

EFFECTS OF CALCIUM IONS AND QUINOLINIC ACID ON RAT
KIDNEY MITOCHONDRIAL KYNURENINE AMINOTRANSFERASE

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SUMMARY: At pH 6.4, rat kidney mitochondrial kynurenine aminotransferase activity is enhanced several-fold by the addition of CaCl_2 , apparently because Ca^{++} facilitates the translocation of α -ketoglutarate, one of the substrates, across the mitochondrial inner membrane. Chloride salts or Mg^{++} , Mn^{++} , Na^+ , K^+ , and NH_4^+ did not have this effect. At pH 6.8, the enzyme activity was near maximal even without added Ca^{++} but was strongly depressed by either of two calcium chelating agents, quinolinic acid (Q.A.) and ethyleneglycol-bis-(β -aminoethyl ether)N,N'-tetraacetic acid (EGTA). These observations support the view that Ca^{++} is involved in regulating kidney mitochondrial translocation of α -ketoglutarate and that the reported interference of polycarboxylate anion translocation by Q.A. in vivo depends on the ability of that agent to chelate Ca^{++} .

In previous studies (1) of the mitochondrial kynurenine aminotransferase of rat kidney, we observed that disruption of the mitochondrial membranes resulted in several-fold increases in enzyme activity. Similar increases were obtained without membrane disruption simply by adding 1 mM CaCl_2 to the reaction mixture. Since CaCl_2 had no effect on the activity of the solubilized mitochondrial enzyme, this enhancement was attributed to an ability of Ca^{++} to facilitate the translocation of the substrate, α -ketoglutarate, across the mitochondrial inner membrane. It was also noted that Q. A., which was reported by Spydevold et al. (2) to interfere in vivo with α -ketoglutarate translocation in rat liver mitochondria, interfered with this effect of Ca^{++} . These observations suggested that Q. A. may interfere in vivo with α -ketoglutarate translocation by complexing Ca^{++} involved in the translocation process.

In the present report, we have compared the effects of the chloride salts of Mg^{++} , Mn^{++} , Na^+ , K^+ , and NH_4^+ with that of Ca^{++} on the activity of the mitochondrial kynurenine aminotransferase and have demonstrated that the effect of Ca^{++} is relatively specific. We have also found conditions under which Q.A.

Abbreviations: Q.A., Quinolinic acid; EGTA, ethyleneglycol-bis-(β -aminoethyl ether)N,N'-tetraacetic acid.

and EGTA, both capable of chelating Ca^{++} , were able to interfere strongly with the enzyme activity in the absence of added Ca^{++} . This suggests that Q.A. can remove endogenous Ca^{++} to hinder α -ketoglutarate translocation and provides a reasonable explanation of the ability of Q.A. to interfere with polycarboxylate anion translocation in vivo.

METHODS

Adult male albino rats of the Sprague-Dawley strain were killed by decapitation and the kidneys were immediately chilled on ice. Homogenization and fractionation were carried out as described previously (3) using 0.25 M sucrose rather than sucrose- α -ketoglutarate solution. The "mitochondrial" fraction was usually used immediately but retained its responsiveness to Ca^{++} for at least 4 to 5 hours of storage on ice. Solubilized mitochondrial enzyme was prepared by centrifuging freeze-thawed "mitochondrial" fraction for 20 minutes at 30,000xg.

All solutes of the incubation mixtures were purchased from Sigma Chemical Co. Initially 0.5 ml of mixture was made up containing 100 mM imidazole-HCl, 1.5 mM α -ketoglutarate, and 30 μg s pyridoxal phosphate. Other constituents were included as indicated. One-tenth ml mitochondrial preparation was then added, and after 20 minutes at 25°, the reaction was started by the addition of 600 μg s L-kynurenine sulfate dihydrate in a volume of 0.1 ml. After 30 minutes at 37°, the reaction was terminated by the addition of 10 ml of 1% boric acid in 95% ethanol. After 20 minutes with occasional shaking, the precipitate was removed by centrifugation at 5000xg for 5 minutes. Kynurenic acid formation was estimated by the spectrophotometric method described earlier (4).

RESULTS AND DISCUSSION

The effects of the chloride salts of Ca^{++} , Mg^{++} , Mn^{++} , K^+ , Na^+ , and NH_4^+ on mitochondrial kynurenine aminotransferase activity are compared in Table I. Only the calcium salt significantly enhanced the activity. Since NH_4^+ is considered to penetrate mitochondrial membranes by a passive process and

TABLE I. Effects of 1.5 mM CaCl_2 , MnCl_2 , MgCl_2 , KCl , NaCl , and NH_4Cl on Mitochondrial Kynurenine Aminotransferase.

| Salt ¹ | Activity ² | Salt ¹ | Activity ² |
|-------------------|-----------------------|------------------------|-----------------------|
| None | 0.154 | NaCl | 0.159 |
| CaCl_2 | 0.404 | KCl | 0.169 |
| MnCl_2 | 0.155 | NH_4Cl | 0.176 |
| MgCl_2 | 0.146 | | |

¹ Salts were included in the incubation mixture before preincubation with the mitochondrial fraction.

² Enzyme activity was measured as described in the text.

Ca^{++} , Mg^{++} , and Mn^{++} by energy dependent processes the unique effect of Ca^{++} does not seem to depend on a unique ability to enter the mitochondria as a counter-ion to the α -ketoglutarate anion.

