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INTERDEPENDENCE BETWEEN MITOCHONDRIAL MATRIX VOLUME AND THE EXTRAMITOCHONDRIAL RATIO OF [ATP]/[ADP] [INORGANIC PHOSPHATE]

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SUMMARY: ATP or combinations of ATP with EDTA and EGTA can act as chelators to support succinate-driven, phosphate-requiring expansion of mitochondrial inner membrane-matrices. Contraction of these swollen mitochondria can be induced with antimycin, $MgCl_2$ and ADP. The magnitude of ADP-induced contraction of mitochondria, swollen in the presence of ATP, is dependent on [ADP] and may be altered by the extramitochondrial concentrations of both P_i and ATP. In fact, the extent of contraction ($+\Delta A_{520}$) is a linear function of the thermodynamic parameter, $-\Delta Gp$ (free energy of hydrolysis of ATP), provided excessive concentrations of reactants are not present and the extents of matrix swelling are similar (e.g. ΔA_{520} is about 0.250) before starting contraction with ADP.

Reversible volume changes of the inner membrane matrices of isolated mitochondria, generated during changes in oxidative metabolism, have received considerable attention (1-5) since low amplitude volume changes were first described in heart mitochondria (1). The early interpretations portrayed a central involvement of respiration-dependent volume and morphological changes for energy capture and transduction. However, the experiments of recent years (5-8) indicate that volume and morphological changes probably are not directly responsible for energy transduction during respiratory chain-linked oxidative phosphorylation even though they accompany induced changes in respiration and ion movements.

The magnitude of swelling and shrinking of the inner membrane-matrices of isolated rat liver mitochondria under essentially isoosmotic conditions at pH = 7.2 is dependent on several factors. A chelator (e.g. EDTA), penetrative anion (e.g. phosphate), monovalent cation (e.g. Na^+) and oxidizable substrate (e.g. succinate) generally are required to promote swelling, whereas dinitrophenol, anaerobiosis, antimycin and ADP promote shrinking or reversal of the induced swelling of mitochondrial inner membrane-matrices. Invariably the volume changes are accompanied by movements of anions, cations or neutral dis-

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sociable molecules across membranes and by unfolding and refolding of the inner membrane-cristae continuum (8-10).

Several investigators (11-15) have considered the extramitochondrial concentrations of phosphorylation reactants (ATP, ADP and P_i) in the regulation of metabolic rates. Strong evidence has been presented that the adenine nucleotide translocase is the rate-limiting step for the overall rate of oxygen uptake when the [ATP]/[ADP] is high (16). Other evidence indicates respiration may be correlated with the [ATP]/[ADP][P_i] under physiological conditions (17). The figures to be presented on mitochondrial absorbance changes show an interrelationship between volume changes and respiratory chain-linked energy transductions coupled to phosphorylation reactions when extents of inner membrane matrix swelling and shrinking are varied by extramitochondrial concentrations of phosphorylation reactants.

MATERIALS AND METHODS

Rat liver mitochondria were isolated by a procedure which uses 250 mM sucrose only (no chelator or buffer is added) in the homogenization, isolation and suspending medium (18). The protein concentration of mitochondrial suspensions was estimated by a biuret procedure (19) after solubilizing the membranes with sodium deoxycholate. All batches of mitochondria used in the experiments for this report had respiratory control ratios greater than seven when measured under appropriate conditions.

Expansion and contraction of mitochondrial inner membrane matrices was estimated by absorbance at 520 nm (A_{520}) in a Beckman DU Spectrophotometer. Changes in A_{520} are considered to estimate changes in inner membrane-matrix volume (20). A decrease in A_{520} reflects matrix expansion and an increase in A_{520} reflects matrix contraction. Specific reaction reagents, incubation conditions, experimental procedures and estimation methods are described in figure legends.

