OPPOSING ACTIONS OF Ca^{++} AND ATP PLUS Mg^{++} IN CONTROLLING THE KYNURENINE AMINOTRANSFERASE ACTIVITY OF ISOLATED RAT KIDNEY MITOCHONDRIA

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Summary: A new method of monitoring changes in the permeability of the mitochondrial inner membrane to substrates, based on mitochondrial kynurenine aminotransferase activity, is applied in this study of a requirement for Ca^{++} for optimal translocation. At pH 7.25, the Ca^{++} requirement was satisfied by endogenous (non-added) Ca^{++}. The endogenous activity was decreased by 1.4 mM $SrCl_2$, $MnCl_2$, $MgCl_2$, $BaCl_2$, and $LaCl_3$ and was unaffected by 1.4 mM NaCl, KCl, and NH_4Cl. It was depressed to a minimum by 70 μM EGTA and rapidly restored by 70 μM $CaCl_2$. The restored level was again depressed (rapidly) by added ATP + Mg^{++}. The observations are discussed as a possible reflection of a known role of the Ca^{++}/Mg^{++} ratio in determining mitochondrial membrane permeability.

In earlier studies (1,2) directed toward characterization of kynurenine aminotransferase (EC 2.6.1.7, L-kynurenine:2-oxoglutarate aminotransferase) of intact rat kidney mitochondria, we observed a strong stimulatory action of Ca^{++} that did not occur with the extracted enzyme and thus appeared to reflect limited access to substrates. The effect was remarkably specific for Ca^{++} and therefore could not be rationalized as the action of a permeant cation serving as a counterion for the entering α-ketoglutarate. Since there is substantial current interest in mechanisms by which Ca^{++} can influence cellular functions (3), we have sought to further characterize this action. In the present study we have outlined in greater detail the specificity and sensitivity of the Ca^{++} effect and have characterized its previously-noted (1) dependence on pH. The action is discussed in relation to previously reported opposing effects of Ca^{++} and Mg^{++} on the permeability of the mitochondrial inner membrane.

Abbreviation: EGTA, Ethyleneglycol bis(β-aminoethyl ether)-N,N'tetraacetic acid.

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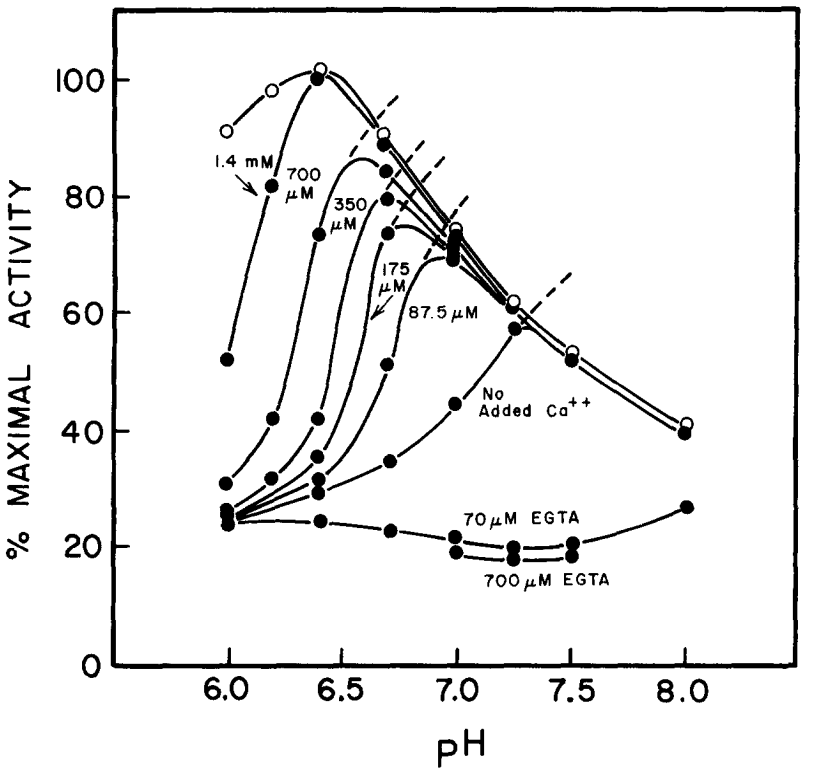

Fig. 1. Kynurenine aminotransferase activity of extracted (-o-) and intact (-●-) isolated rat kidney mitochondria and the effect of various levels of Ca^{++} at various pHs. Final concentrations of added $CaCl_2$ and EGTA are shown.

