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REVERSAL OF PALMITOYL COENZYME A-CAUSED INHIBITION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE BY POLYAMINES

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SUMMARY: Spermine and spermidine released yeast glucose-6-P dehydrogenase from palmitoyl-CoA-induced inhibition. Spermidine was less effective for canceling the inhibition of the enzyme than spermine. Putrescine hardly released the enzyme from the inhibited state. Spermine enhanced slightly the enzyme activity, whereas spermidine and putrescine exerted no activating effect on the enzyme activity.

INTRODUCTION: It has been demonstrated that long chain fatty acyl-CoA thioesters, such as palmitoyl-CoA inhibit glucose-6-P dehydrogenase and also several other enzymes (1,2). It has also been found that long chain fatty acyl-CoA thioester concentrations are high in fasted animals, in which cell function is probably depressed (3-6). Thus, it is supposed that these thioesters are natural inhibitors of these enzymes and play a role in the regulation of several cell function. On the other hand, it has also been demonstrated that polyamine concentrations are high in the tissues with high rates of nucleic acids and protein synthesis (7-9). Polyamines have been found to exert stimulating effect on the activities of several enzymes, such as DNA polymerase and RNA polymerase (10,11). It is also known that phosphorylase remains in a form in the presence of polyamines (12). Hence, polyamines are also supposed to be physiological modulators controlling cell functions. These two groups of modulators seem to play respective roles antagonistic with each other in the regulation of cell functions.

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In the present study, we found canceling effect of polyamines on palmitoyl-CoA caused inhibition of yeast glucose-6-P dehydrogenase. This seems to suggest that polyamines and long chain fatty acyl-CoA thioesters are respectively positive and negative modulators to control the activity of glucose-6-P dehydrogenase.

MATERIALS AND METHODS: Glucose-6-P dehydrogenase (EC 1.1.1.49) (ca. 140 units/mg protein), glucose-6-P and $NADP^+$ were purchased from Boehringer Mannheim Co., Germany. Palmitoyl-CoA, putrescine, spermidine and spermine were obtained from Sigma, St. Louis. Glucose-6-P dehydrogenase activity was measured spectrophotometrically at 20°C. Temperature was controlled by a thermostat using thermoelement (Coolnics CTR-120 and CTE-120, Komatsu electronics Inc., Japan). The increase in absorbance at 340 nm was recorded on a dual beam spectrophotometer (Hitach 200-10) equipped with a recorder (Hitachi 200). The reaction mixture contained 0.10 mmol Tris-HCl buffer, pH 7.4, 0.50 μmol glucose-6-P, 0.20 μmol $NADP^+$ and 0.25 μg of enzyme in a total volume of 2.0 ml. The reaction was started by addtion of enzyme.

RESULTS: Fig. 1 shows a trace of the change in absorbance at 340 nm due to $NADP^+$ reduction caused by yeast glucose-6-P dehydrogenase, recorded on a dual beam spectrophotometer. The rate of $NADP^+$ reduction was very low in the presence of 10 μM palmitoyl-CoA as compared with that observed in the absence of palmitoyl-CoA. Glucose-6-P dehydrogenase is inhibited by palmitoyl-CoA, as has been demonstrated by Taketa and Pogell (1). Adding either spermine or spermidine at 80 seconds of the inhibition of the reaction resulted in reversal of palmitoyl-CoA induced inhibition of glucose-6-P dehydrogenase, but putrescine failed to reverse the inhibition (Fig. 1B, C and D). Polyamines exerted very slight effect on the enzyme activity in the absence of palmitoyl-CoA. Extent of spermine-induced reversal of the inhibition decreased slightly during the prolonged enzyme reaction in the presence of palmitoyl-CoA (Table 1). By adding spermine at the start of reaction, the enzyme activtity in the presence of palmitoyl-CoA was almost the same as that

