Coupling of Adenosine Triphosphate Formation in Mitochondria to the Formation of Nucleoside Triphosphates

INVolVEMENT OF NUCLEOSIDE DIPHOSPHOKINASE*

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SUMMARY

Addition of the nucleoside diphosphates CDP, UDP, TDP, and IDP to intact, respiring, rat liver mitochondria immediately following ATP formation results in a marked stimulation of respiration at all concentrations of nucleotide tested. Addition of GDP stimulates respiration when added at concentrations below 150 μM, whereas at higher concentrations this nucleotide has much less effect on respiration and prevents stimulation given by other nucleoside diphosphates. Half-maximal stimulation of respiration is provided by less than 150 μM of each nucleotide.

Nucleoside triphosphate formation is associated directly with stimulation of respiration by nucleoside diphosphates. CTP, UTP, TTP, and ITP are formed in good yield at all concentrations of nucleoside diphosphate tested, whereas GTP is formed in best yield at concentrations of GDP below 150 μM. Concentrations of nucleoside diphosphates which give half-maximal rates of nucleoside triphosphate formation correlate well with concentrations necessary to give half-maximal stimulation of respiration. Concentrations of GDP higher than 150 μM inhibit both the formation of GTP and the formation of other nucleoside triphosphates. Oligomycin and 2,4-dinitrophenol, inhibitors of oxidative phosphorylation, also inhibit nucleoside triphosphate formation.

Partial removal of nucleoside diphosphokinase localized in the outer mitochondrial compartment markedly reduces both nucleoside diphosphate stimulation of respiration and nucleoside triphosphate formation. Kinetic analysis of the solubilized preparation reveals typical Michaelis-Menten kinetics for the substrate pairs ATP-CDP, ATP-UDP, and ATP-TDP, a slight deviation from normal kinetic behavior for the substrate pair ATP-IDP, and a marked deviation from normal behavior for the substrate pair ATP-GDP. GDP is a potent inhibitor of the enzyme at concentrations exceeding 150 μM and, as a result, biphasic kinetic response curves are obtained when the substrate pair is ATP-GDP.

Taken together these results show that ATP formation in intact rat liver mitochondria can be readily coupled to nucleoside triphosphate formation when nucleoside diphosphates are supplied exogenously, that nucleoside diphosphokinase localized in the outer mitochondrial compartment is intimately associated with the coupling process, and that GDP via its action on nucleoside diphosphokinase is a potent inhibitor of nucleoside triphosphate formation.

Herbert et al. (1) first showed that mitochondria contain nucleoside diphosphokinase (ATP : nucleoside diphosphate phosphotransferase, EC 2.7.4.6), an enzyme which catalyzes the transfer of a phosphate group from ATP and other nucleoside triphosphates to nucleoside diphosphates according to the following scheme:

\[ \text{NTP} + \text{NDP} \rightarrow \text{NDP} + \text{NTP} \]

Studies carried out shortly thereafter by Sanadi et al. (2) with a partially purified preparation of nucleoside diphosphokinase from bovine heart mitochondria emphasized the phosphorylation of ADP by GTP in connection with the succinic thiokinase reaction (succinate : coenzyme A ligase, EC 6.2.1.5), which requires the transphosphorylation product GDP as a substrate. Subsequent experiments, however, carried out by Glaze and Wadkins (3) and Goffeau et al. (4) with a homogeneous preparation of nucleoside diphosphokinase from bovine liver mitochondria showed that the enzyme is a nonspecific phosphotransferase as indicated by the reaction scheme noted above and will catalyze transphosphorylations not only between ADP and GTP but between other nucleoside di- and triphosphates as well.