RESULTS AND DISCUSSION

The inner membrane matrices of rat liver mitochondria undergo "reversible" low amplitude swelling in a 250 mM sucrose solution containing sodium salts of succinate (plus rotenone), inorganic phosphate (P_i) and a chelator of divalent cations, especially Mg^{2+}. Both EDTA and ATP serve as chelators in support of succinate-driven "reversible" swelling, whereas EGTA (20 to 1000 μM) does not support swelling but can interact with ATP to lower the effective ATP concentration required to promote inner membrane-matrix expansion (Fig. 1). 60 and

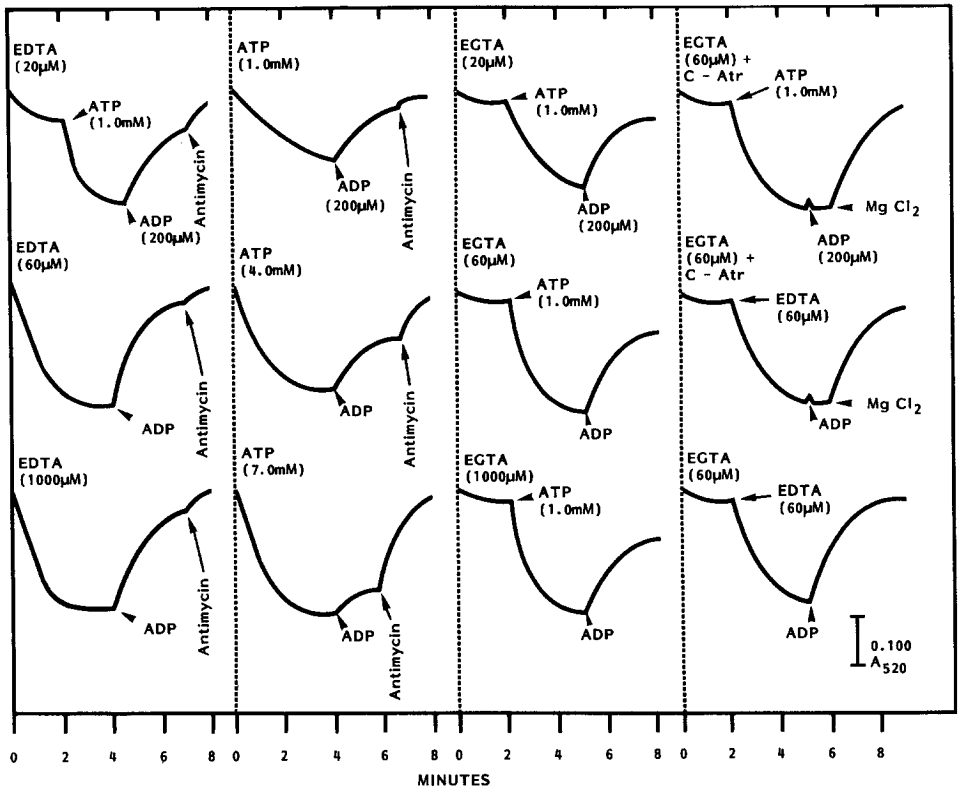

Fig. 1. Main effects and interactions of EDTA, EGTA and ATP on reversible swelling of rat liver mitochondria. Absorbance at 520 nm (A_{520}) was recorded from a modified direct readout Beckman DU Spectrophotometer equipped with a water-jacketed cell (1-cm light path) maintained at 30° C. Rat liver mitochondrial protein (600 μg) was added to a reaction mixture containing 250 mM sucrose, 5 mM phosphate, 15 mM succinate (all adjusted to pH = 7.2 with NaOH), 500 ng of rotenone and the cation chelators indicated at the start of the A_{520} traces. All aqueous reagents to be added in small aliquots were adjusted to pH = 7.2 and the total reaction volume, after making all additions, was 3 ml. Added ADP was initially 200 μM, antimycin was 500 ng/ml and $MgCl_2$ was 1000 μM. The incipient A_{520} of the mitochondria was about 0.950. Carboxyatractyloside (C-Atr) was added at a concentration of 30 μM in the two reactions indicated.

1000 μM EDTA promote essentially the same extent of expansion. A low level of chelator (less than 20 μM EDTA or EGTA) is necessary to prevent large amplitude "irreversible" swelling of mitochondria; perhaps this level of chelator is tying up "contaminating" Ca^{2+} which is known to cause swelling.

