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STIMULATION OF PYRUVATE DEHYDROGENASE PHOSPHATASE ACTIVITY BY POLYAMINES

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Pyruvate dehydrogenase phosphatase requires Mg^{2+} or Mn^{2+}, and its activity in the presence of Mg^{2+} is markedly stimulated by Ca^{2+}. At saturating Mg^{2+} and Ca^{2+} concentrations, the polyamines spermine, spermidine and putrescine stimulated the activity of pyruvate dehydrogenase phosphatase 1.5- to 3-fold. Spermine was the most active of the polyamines. At a physiological concentration of Mg^{2+} (1 mM) and saturating Ca^{2+} concentration, the stimulation by 0.5 mM spermine was 4- to 5-fold, and at 0.3 mM Mg^{2+}, the stimulation was 20- to 30-fold. In the absence of Mg^{2+} or Ca^{2+}, spermine had no effect. These results suggest that a polybasic factor may be involved in the regulation of pyruvate dehydrogenase phosphatase activity.

Pyruvate dehydrogenase phosphatase, a mitochondrial phosphoprotein phosphatase, consists of two subunits with M_r of 50,000 and 97,000 (1). Phosphatase activity is associated with the former subunit. The latter subunit is a flavoprotein of unknown function. Pyruvate dehydrogenase phosphatase requires Mg^{2+} (apparent K_m about 2 mM) or Mn^{2+}, when acting on both its physiological substrate and phosphopeptide substrates (2, 3). At saturating Mg^{2+} concentration (about 10 mM), phosphatase activity toward phosphorylated pyruvate dehydrogenase is stimulated about 10-fold by micromolar concentrations of Ca^{2+} (4), provided the dihydrolipoamide acetyltransferase core enzyme of the pyruvate dehydrogenase complex is present (5). However, phosphatase activity toward phosphopeptide substrates is not affected by Ca^{2+}, whether or not the acetyltransferase is present (3). It appears that Ca^{2+} is not directly involved in pyruvate dehydrogenase phosphatase catalysis, but rather in specific positioning of the phosphatase on the acetyltransferase with respect to pyruvate dehydrogenase to facilitate the Mg^{2+}-dependent dephosphorylation.

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By contrast with pyruvate dehydrogenase phosphatase, the branched chain α-keto acid dehydrogenase phosphatase, also a mitochondrial phosphoprotein phosphatase, is active in the absence of divalent cations (6). Its activity is stimulated by protamine, but polyamines have no effect. In this communication, we report that polyamines markedly stimulate pyruvate dehydrogenase phosphatase activity at physiological concentrations of Mg^{2+}, and we discuss the possible physiological significance of this finding.