More recently, localization studies carried out in our laboratory (9, 6) using the Digitonin-Lubrol method (7, 8) and in other laboratories with different methods (9, 10) have shown that the bulk of the nucleoside diphosphokinase of rat liver mitochondria is

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localized in the outer compartment (outer membrane + intercristal space) whereas enzymes associated with the oxidative phosphorylation of ADP are localized exclusively in the inner compartment. This compartmentation of enzymes involved in ATP formation and in nucleoside triphosphate formation led us to consider the possibility that ATP once formed in the inner mitochondrial compartment of rat liver may be readily coupled to the formation of nucleoside triphosphates via the nucleoside diphosphokinase localized in the outer mitochondrial compartment. Experiments described in this paper were carried out with the purpose of testing this possibility and are summarized in detail below.

**EXPERIMENTAL PROCEDURE**

**Materials**

Adult male Sprague-Dawley albino rats were obtained from Carworth Farms and provided with a Rockland rat diet purchased from Teklad, Inc., Winfield, Iowa. Hepatoma-bearing rats were obtained from Dr. Harold P. Morris, Howard University, School of Medicine, Washington, D. C. All nucleotides were products of P-L Biochemicals, Inc. d-(+)-Mannitol was obtained from Schwarz-Mann and sucrose from Baker Chemical Co. Defatted bovine albumin (Fraction V) was purchased from Nutritional Biochemicals Corp. 32P-Labeled orthophosphate in 0.02 N HCI was purchased from New England Nuclear Corp. and heated 3 hours prior to use in a boiling water bath. Lactic dehydrogenase (bovine heart, type III), hexokinase (yeast, type C-300), phosphoenolpyruvate, oligomycin, and HEPES were obtained from Sigma Chemical Co. Pyruvate kinase (rabbit muscle, Grade A) and glucose 6-phosphate dehydrogenase (yeast, Grade A) were obtained from Calbiochem and Lubrol WX from ICI America, Inc. Sephadex G-100 was purchased from Pharmacia Fine Chemicals and prepared for use according to their instruction manual. Thin layer sheets of cellulose (6064), and polyethyleneimine-impregnated cellulose (Polygram Cel PEI) were obtained from Eastman Kodak Co. and Brinkmann Instruments, Inc., respectively. All other reagents used in these studies were of the highest commercial purity available.

**Methods**

**Isolation of Mitochondria**—Rat liver, rat kidney, and rat hepatoma mitochondria were isolated by differential centrifugation as described by Sahnaint and Greenawalt (7). Mitochondria from crab hepatopancreas were prepared by the method of Chen and Lehninger (11), from rat and bovine heart mitochondria prepared by the methods of Pande and Blanchaer (12) and Settemire et al. (13), respectively, and from yeast (Saccharomyces cerevisiae) prepared by the method of Mattoon and Balcavage (14) were kindly supplied by Drs. Chen, Jacobus, and Mattoon of this department.

**Respiration Measurements**—Oxygen uptake was monitored at 25° in a closed 3.0 ml reaction vessel equipped with a Clark oxygen electrode. The basic medium contained the following components in a total volume of 2.8 ml: 220 mM d-mannitol, 70.0 mM sucrose, 1.8 mM MgCl₂, 0.5 mM EDTA, 3.6 mM sodium succinate, 2.5 mM potassium phosphate, 2.0 mM HEPES, pH 7.4, and 2.5 mg of mitochondrial protein.

**Preparation of Nucleoside Diphosphokinase Free of Adenylate Kinase**—Freshly isolated mitochondria from five rat livers were suspended at a protein concentration of 20 mg per ml in a medium containing 220 mM d-mannitol, 70 mM sucrose, 2.0 mM HEPES, 0.5 mg of defatted bovine albumin per ml, and 0.3 mM EDTA, pH 7.4. The suspension was allowed to stand 5 hours at 0-4° during which time about 60% of the nucleoside diphosphokinase activity is released into the suspending medium (Fig. 4). After centrifugation at 10,000 × g for 15 min the resultant supernatant was concentrated to about 5 ml by using an Amicon Diaflo apparatus equipped with a 10M-10 filter. The concentrated fraction was placed on a Sephadex G-100 column (1.5 × 57 cm) pre-equilibrated with 0.1 mM potassium phosphate, pH 7.5, and chromatographed with the same buffer. Nucleoside diphosphokinase required 25 ml of buffer for complete elution, whereas adenylate kinase, well separated from nucleoside diphosphokinase, was not removed completely until 40 ml of buffer had passed through the column. The peak fractions containing nucleoside diphosphokinase activity were lyophilized to dryness and stored at -20°, at which they were stable for several months.