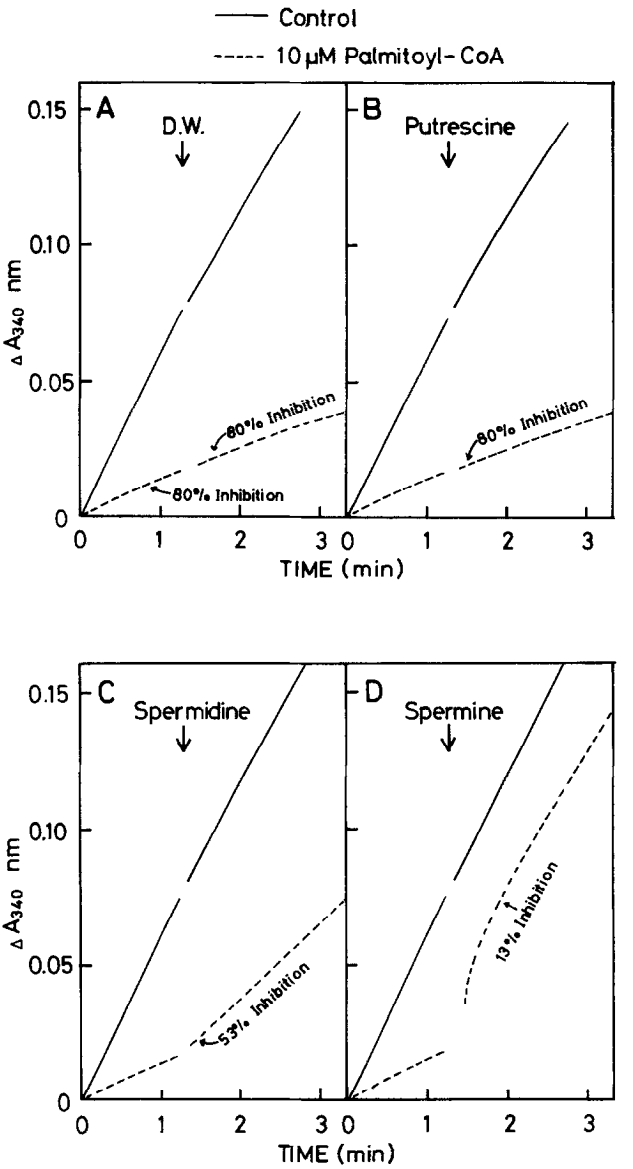

Fig. 1. Reversal by polyamine of the inhibition of yeast glucose-6-P dehydrogenase by palmitoyl-CoA. At 80 seconds from the start of the reaction, 0.05 ml of 40 mM each polyamine (putrescine, spermidine and spermine) was added to the reaction mixture. Solid and broken lines respectively show the chenges in the absorbance at 340 nm in the absence and the presence of palmitoyl-CoA. Concentration of palmitoyl-CoA in the reaction mixture was 10 μM. Arrow mark shows the time of polyamine addition. D.W.: distilled water.

Table 1. Reversal of palmitoyl-CoA induced inhibition by spermine. The reaction was started by adding enzyme. Palmitoyl-CoA (2.5 μM) was added prior to the start of the reaction. At indicated time, 0.05 ml of 40 mM spermine was added to the reaction mixture. Values in parentheses indicate ratios (in percent) of the activity in the presence of palmitoyl-CoA to control one.

Palmitoyl-CoA (2.5 μM)	Addition	Time of spermine addition after start of reaction	Glucose-6-P dehydrogenase activity	
			Before addition of spermine	After addition of spermine
		sec	$\Delta A_{340}/min$	
-	Water	80	0.061 (100)	0.055 (100)
+	Water	80	0.038 (61)	0.031 (56)
+	Spermine	0		0.062 (113)
+	Spermine	40	0.038 (61)	0.056 (102)
+	Spermine	80	0.038 (61)	0.050 (91)
+	Spermine	120	0.038 (61)	0.045 (82)
+	Spermine	200	0.038 (61)	0.042 (76)

observed without the compound. However, the extent of the inhibition-reversal decreased gradually during 200 seconds of the inhibited reaction. Fig. 2 shows the effect of polyamines on the glucose-6-P dehydrogenase activity in the presence and the absence of palmitoyl-CoA. The enzyme reaction was initiated in the presence of palmitoyl-CoA, and polyamines were added just before the start of the enzyme reaction. In the absence of palmitoyl-CoA, spermine caused very slightly elevation of the activity (Fig. 2C). Adding spermine at concentrations above 0.2 mM, the enzyme activity observed in the presence of palmitoyl-CoA was similar to that observed without the inhibitor. Spermidine also canceled, to some extent, the inhibition of the enzyme caused by palmitoyl-CoA, but was not so effective to release the palmitoyl-CoA induced inhibition as spermine was. Putrescine failed to cancel the inhibition of