MATERIALS AND METHODS

Highly purified preparations of the pyruvate dehydrogenase complex, pyruvate dehydrogenase phosphatase, the branched chain α-keto acid dehydrogenase complex and branched chain α-keto acid dehydrogenase phosphatase were obtained from bovine kidney or heart as described (1, 6, 7). ^{32}P-labeled pyruvate dehydrogenase complex was prepared by incubating for 15 min at 30°C 0.5 ml of a solution containing 5-10 mg of the complex, 0.1 mM [γ-^{32}P]ATP (Amersham, ∿ 200,000 cpm/nmol), 2 mM $MgCl_2$, buffer A (50 mM imidazole, pH 7.3, 10% glycerol, 0.1 mM EDTA, 0.1 mM ethylene glycol bis(β-aminoethyl ether)-N,N'-tetraacetate, 1 mM benzamidine, 0.1 mM phenylmethanesulfonyl fluoride, 1 mM dithiothreitol). The solution was filtered through a column (10 x 1.5 cm) of fine Sephadex G-50 equilibrated and developed with buffer A. The pyruvate dehydrogenase complex contained 7-9 nmol of ^{32}P-labeled phosphoryl groups per mg of protein. ^{32}P-labeled branched chain α-keto acid dehydrogenase complex was prepared in a similar manner (6). Assay of pyruvate dehydrogenase phosphatase was based on the initial rate of release of ^{32}Pi from the phosphorylated pyruvate dehydrogenase complex. Prior to assay, the phosphatase was diluted with buffer A containing 0.2% bovine serum albumin. The standard assay mixture contained diluted phosphatase, 10 mM $MgCl_2$, 0.1 mM $CaCl_2$ and additions as noted in the text in a volume of 40 µl. The solution was incubated for 2 min at 30°C in a plastic microcentrifuge tube. The reaction was initiated with 10 µl of ^{32}P-labeled pyruvate dehydrogenase complex (5-10 mg/ml). After 4 min at 30°C, 0.25 ml of 10% trichloroacetic acid and 0.1 ml of 0.2% bovine serum albumin were added. The mixture was centrifuged for 2 min in an Eppendorf microcentrifuge. A 0.2-ml aliquot of the supernatant fluid was removed, added to 10 ml of aqueous counting scintillant (Amersham), and counted. One unit of pyruvate dehydrogenase phosphatase is the amount of enzyme that releases 1 nmol of ^{32}Pi per min at 30°C. The extent of release of total phosphoryl groups was kept below 20% to ensure linearity. Protein was determined as described by Bradford (8). In certain experiments pyruvate dehydrogenase phosphatase activity was determined by measuring the initial rate of reactivation of phosphorylated inactive pyruvate dehydrogenase complex (1).

RESULTS AND DISCUSSION

At saturating concentrations of Mg^{2+} (10 mM) and Ca^{2+}(0.1 mM), the polyamines spermine, spermidine and putrescine stimulated the activity of highly purified pyruvate dehydrogenase phosphatase 1.5- to 3-fold (Fig. 1). Spermine (Fig. 1A) was more effective than spermidine (Fig. 1B) and putrescine (data not shown). At a physiological concentration of Mg^{2+} [about 1 mM

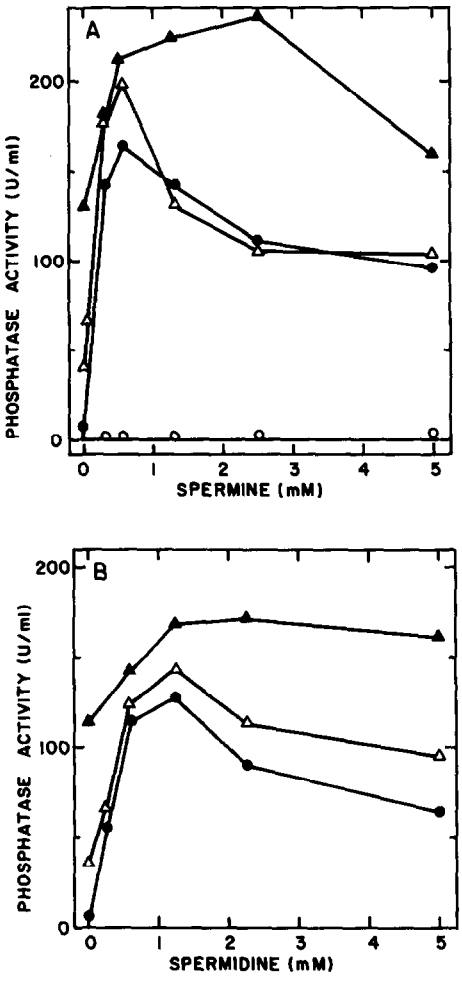

Fig. 1. Effect of spermine (A) and spermidine (B) on pyruvate dehydrogenase phosphatase activity at different concentrations of Mg^{2+}. The assay mixtures contained phosphatase, 0.1 mM $CaCl_2$, $MgCl_2$, and the indicated concentrations of polyamine in 40 µl of buffer A.[2] The mixtures were incubated at 30°C for 2 min, and then 10 µl of ^{32}P-labeled pyruvate dehydrogenase complex (5 mg/ml) was added. Incubation was continued for 4 min, and then the amount of ^{32}Pi released was determined. The Mg^{2+} concentrations were 10 mM (▲), 1 mM (△), 0.3 mM (●) and 0 mM (○).