**Nucleoside Diphosphokinase Assays**—Nucleoside diphosphokinase activity was assayed by three different procedures. In the first, the reaction dCTP + ADP → dCDP + ATP was measured spectrophotometrically at 340 nm and 25° in a system containing in 1.0 ml: 3.0 mM dCTP, 40 μM ADP, 0.5 mM NADP, 10.0 mM glucose, 7.5 units of hexokinase, 0.4 unit of glucose 6-phosphate dehydrogenase, 0.45 mM KCN, 5.0 mM MgCl₂ and 70.0 mM glycylglycine, pH 8.0. When mitochondria were assayed, 1.0 mg of Lubrol WX per mg of protein was included in the assay to disrupt the mitochondria and 0.10 mM AMP to inhibit adenylate kinase activity (7). The rate observed with the complete assay system was corrected for the slow reaction observed in the absence of enzyme due to the direct reaction of dCTP with hexokinase and glucose.

In the second assay procedure, the reaction ATP + NDP → ADP + NTP was measured spectrophotometrically at 340 nm and 25° in a system containing in 1.0 ml: 4.8 mM MgCl₂, 0.14 mM NADH, 0.60 mM phosphoenolpyruvate, 1.0 unit of lactate dehydrogenase, 1.0 unit of pyruvate kinase, 65 mM Tris-Cl, pH 7.5, and concentrations of ATP and NDP indicated in the figure legends. The rate observed for the complete system was corrected for the reaction observed in the absence of enzyme due to direct reaction of nucleoside diphosphates with pyruvate kinase and phosphoenolpyruvate. In the case of GDP and IDP, the latter reaction was too rapid to permit meaningful data to be obtained.

In the third assay procedure, the reaction ATP + NDP → ADP + NTP was followed at 25° by assessing the incorporation of [γ³²P]orthophosphate from γ labeled ATP into NTP. The reaction system contained in a final volume of 1.0 ml: 150 μM ATP, 1.0 μCi of [γ³²P]ATP labeled in the γ position according to the procedure of Glynn and Chappell (15), 4.8 mM MgCl₂, 65.0 mM Tris-Cl, pH 7.5, and concentrations of GDP or IDP as indicated in the legends. After 5.0 min the reaction was stopped by addition of 0.10 ml of 2.5 M perchloric acid and allowed to sit 15 min at 0-4°. The mixture was neutralized with 0.10 ml of KOH and centrifuged for 10 min at 2000 × g. Aliquots (5.0 μl) of the reaction mixture were spotted on thin layer sheets of polyethyleneimine-substituted cellulose and separations of ATP from NTP were carried out as described by Randerath and Randerath (16). Separation of ATP from GTP was effected in a solvent system containing 2.0 M sodium formate, pH 3.4, and ATP from ITP in a solvent system containing 1.0 M LiCl + 0.1 M formic acid. Radioactivity was assessed as outlined below.

**Monoamine Oxidase and Adenylate Kinase Assays**—Monoamine oxidase and adenylate kinase were assayed exactly as...
using unsubstituted cellulose was employed. Pi was first separated, pH 3.4, for the GTP-containing mixture. For the CTP-solvent, for the ITP-containing mixture, and 2.0 M sodium for-UTP-containing mixtures, 1.0 M LiCl + 1.0 M formic acid as and Randerrath (16) using 1.0 M LiCl as solvent for the TTP- and ethyleneimine-substituted cellulose as described by Randerrath Nucleoside triphosphate, ATP, and Pi were separated on poly- wait (7), respectively.

described by Schnaitman et al. (17) and Schnaitman and Greena-

benzene, and 625 ml of toluene.

terminated using a liquid scintillation spectrometer of the Beckman 100 series. Samples adsorbed to solid thin layer supports were counted in 10.0 ml of a scintillation mixture consisting of 2.51 g of 2,5-diphenyloxazole, 63.0 mg of 1,4-bis (2.phenyloxazolyl) -benzene, and 625 ml of toluene.

