA decarboxylase, and a second "classical" Mn^{++}-dependent OAA decarboxylase in the cytoplasm (4). The membrane bound decarboxylase was present only in citrate grown cells and was absent from cells grown on L-malate or glycerol. The soluble Mn^{++}-dependent OAA decarboxylase was present after growth on all three carbon sources. Thus citrate uptake correlated with induction of the Na^+-dependent OAA decarboxylase, and we now find (unpublished experiments) that citrate uptake by citrate grown cells requires Na^+ specifically.

(f) Kinetic studies showed that Na^+, which does not affect citritase activity, increased the affinity of the Aerobacter citrate transport system for citrate without affecting V_{max}.

(g) Citrate uptake by washed cells of S. faecalis grown on citrate

plus yeast extract required Ca^{++} and biotin and this correlated with the presence of a membrane-bound OAA decarboxylase activated specifically by Ca^{++} and sensitive to avidin (7).

The experiments reported here showing that citrate is a competitive inhibitor of OAA decarboxylase demonstrate unequivocally that the decarboxylase can bind citrate as required of a transport protein.

We propose the following mechanism for citrate transport (Fig. 3) in which OAA decarboxylase functions as the carrier protein or "transporter" of citrate. OAA decarboxylase (E) on the outer surface of the membrane binds citrate from the medium. The fact that Na^+ increases the affinity of citrate for its transport system suggests that this binding may require Na^+, but this remains to be established. The E-citrate complex turns to the inside of the membrane. There it can (Reaction 3A) dissociate to E and citrate, the citrate being cleaved by soluble citritase to OAA and acetate

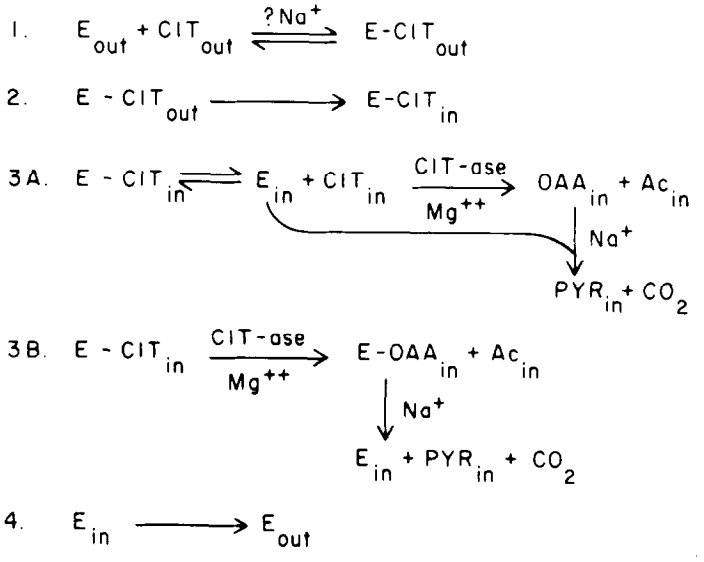

Figure 3. A Model for OAA Decarboxylase as the Carrier Protein in Citrate Transport.

and the OAA being decarboxylated (on the inner surface of the membrane) in presence of Na^+. Alternatively (Reaction 3B), when the E-citrate complex reaches the inner surface, the complex itself may serve as the "true" substrate of soluble citritase, giving rise to OAA still bound to the decarboxylase and acetate. This removes citrate from the decarboxylase, thereby permitting it to function as a decarboxylase in presence of Na^+ and releasing pyruvate on the inner surface of the membrane. The enzyme then "returns" to the outer surface completing the cycle. Energy may be required for Reactions 2 and 4.

Since the dissociation constant of Reaction 3A is 6.3×10^{-4} and the K_m of citrate for citritase is 2.1×10^{-4} (8) it is conceivable that the Reaction sequence 3A can account for the high rates of citrate transport (859 μmoles per min per g dry wt of cells) by A. aerogenes. Reaction 3A or 3B can each explain how the transport of citrate is accomplished, when the catabolism of citrate requires that it must first be attacked by a soluble citritase in order to generate the substrate (OAA) of the membrane bound decarboxylase. However Reaction 3B can better explain our finding that inhibitors of citritase (or OAA decarboxylase) do not result in internal accumulation of citrate (or of OAA from citrate).

We propose the term metabolic transport to describe the dynamics of this transport system wherein the carrier protein in the membrane is an enzyme involved in the catabolism (or anabolism) of the compound being transported.

This model for citrate transport is applicable to microorganisms such as A. aerogenes, S. typhimurium and S. faecalis where citritase and OAA decarboxylase are induced by growth on citrate. It is clearly not the

only possible mechanism of citrate transport among bacteria. For example, recent studies of an inducible citrate transport system in B. subtilis (9) have shown that these enzymes are not present and that citrate transport can be separated from citrate catabolism in aconitase-less mutants of B. subtilis.

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