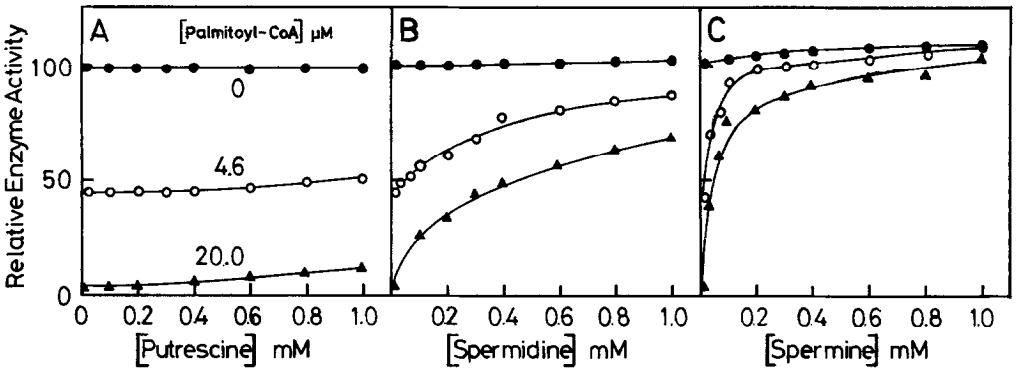

Fig. 2. Protection of glucose-6-P dehydrogenase from palmitoyl-CoA-caused inhibition by polyamine. The reaction was started by addition of enzyme to the complete reaction mixture containing polyamine and/or palmitoyl-CoA. Initial rate of $NADP^+$ reduction was calculated from the record of the change in absorbance at 340 nm. Solid circules indicate relative activity of glucose-6-P dehydrogenase in the absence of palmitoyl-CoA. Open circules and solid triangles respectively indicate relative activity in the presence of palmitoyl-CoA at concentrations of 4.6 and 20.0 mM. Relative enzyme activity is expressed as percentage of the activity to that estimated without palmitoyl-CoA and polyamines.

glucose-6-P dehydrogenase as far as examined. In some enzymes, which have been known to be inhibited by palmitoyl-CoA (1), reversal of the inhibition occured in the presence of polyamines. Canceling effect of polyamines on the inhibition of several other enzymes by palmitoyl-CoA will be published elsewhere.

DISCUSSION: Canceling effect of polyamines on palmitoyl-CoA-induced inhibition of glucose-6-P dehydrogenase was observed at concentrations of polyamines within physiological range (13). This suggests that polyamines cause an elevation of glucose-6-P dehydrogenase activity in the cells with high intracellular concentrations of long chain fatty acyl-CoA thioesters.

Recently, it has been demonstrated that polyamines play an important role in mitosis, especially in DNA synthesis (10,13-15). The inhibitors for polyamine synthesis, such as α-hydrazinoornitine,

exerts harmful effect on DNA synthesis and mitosis (13-15). DNA polymerase activity is reportedly enhanced by polyamines (10). It can be easily supporsed that deoxyribonucleotides, DNA precoucers, are indispensable in the cells, in which DNA synthesis is going to occur. The levels of deoxyribonucleotides, which have been found to be very low in the resting cells, should be elevated due to their production by the reduction of ribonucleotides (16-18). In the reaction catalyzed by ribonucleoside diphosphate reductase, NADPH is indispensable as the reductant (19,20). The reaction catalyzed by thymidylate synthase is known to be sensitive to depressed level of tetrahydrofolate, which is produced due to the reduction of dihydrofolate using NADPH as the reductant. In the reaction catalyzed by glucose-6-P dehydrogenase, $NADP^+$ is reduced, and hence increase in intracellular concentrations of polyamines probably caused an elevation of deoxyribonucleotide levels as well as the rate of DNA synthesis, even in the cells with high concentrations of long chain fatty acyl-CoA thioesters.

The mechanism for canceling the palmitoyl-CoA caused inhibition by polyamines is not known at present and is now under investigation.

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