Protein Determination—Protein was estimated by the biuret reaction using crystalline bovine albumin as standard (19).

Measurement of Radioactivity—Radioactivity was routinely determined using a liquid scintillation spectrometer of the Beckman 100 series. Samples adsorbed to solid thin layer supports were counted in 10.0 ml of a scintillation mixture consisting of 2.51 g of 2,5-diphenyloxazole, 63.0 mg of 1,4-bis(2-phenyloxazolyl)benzene, and 625 ml of toluene.

Results of experiments summarized in Fig. 2 show that the stimulatory effect of GDP on respiration is biphasic in nature. Whereas low concentrations of this nucleotide markedly stimulate respiration (<150 μM), higher concentrations are much less effective. At a concentration of 600 μM, GDP has essentially no effect on respiration and completely prevents stimulation given by other nucleoside diphosphates (CDP, UDP, and TDP) (Figs. 1 and 2).

To some extent the effect of IDP on respiration is similar to that of GDP. As shown in Fig. 2, the effect of IDP concentra-
tion on the stimulation of respiration is also biphasic in nature. However, the biphasic response pattern observed with IDP is much less pronounced than that observed with GDP, and, at all concentrations tested, IDP is much more effective than GDP in enhancing the rate of respiration.

Fig. 1. A, effect of CDP on the respiration rate of rat liver mitochondria (RLM). Oxygen consumption was measured exactly as described under “Methods” with 2.5 mg of mitochondrial protein in the respiration medium. Where indicated, 160 μM ADP and 600 μM CDP were added to the respiration medium. B, effect of other nucleoside diphosphates on the respiration rate of rat liver mitochondria. Where indicated, 160 μM ADP, 600 μM UDP, 600 μM TDP, 107 μM IDP, and 107 μM GDP were added to the respiration medium. C, inhibition of nucleoside diphosphate-stimulated respiration by GDP. Where indicated, 160 μM ADP and 600 μM of other nucleoside diphosphates were added to the respiration medium. Numbers on the traces refer to the oxygen consumption rate in nanomoles per min.

Fig. 2. Effect of nucleoside diphosphate concentration on the respiration rate of rat liver mitochondria. Conditions are identical with those described in Fig. 1A with ADP present at 160 μM and other nucleoside diphosphates present at the concentrations indicated. The percent maximal stimulation of respiration was calculated from the expression, (NDP rate - State 4 rate)/(State 3 rate - State 4 rate) × 100, where State 3 rate refers to the rate of respiration in the presence of ADP, State 4 rate to the rate of respiration after the consumption of added ADP, and NDP rate to the rate of respiration obtained when a nucleoside diphosphate other than ADP is added immediately following the State 3 to State 4 transition.
Formation of Nucleoside Triphosphates Accompanying Respiratory Stimulation by Nucleoside Diphosphates—In order to determine whether enhancement of respiration by nucleoside diphosphates is associated directly with nucleoside triphosphate formation, mitochondria were incubated under conditions identical with those used to obtain respiratory stimulation with the exception that 32P-labeled phosphate was used in place of unlabeled phosphate. After a 5-min incubation period, the reaction was stopped and aliquots of the reaction mixture were spotted on PEI cellulose or cellulose. Separation of labeled nucleoside triphosphate from labeled ATP and Pi were carried out in solvent systems described under "Methods." As shown in Fig. 3, nucleoside triphosphate formation is observed in all reaction mixtures containing nucleoside diphosphate. The velocity versus nucleoside diphosphate concentration response curves resemble very closely the respiratory stimulation versus nucleoside diphosphate concentration response curves summarized in Fig. 2. When CDP, UDP, or TDP is substrate, velocity versus substrate response patterns are of the Michaelis-Menten type, whereas when either GDP or IDP is present such patterns are biphasic in nature.