METHODS AND RESULTS

The methods of mitochondrial isolation and extraction and of enzyme assay have been described (1,2). Specific variations of the methods are presented with the tables.

Fig. 1 compares the kynurenine aminotransferase activities at various pHs in the presence of various levels of $CaCl_2$ and in the absence of added Ca^{++} both before and after mitochondrial membrane disruption. Activity levels that were below those obtained following membrane disruption were considered to reflect limited access to substrates. $CaCl_2$ at 1.4 mM gave essentially complete activity with the intact mitochondria at the pH optimum (6.4) and above, although the activity was incompletely expressed at lower pHs. Decreasing

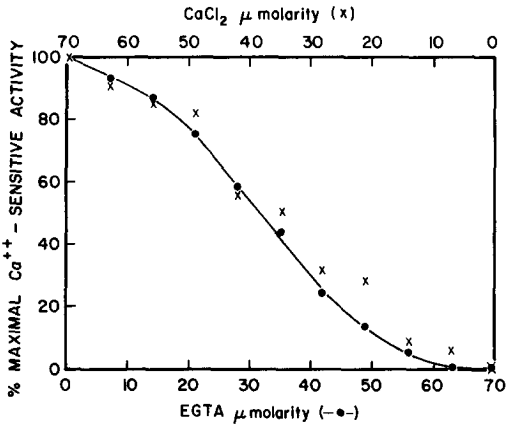

Fig. 2. Kynurenine aminotransferase activity of isolated rat kidney mitochondria at pH 7.25 in the presence of 70 μM EGTA (-●-) and in the presence of 70 μM EGTA plus various concentrations of $CaCl_2$ (X).

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the $CaCl_2$ levels (Fig. 1) caused shifts of the pH optimum to higher pH values concommitant with decreases in the level of the optimal activity to values which corresponded well with those obtained with the extracted enzyme at those pHs. It appears thus that each curve is a composite of a segment to the right of the optimum whose activities are limited by the V_{max} of the enzyme and a segment to the left whose activities reflect limited access to the substrate. The dotted lines in the figure are extrapolations of the translocation-sensitive segment and suggest the existence of optimal translocation conditions at higher pH values which would not be observable through measurement of the enzyme activities.

The view that the activity *vs* pH profile obtained without added Ca^{++} represents the action of Ca^{++} already present in the mitochondria and reaction media, i.e., endogenous Ca^{++}, is supported by the strong depression of activity in the presence of 70 and 700 μM EGTA. Since the two levels gave similar depressions, we concluded that 70 μM EGTA reduced the endogenous free Ca^{++} level to ineffective concentrations.