(9, 10)], pyruvate dehydrogenase phosphatase activity was stimulated 4- to 5-fold by 0.5 mM spermine (Fig. 1A), and at 0.3 mM Mg^{2+}, stimulation by 0.5 mM spermine was 20- to 30-fold. The spermine-stimulated release of ^{32}Pi correlated with reactivation of the pyruvate dehydrogenase complex (data not shown). L- Arginine was inactive at concentrations up to 10 mM. In the absence of Mg^{2+}, spermine was ineffective. Thus spermine can spare but not completely replace Mg^{2+}. Spermine also did not replace Ca^{2+} (data not shown).

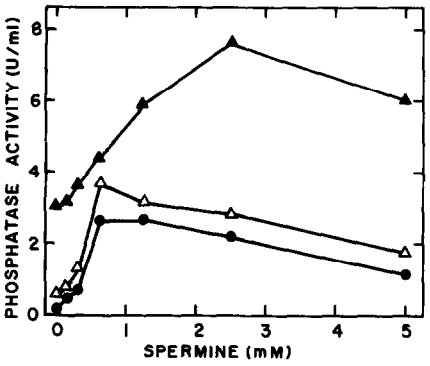

Fig. 2. Effect of spermine on pyruvate dehydrogenase phosphatase activity toward ^{32}P-labeled branched chain α-keto acid dehydrogenase complex. The assay mixture contained 10 μl of ^{32}P-labeled branched chain α-keto acid dehydrogenase complex (5 mg/ml) instead of ^{32}P-labeled pyruvate dehydrogenase complex, and 10 mM (▲), 1 mM (△) or 0.3 mM (●) Mg^{2+}. Other components and conditions were as described in Fig. 1.

Pyruvate dehydrogenase phosphatase exhibited slight activity with ^{32}P-labeled branched chain α-keto acid dehydrogenase complex, i.e., 0.5-1.0% of the activity observed with ^{32}P-labeled pyruvate dehydrogenase complex (6). This activity was Mg^{2+}-dependent and Ca^{2+}-independent. Thus, phosphorylated branched chain α-keto acid dehydrogenase complex is an alternate substrate for pyruvate dehydrogenase phosphatase. The effect of spermine on pyruvate dehydrogenase phosphatase activity with ^{32}P-labeled branched chain α-keto acid dehydrogenase complex as substrate (Fig. 2) was similar to that observed with ^{32}P-labeled pyruvate dehydrogenase complex (Fig. 1A). By contrast with pyruvate dehydrogenase phosphatase, branched chain α-keto acid dehydrogenase phosphatase activity toward phosphorylated branched chain α-keto acid dehydrogenase complex was not affected by spermine or spermidine (6). Branched chain α-keto acid dehydrogenase phosphatase exhibited about 10% of maximal activity with ^{32}P-labeled pyruvate dehydrogenase complex as substrate (6), but this activity was not affected by spermine (data not shown). These results suggest that polyamines act, at least in part, directly on pyruvate dehydrogenase phosphatase.

Although there are many reports that polyamines stimulate or inhibit various enzymes, the physiological role of the polyamines and the mechanism of their action is still obscure (11). The stimulatory effect of polyamines on

pyruvate dehydrogenase phosphatase activity may be relevant to the insulin stimulation of pyruvate dehydrogenase complex activity in adipose tissue. Several laboratories have reported that insulin stimulated release from plasma membranes of a substance that increased pyruvate dehydrogenase complex activity of mitochondrial preparations (12-15); this putative chemical mediator is proposed to act by stimulating pyruvate dehydrogenase phosphatase activity (16). It is interesting to note that the assay for the phosphatase was carried out in the presence of a suboptimal concentration of Mg^{2+} (50 μM) (16). In view of the marked stimulation of pyruvate dehydrogenase phosphatase activity by polyamines at physiological Mg^{2+} concentrations, it is possible that the putative insulin mediator is polybasic in character.

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