Since a high concentration of GDP (900 μM) had been shown to prevent stimulation of respiration by other nucleoside diphosphates (Fig. 1), this nucleoside was tested for its capacity to prevent nucleoside triphosphate formation. Results of experiments summarized in Table I show that 600 μM GDP inhibits the formation of CTP, UTP, and TTP more than 75%.

On the basis of results described here, it seems clear therefore that stimulation of respiration by nucleoside diphosphates is directly associated with nucleoside triphosphate formation and that GDP is a potent inhibitor of both processes.

Inhibition of Nucleoside Triphosphate Formation by Inhibitors of Oxidative Phosphorylation—Although the results described above would strongly suggest that ATP provided by oxidative phosphorylation is coupled to the formation of nucleoside triphosphates, they do not exclude the possibility that a major source of ATP may be provided also by the adenylyl kinase reaction: 2 ADP = AMP + ATP. For this reason, the effects of oligomycin and 2,4-dinitrophenol, inhibitors of oxidative phosphorylation (20), and atracyloside, an inhibitor of adenine nucleotide permeation of the inner mitochondrial compartment (21, 22), were tested for their effectiveness as inhibitors of nucleoside triphosphate formation. Neither nucleoside diphosphokinase nor adenylyl kinase, which is localized in the intracristal space between the inner and outer membranes (7) is affected by these agents (3, 23, 24).

Results summarized in Table II show that oligomycin, 2, 4-dinitrophenol, and atracyloside inhibit nucleoside triphosphate formation more than 90% in all cases, indicating that the oxidative phosphorylation system rather than the adenylyl kinase reaction provides the major source of ATP for the formation of nucleoside triphosphates. This finding is consistent with the well-established facts that both the adenine nucleotide permeation system and the terminal enzyme of oxidative phosphorylation in rat liver mitochondria have affinities for ADP which are many fold greater than the affinity of adenylyl kinase for ADP (25, 26).

Effect of Partial Removal of Nucleoside Diphosphokinase on Nucleoside Diphosphate Enhancement of Respiration and on Formation of Nucleoside Triphosphates—When freshly isolated mitochondria are suspended for 6 hours at 0–4°C in a system containing isolation medium and EDTA, a large fraction of the total nucleoside diphosphokinase activity (65%) is rather selectively released into the supernatant (Fig. 4). The mitochondrial fraction remaining is structurally and functionally intact as evidenced by the retention of monoamine oxidase, a marker for the outer membrane.
Table II

Inhibition of nucleoside triphosphate formation by inhibitors of oxidative phosphorylation

Assay conditions were exactly as described in Table I with GDP present where indicated at 600 μM instead of 2.5 μM of oligomycin, 2.5 μM 2,4-dinitrophenol, and 10.5 μM atrantyloside were included in the assay.

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<tr>
<th>Nucleoside diphosphate in assay</th>
<th>Nucleoside triphosphate formation</th>
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<tr>
<td></td>
<td>Without inhibitor</td>
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<td></td>
<td>nmoles X min⁻¹</td>
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<td>CDP</td>
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<td>TDP</td>
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Fig. 5. Effect of removal of mitochondrial nucleoside diphosphokinase on the enhancement of respiration by CDP and on the formation of nucleoside triphosphates. Nucleoside diphosphokinase was removed exactly as described in Fig. 4 by allowing the mitochondrial suspension to sit 6.0 hours at 0-4°C. Where indicated, 67% of the nucleoside diphosphokinase activity was released. Oxograph traces were obtained in the system described under “Methods” with 2.5 mg of mitochondrial protein. Where indicated, 160 μM ADP and 600 μM GDP were added to the system. Nucleoside triphosphate formation was measured exactly as described in Fig. 3. CDP, UDP, and TDP were present at 600 μM whereas IDP and GDP were present at 167 μM. Numbers on the oxograph traces refer to oxygen consumption in nanomols per min. RLM, rat liver mitochondria.