To serve as a counter-ion, Ca^{++} would need to be present in amounts at least equivalent to the α -ketoglutarate translocated unless it served a catalytic function, recycling as the α -ketoglutarate was consumed in the reaction. Fig. 1 provides an indication that even endogenous levels of Ca^{++} may be sufficient to support the translocation process. It compares the actions of Ca^{++} and Q.A. at pH 6.4 and pH 6.8. At the lower pH there was a strong dependency on Ca^{++} for maximal activity. Q.A. interfered with this action of added Ca^{++} , but had little effect on the preparations lacking added Ca^{++} . At pH 6.8, however, the activity was almost maximal without added Ca^{++} , whereas a strong dependence on added Ca^{++} was created by the presence of Q.A.

Since Q.A. may have caused the decreased activity in mitochondria lacking added Ca^{++} by complexing endogenous Ca^{++} , it was of interest to test the more conventional Ca^{++} chelator, EGTA. Fig. 2 compares the effects of various concentrations of Q.A. and EGTA on activity and demonstrates a substantial similarity in the actions of these two agents. Table 2 shows that neither Q.A. or EGTA significantly inhibits the solubilized mitochondrial enzyme at pH 6.8.

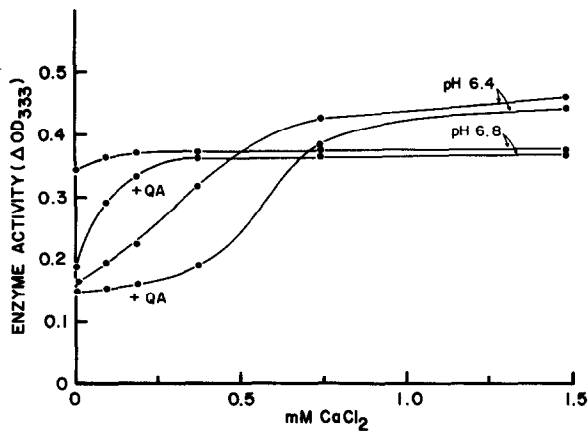


Fig. 1. Effects of 9 mM quinolinic acid and various concentrations of CaCl_2 on the activity of kidney mitochondrial kynurenine aminotransferase at pH 6.4 and 6.8. Tissue fractionation and assay conditions are described in the text.

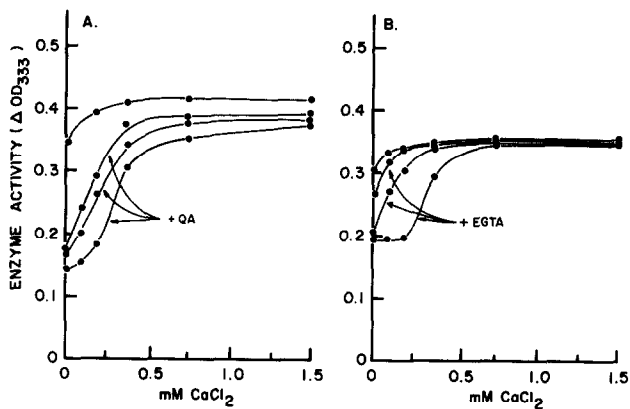


Fig. 2. (A) Effects of various concentrations of quinolinic acid and CaCl_2 on kidney mitochondrial kynurenine aminotransferase at pH 6.8. Q.A. concentrations were 6.75, 9.0, and 13.5 mM . (B) Effects of various concentrations of EGTA and CaCl_2 on kidney mitochondrial kynurenine aminotransferase at pH 6.8. EGTA concentrations were 0.75, 1.5, and 3.75 mM .

A role of Ca^{++} in the translocation of polycarboxylate anions in mitochondria has been suggested previously. Robinson and Chappell (5) and Haslam and Griffiths (6) reported that rat liver mitochondria prepared in a medium containing 1 mM EGTA were almost lacking the ability to translocate oxaloacetate under certain conditions and that this ability was restored by the additions of small amounts of CaCl_2 . Harris and Berent (7) showed that substrate-depleted

TABLE II. Effects of Various Concentrations of Q.A. and EGTA on the Activity of Solubilized Kidney Mitochondrial Kynurenine Aminotransferase

| Agent added | conc. (mM) | Activity | % of Control |
|-------------|------------|----------|--------------|
| None | | 0.363 | 100 |
| Q.A. | 4.50 | 0.352 | 97 |
| | 6.75 | 0.349 | 96 |
| | 9.00 | 0.336 | 92 |
| | 13.50 | 0.322 | 90 |
| EGTA | 0.75 | 0.346 | 95 |
| | 1.50 | 0.357 | 98 |
| | 3.75 | 0.362 | 100 |

rat liver mitochondria reaccumulate malate, succinate, α -ketoglutarate, β -hydroxybutyrate, and glutamate if provided with an energy source and Ca^{++} . In working with liver mitochondria, we have observed a Ca^{++} effect on kynurenine aminotransferase somewhat similar to, but less prominent than, that described for kidney.

Although the application of our observations to liver and other tissues remains to be investigated fully, the observed interactions of Q.A., Ca^{++} , and α -ketoglutarate translocation in kidney mitochondria provides a mechanistic model to rationalize the effects of Q.A. in vivo on polycarboxylate anion translocation as reported by Spydevold et al. (2). Those authors cited evidence that a metal ion chelated by Q.A. might be of regulatory importance in the reported in vivo effect. Our results suggest such a regulatory role for Ca^{++} through its effects on polycarboxylate anion translocation.

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