60 μM EDTA, 7000 μM ATP and 60 μM EGTA plus 1000 μM ATP support virtually the same magnitude of succinate-driven "reversible" inner membrane-matrix

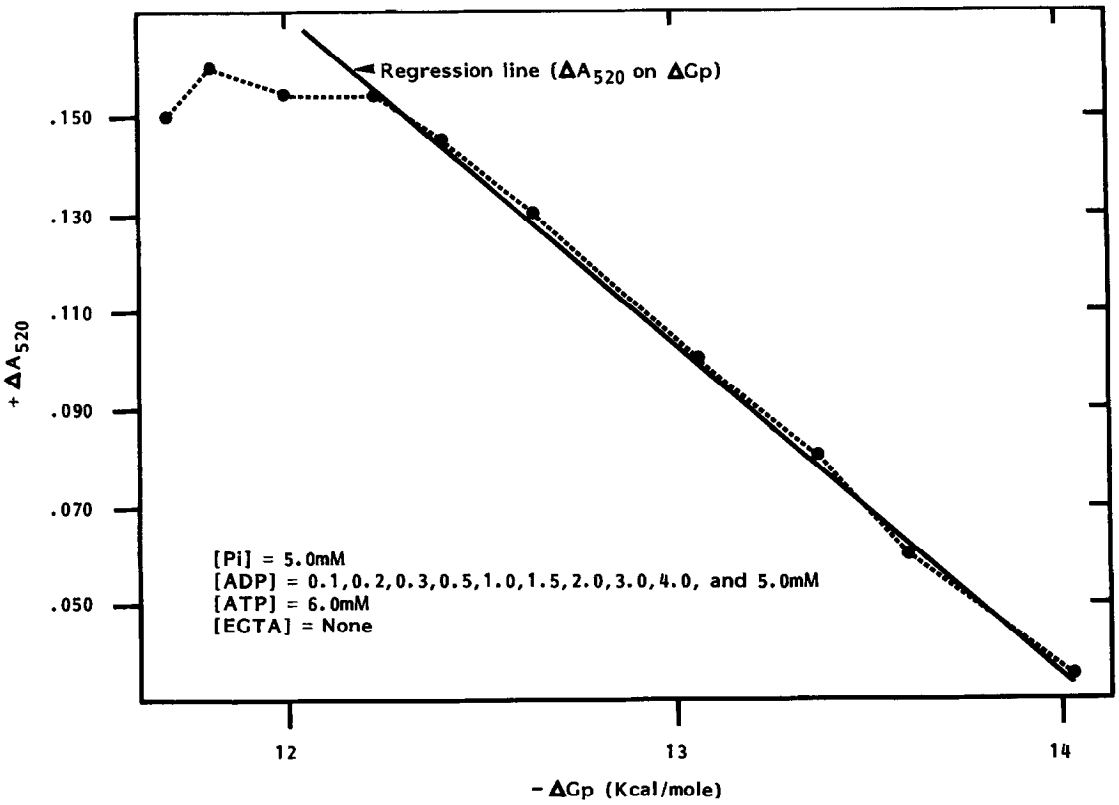

Fig. 2. Contraction of mitochondrial inner membrane matrices as a function of the ADP concentration after swelling the matrices at a constant ATP and phosphate concentration. Experimental procedures, incubation conditions and basic reagents are the same as described for Figure 1. The linear regression line was calculated by the least squares method on only A_{520} observations where ADP concentration was less than 3.0 mM because maximal contraction was essentially achieved by addition of 2.0 mM ADP. The standard free energy change for hydrolysis of ATP was taken to be -8.4 kcal/mole.

swelling (ΔA_{520} is about 0.250). Contraction of the expanded matrices, supported by chelators or the chelator combinations, can be accomplished with antimycin, $MgCl_2$ and ADP. But the extent of ADP-induced contraction is modulated by the extramitochondrial ATP concentration - as the ATP concentration is increased the extent of ADP-induced contraction is decreased when the amount of ADP added is constant (Fig. 1, second panel). The extent of ATP-supported swelling is also concentration dependent within the limits shown in Figure 1. The ATP-supported swelling is not prevented by carboxyatractyloside, a noncom-