Kinetic Analysis of Soluble Nucleoside Diphosphokinase—After removal of small amounts of adenylate kinase by gel filtration chromatography as described under “Methods,” the solubilized nucleoside diphosphokinase fraction was examined for its capacity to catalyze transphosphorylation reactions between ATP and nucleoside diphosphates. Results of experiments presented in Fig. 6 show that the solubilized enzyme catalyzes transphosphorylations between ATP and all nucleoside diphosphates tested (CDP, UDP, TDP, IDP, and GDP). Michaelis-Menten type kinetic response patterns are obtained with the substrate pairs ATP-CDP, ATP-UDP, and ATP-TDP. The kinetic response pattern obtained with the substrate pair ATP-IDP shows a slight deviation from normal behavior, whereas the ATP-GDP response pattern is clearly biphasic in nature. Half-maximal rates are obtained with less than 150 μM nucleoside diphosphate in all cases.

The similarities between the kinetic response patterns obtained with the isolated enzyme (Fig. 6) and the response patterns depicting the stimulatory effect of nucleoside diphosphates on respiration (Fig. 2) and on nucleoside triphosphate formation (Fig. 3) should be noted. With the exception of those response patterns obtained with IDP as substrate, striking similarities are noted in all cases.

Rates obtained with the isolated enzyme when ATP-IDP is the substrate pair (Fig. 6) are somewhat lower than would be

**Fig. 5.** Percentage total activity or protein released.
predicted on the basis of stimulation of respiration (Fig. 2) and nucleoside triphosphate formation (Fig. 3) by IDP. However, this finding is consistent with the observations noted above that less of the nucleoside diphosphokinase activity associated with ITP formation is released from the membrane than that associated with the formation of other nucleoside triphosphates.

In any case, the results described above on the respiratory and kinetic properties of nucleoside diphosphokinase-deficient mitochondria and on the kinetic properties of the solubilized enzyme are consistent with a direct involvement of nucleoside diphosphokinase in nucleoside triphosphate formation coupled to the formation of ATP.

Nucleoside Diphosphokinase Activity of Mitochondria from Other Sources—Should mitochondria in general have the enzymatic capacity to couple ATP formation via oxidative phosphorylation to the formation of nucleoside triphosphates, one might expect results similar to those summarized here for rat liver mitochondria to be found with mitochondria from other sources as well. Although we have not examined this possibility since it is clearly outside the scope of the present investigation, we have assayed mitochondrial preparations from a number of different sources for nucleoside diphosphokinase activity.

Results tabulated in Table III show that nucleoside diphosphokinase activity is detectable in all mitochondrial preparations examined: rat liver, rat hepatoma, rat heart, bovine heart, rat kidney, crab hepatopancreas, and yeast. In no case is the activity level of nucleoside diphosphokinase significantly greater than that observed in rat liver mitochondria, and only in the case of heart mitochondria is the activity less than one-half of the rat liver value. Thus, nucleoside diphosphokinase activity appears to be a fairly common property of mitochondrial preparations.

**Discussion**

Results of experiments described here show that once ATP is produced via oxidative phosphorylation in the inner mitochondrial compartment, it can be used immediately for the rapid phosphorylation of exogenously added nucleoside diphosphates. Several lines of evidence indicate that the ATP-NDP transphosphorylation process is mediated by nucleoside diphosphokinase, which is known to be localized predominantly in the outer mitochondrial compartment (5, 6, 9, 10). First, the specificity of the transphosphorylation process for nucleoside diphosphates in intact mitochondria corresponds to the specificity of the soluble enzyme for nucleoside diphosphates (CDP > UDP > TDP > IDP > GDP) (Figs. 3 and 6). Second, half-maximal rates for both the transphosphorylation process in intact mitochondria and the activity of the soluble enzyme are obtained with less than 150 μM nucleoside diphosphate in all cases (Figs. 3 and 6). Third, and perhaps most important, removal of nucleoside diphosphokinase from mitochondria results in a marked decrease in the capacity of nucleoside diphosphates to stimulate respiration and nucleoside triphosphate formation (Fig. 5).