Although a significant level of Ca^{++}-insensitive enzyme activity remained

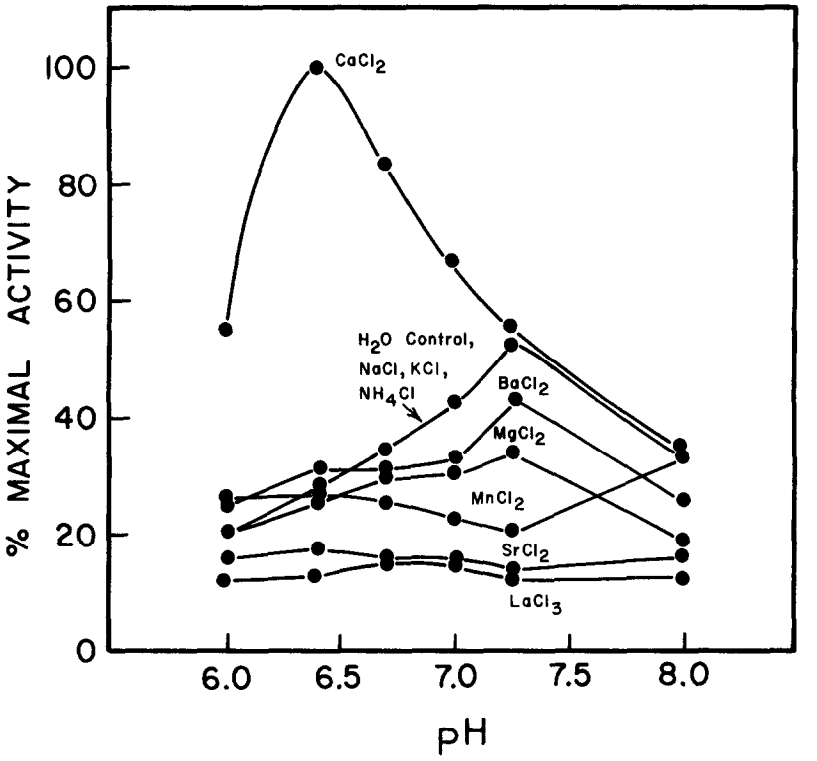

Fig. 3. Kynurenine aminotransferase activities of isolated rat kidney mitochondria at various pHs and in the presence of 1.4 mM levels of various metal chloride salts.

in the presence of EGTA, it was clear that one should be able to titrate the endogenous Ca^{++} effect with EGTA to approach a minimal Ca^{++}-sensitive activity at 70 μM EGTA or less. Fig. 2 represents such a titration and also shows a back-titration with $CaCl_2$. These responses to Ca^{++} depletion and replenishment strongly suggest that Ca^{++} specifically increases the availability of substrate(s) to the mitochondrial enzyme.

The specificity of the Ca^{++} effect was further examined by comparing the actions of various metal chloride salts on the endogenous enzyme activities at various pHs (Fig. 3). Na^{+}, K^{+}, and NH_4^{+} were without effect at 1.4 mM levels. Multivalent cations (1.4 mM) other than Ca^{++} inhibited the activities. When tested at lower cation concentrations, at pH 7.25, La^{+++}, caused 50% inhi-

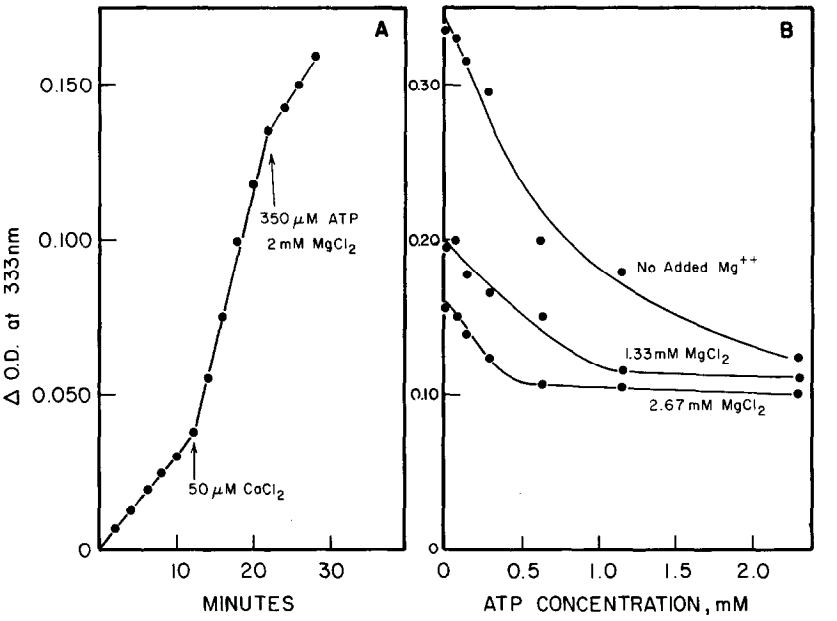

Fig. 4A. Opposing actions of Ca^{++} and ATP + Mg^{++} in controlling the kynurenine aminotransferase activity of isolated rat kidney mitochondria. The incubation mixture (10.4 ml) contained 70 μM EGTA. Aliquots (0.6 ml) were removed and deproteinized at 2 min. intervals. $CaCl_2$ and ATP + Mg^{++} were added in 0.05 ml volumes to give the concentrations shown.

Fig. 4B. The effect of various levels of ATP on the endogenous Ca^{++} effect (pH 7.25) in the presence of various levels of Mg^{++}.

bition at 1.4 μM levels; none of the other cations inhibited significantly at that concentration. Of all the cations tested, only Ca^{++} elevated the activities above the endogenous level (Fig. 1).

As shown in Fig. 4A, the response of the mitochondria to Ca^{++} was immediate and sustained but was reversed rapidly by the subsequent addition of ATP and Mg^{++}. A dependence on ATP for diminishing the Ca^{++}-dependent enzyme activity was observed at endogenous levels of Mg^{++} and with two different levels of added $MgCl_2$ (Fig. 4B). Dependence on ATP decreased sharply as the $MgCl_2$ level increased, suggesting a secondary role for ATP.