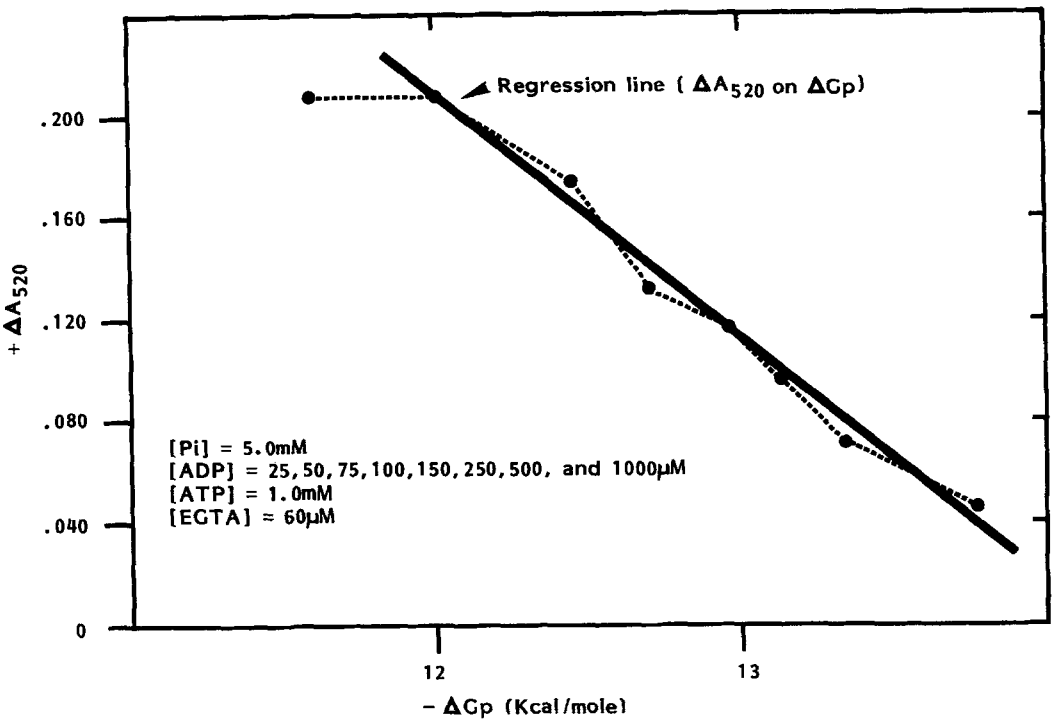

Fig. 3. Contraction of mitochondrial inner membrane matrices as a function of the ADP concentration after swelling at a constant ATP and phosphate concentration with 60 μM EGTA present. Experimental procedures, incubation conditions and basic reagents are the same as described for Figure 1. The linear regression line was estimated by the least squares method on only A_{520} observations where ADP concentration was 500 μM or less.

petitive inhibitor of the adenine nucleotide translocase, whereas the ADP-induced shrinking is blocked (Fig. 1, fourth panel).

It seems probable from these absorbance recordings, reflecting volume changes in mitochondrial inner membrane-matrices, that the chelator function in promoting "reversible" swelling is removal of Mg^{2+} from outside the inner membrane-matrices; perhaps from a component possessing high affinity for Mg^{2+}, probably a protein (18,21) associated with the outer surface of the inner mitochondrial membrane-cristae continuum. It is also evident that the magnitude of ADP-induced contraction is decreased at the higher ATP concentrations and that this type of induced contraction might be related to the extramitochondrial phosphorylation potential ($\Delta Gp = \Delta G\overset{0'}{p} + 1.38 \log [ATP]/[ADP][P_i]$).