Additional results summarized here show that GDP is a potent inhibitor of nucleoside triphosphate formation and associated nucleoside diphosphate stimulation of respiration (Figs. 2 and 3). GDP at 600 μM is sufficient to inhibit nucleoside triphosphate formation (CTP, UTP, and TTP) more than 75% (Table I). The inhibitory effect of GDP can be best explained by a direct effect of this nucleoside diphosphate on nucleoside diphosphokinase. This suggestion is supported by the findings that the kinetic response curves of the soluble enzyme (Fig. 6), just as the response curves summarizing the effect of GDP on both respiration (Fig. 2) and GTP formation in intact mitochondria (Fig. 3), are biphasic in character. At low concentration (≤150 μM) GDP stimulates nucleoside diphosphokinase activity whereas at higher concentrations it is a potent inhibitor of enzymatic activity (Fig. 6).

In previous studies of nucleoside diphosphokinases (3, 4, 27-29), GDP concentration has not been emphasized as an important factor in the regulation of its enzymatic activity and hence in the regulation of nucleoside triphosphate formation. Rather, either the ATP:ADP ratio or the energy charge of the adenylate pool as defined by the expression ([ATP] + 1/2 [ADP]) / ([ATP] + [ADP] + [AMP]) (30) has been considered the major factor in the regulation of nucleoside diphosphokinase activity (31). These suggestions have been derived in part from studies which show that most nucleoside diphosphokinases are inhibited by either ADP (3, 4, 27-29) or AMP (27, 29) and that the relative
degree of inhibition is altered by the concentration of ATP in the assay system. The finding described here that GDP is just as potent an inhibitor of mitochondrial nucleoside diphosphokinase from liver as is ADP (3, 4), together with the previous finding that the activity of the liver enzyme is not affected by AMP (4), would suggest that the ATP: (ADP + GDP) ratio may be one of the more important factors regulating the transphosphorylation activity of this enzyme.

It would be of considerable interest to know the metabolic significance of the transphosphorylation of nucleoside diphosphates by ATP catalyzed by mitochondrial nucleoside diphosphokinase. Results presented here and elsewhere (5, 6, 8, 9) indicate that the mitochondrial enzyme has the necessary properties for transducing phosphate bond energy from mitochondrial ATP to the cytosol in the form of nucleoside triphosphates other than ATP. Thus, the enzyme is localized predominantly in the outer mitochondrial compartment (5, 6, 9, 10), it has relatively high affinities for substrates (Km < 150 μM) (Fig. 6), it reacts immediately and rapidly with ATP generated via oxidative phosphorylation (Figs. 1 and 3), and it is present in a wide variety of mitochondrial preparations (Table III). Such a role for mitochondrial nucleoside diphosphokinase is more easily justified than one in which the enzyme is depicted as simply furnishing nucleoside triphosphates by ATP catalyzed by the nucleoside diphosphokinase located in the outer mitochondrial compartment and on the small amount of nucleoside triphosphate-dependent processes may well depend on the ATP and nucleoside diphosphokinase pools endogenous to the inner compartment and on the small amount of nucleoside diphosphokinase activity associated with this compartment (<5.0% of the total activity) (5, 6), rather than on reactions catalyzed by the nucleoside diphosphokinase located in the outer compartment.

A role of mitochondrial nucleoside diphosphokinase in the transduction of phosphate bond energy from mitochondria to cytosol should be consistent with the findings that nucleoside diphosphokinase activity is present also in the cytosol of both liver (1, 40) and heart tissue (29). The interesting possibility that the mitochondrial and cytosolic enzymes in the liver cell are kinetically and physically distinguishable species which participate either in different metabolic processes or in similar processes that are subject to different modes of regulation is currently under investigation.

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REFERENCES


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