Ca^{++} and ATP + Mg^{++} also exhibited opposing actions on a swelling-contraction cycle which occurred (Fig. 4) under the conditions of the activity transitions

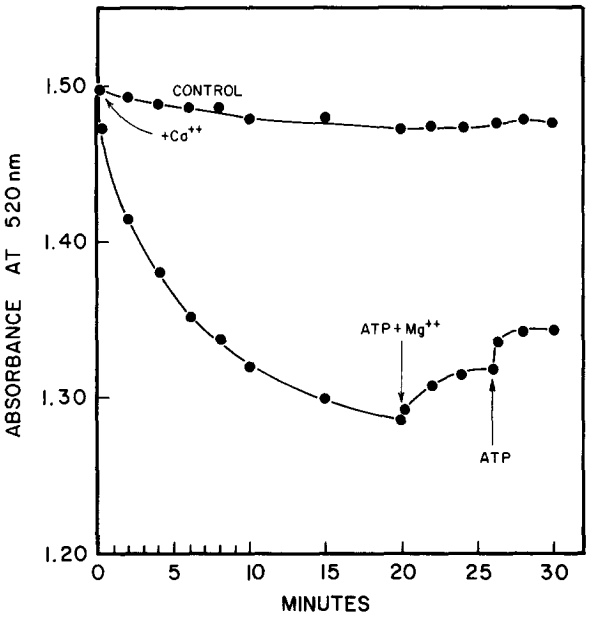

Fig. 5. Opposing actions of Ca^{++} and ATP + Mg^{++} on swelling and contraction of rat kidney mitochondria as determined by absorbance changes at 520 nm. Responses to $CaCl_2$ (50 μM) and to ATP (350 μM and 1 mM) + $MgCl_2$ (2 mM) were determined at 37° (4), using incubation mixtures similar to those used for following activity transitions (Fig. 4A).

(Fig. 4A). Such changes, measured by shifts in optical density at 520 nm (4), did not occur during the 10 minute preincubation at room temperature but did occur slowly and with small amplitude at 37°.

DISCUSSION

The mitochondrial kynurenine aminotransferase has several properties that recommend its use for monitoring changes in kidney and liver mitochondrial inner membrane permeability. The measured product, kynurenic acid, has a strong absorption maximum at 333 nm, is formed irreversibly, and is not significantly further degraded during the incubation period. These properties allow one to observe over relatively long periods of time, using routine spectrophotometry, aminotransferase activity rates that reflect the availability of entering substrate(s). This approach has allowed us to examine in some detail the roles

of Ca^{++}, Mg^{++}, and ATP in controlling substrate availability. Preliminary studies indicate that these characteristics of the assay system will also permit its use in kidney slice experiments which seek to determine whether the observed regulatory actions also occur in intact cells.

The mechanism of the observed opposing actions may be related to previously reported (5-10) opposing actions of Ca^{++} and Mg^{++} in determining membrane permeability. Our data are consistent with other observations which show that increasing the Ca^{++}/Mg^{++} ratio increases the mitochondrial membrane permeability and that decreasing the ratio decreases it. The ability of ATP at low Mg^{++} levels to reverse the endogenous Ca^{++} effect on substrate permeability (Fig. 4B) would appear to be easily explained as a result of a lowered Ca^{++}/Mg^{++} ratio caused by the known ability of ATP to promote the uptake (11) and sequestration of Ca^{++} by mitochondria. However, the interaction of Ca^{++}, Mg^{++}, nucleotides, and other factors in controlling inner membrane permeability appears to be very complex (5-10).

Ca^{++} promotes the orthodox configuration of mitochondria, whereas Mg^{++} promotes the condensed configuration (8). These morphological changes are believed to be the basis of the optical density decrease at 520 nm observed on the addition of Ca^{++} and the partial reversal on the addition of ATP + Mg^{++} (so-called swelling-contraction cycle). Ca^{++}-induced transitions between these two configurations have been associated temporally with non-specific changes in inner membrane permeability and with other functional changes (10). In our studies, however, the rapid activity transitions (Fig. 4A) were associated with a relatively slow swelling-contraction cycle (Fig. 5) of small amplitude. Thus, under our conditions, the swelling-contraction cycle appears not to be concommitant with the activity transitions.

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