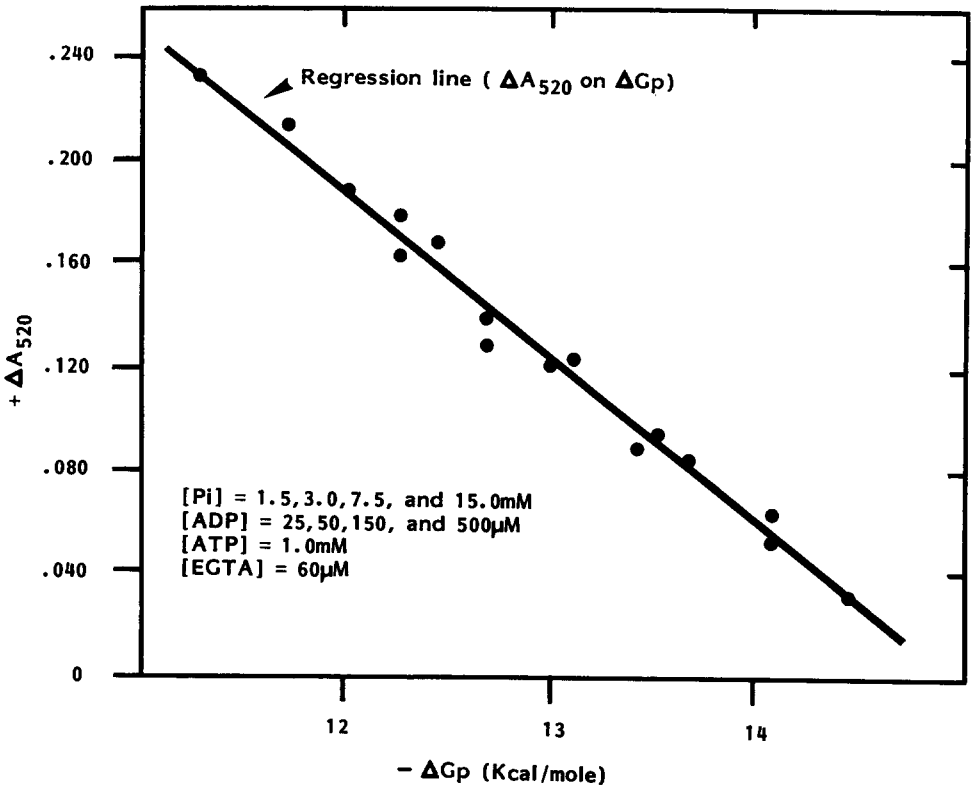

Fig. 4. Relationship among mitochondrial inner membrane matrix contraction, extramitochondrial concentrations of phosphorylation reactants and the phosphorylation reactions. Experimental procedures, incubation conditions and basic reagents are the same as described for Figure 1. The linear regression was estimated by the least squares method on all A_{520} observations.

Experiments were designed to examine this notion. When ATP is used to support swelling the shrinking promoted by low levels of ADP is sustained for a short time before swelling recommences. Therefore, several concentrations of ADP were used to induce contraction of mitochondrial inner membrane-matrices swollen in 6 mM ATP and 5 mM P_i. Figure 2 is a plot of the magnitude of contraction ($+\Delta A_{520}$) as a function of the -ΔGp (phosphorylation potential), calculated from the $\Delta G_p^{0'}$ and the [ATP], [ADP] and [P_i]. Clearly, the extent of contraction has a linear relationship to the -ΔGp until maximal sustained contraction is attained at about 2 mM ADP. A similar relationship is observed when 6 mM ATP is replaced by 1 mM ATP plus 60 μM EGTA (Fig. 3). Notice lower concentrations of ADP are required now to obtain submaximal contractions of

short duration and that maximal sustained contraction is attained at about 500 μM ADP.

Perhaps more convincing data are plotted in Figure 4. The inner membrane-matrices of rat liver mitochondria were expanded with 1000 μM ATP plus 60 μM EGTA at four concentrations of P_i. The extents of swelling were virtually the same at each P_i concentration. The contraction of the expanded matrices was induced independently by 25 to 500 μM ADP. Higher concentrations of ADP were not used because maximal sustained contraction could be attained with 500 μM ADP. Under the described conditions and initial concentrations of extramitochondrial phosphorylation reactants the regression line of $+\Delta A_{520}$ on $-\Delta Gp$ remain linear. Thus, it seems likely that the magnitude of mitochondrial contraction is dependent on the extramitochondrial phosphorylation potential which is thought to play an important role in metabolic regulation under physiological conditions (22). The magnitude of inner membrane-matrix contraction does not appear to be solely dependent on ATP-ADP exchange as may be the case for the respiratory rate (16). It seems that the magnitude of contraction is linked to both P_i and adenine nucleotide movements and their utilization.

Experiments are in progress to determine the actual intra- and extramitochondrial concentrations of ATP, ADP, P_i and monovalent cations at various levels of swelling and contraction induced by the phosphorylation reactants.

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