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3. Effects of Polyamines on the Activities of Corn Glucose-6-Phosphate Dehydrogenase, 6-Phosphogluconate Dehydrogenase, and Protein Kinase

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生體發生 및 分化機構의 細胞生物學的 硏究 Ⅷ

3. Polyamine이 옥수수 胚의 Glucose-6-Phosphate Dehydrogenase, 6-Phosphogluconate Dehydrogenase 및 Protein Kinase 活性에 미치는 影響

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ABSTRACT

Palmitoyl CoA was found to inhibit corn embryo axis glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, which were also inhibited by polyamines. However, reversal of inhibition of both enzymes by palmitoyl CoA was made by spermine. Activity of corn embryo axis protein kinase was found to increase steadily after germination. Activation and inhibition of protein kinase were made by $MgCl_2$ and all polyamines, respectively. Such results suggest that fatty acid biosynthesis and lypolysis could be regulated to some extent by polyamines in corn embryo axis.

INTRODUCTION

Glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (EC 1.1.1.44) are known to get involved in hexose monophosphate shunt and produce NADPH, which is utilized for long chain fatty acid biosynthesis while there is

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increasing interest to whether a similar regulatory mechanism including hormone sensitive lipase phosphorylation operates in plant tissue in view of the importance of protein phosphorylation-dephosphorylation in the regulation of cellular processes in animal tissue. The enzyme responsible for phosphorylation is known to be protein kinase (EC 2.7.1.37), which has been characterized in wheat germ (Davies and Polya, 1983; Rychlik and Zagorski, 1980; Yan and Tao, 1982a). In addition, endogenous substrate for wheat germ protein kinase was also found as T-substrate (Yan and Tao 1982b). As another light-dependent enzyme in plants, protein kinase was found to be dependent in spinach chloroplast (Alfonzo *et. al.*, 1980). Interestingly, protein kinase activity was also found to be c-AMP independent in wheat germ (Rychlik and Zagorski, 1980) as animals.

Possible candidates to regulate the enzyme activities are long chain fatty acyl CoA and polyamines for glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, magnesium ion and polyamines for protein kinase. Long chain fatty acyl CoA such as palmitoyl CoA, inhibits glucose-6-phosphate dehydrogenase (Kawaguchi and Bloch, 1974; Mita and Yasumasu, 1980; Taketa and Pogell, 1966). Polyamines which has been suggested to substitute the role of magnesium ion (Takeda and Igarashi, 1969; Igarashi and Takeda, 1970; Kayne and Cohn, 1972; Pastuszyn and Loftfield 1972). Putrescine, one of polyamines, are found to increase in corn after germination whereas the rest of them, spermidine, and spermine, decrease (Cho *et al.*, 1983). Such fluctuations in each polyamine might have a regulation mechanism as far as limited number of enzyme is concerned.

The present paper deals with inhibition of glucose-6-phosphate dehydrogenase by palmitoyl CoA and reversal by polyamines, and the effect of magnesium ion and polyamines on protein kinase activity and dependence of protein kinase on time after germination. The results presently obtained suggest that polyamine might regulate the pentose monophosphate shunt cycle and lypolytic activity to some extent through regulation of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, and protein kinase.

MATERIALS AND METHODS

Plant materials; Corn (*Zea may* L.) plants were grown in vermiculite in a growth chamber and were harvested when they were between 1 and 7 days old. Embryo axis was washed in distilled water and used in the preparation of enzymes. Chemicals; Palmitoyl CoA, putrescine, spermidine, spermine, $NADP^+$, glucose-6-phosphate, 6-phosphogluconate casein and bovinealbumin were purchased from Sigma Chemical Company. [γ-^{32}P]ATP was purchased from Amersham Corp.. All other reagents were of analytical grade. Preparation of glucose-6-phosphate and 6-phosphogluconate dehydrogenase, and

protein kinase; The first two enzymes were prepared using the method described elsewhere in stead of urchin eggs (Mita and Yasumasu, 1980). Protein kinase was obtained by using the method in wheat germ (Yan and Tao 1980a) except affinity chromatography. Protein determination; Protein was measured with bovine serum albumin standard. Enzyme assays; The enzyme reaction was performed at 20°C in a Hitachi 200-10 double beam spectrophotometer equipped with recorder and the increase in absorbance at 340 nm was recorded in case of both hydrogenases. The other conditions not described are same (Cho *et al.*, 1982; 1983). Protein kinase was assayed by the following method. The standard incubation mixture (0.2 ml) contained 50 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, 1.5 mg/ml of casein bovine serum albumin, 0.2 mM [γ-^{32}P] ATP (1.5~3.0 cpm/pmole), and enzyme protein. The incubation was conducted at 37°C for 5min and the reaction was terminated by the addition of about 2 ml of 10 % trichloroactic acid. The radioactivity incorporated into casein or bovine serum albumin was determined as described elsewhere (Tao *et al.*, 1980). Purification of protein kinase; All procedures were carried out at 0~4°C unless described elsewhere. The other conditions were same except DEAE-cellulose column.

RESULTS AND DISCUSSION

Palmitoyl CoA inhibited glucose-6-phosphate dehydrogenase in corn as shown in Fig. 1 after preincubation for 5 min of the enzyme preparation with the complete reaction

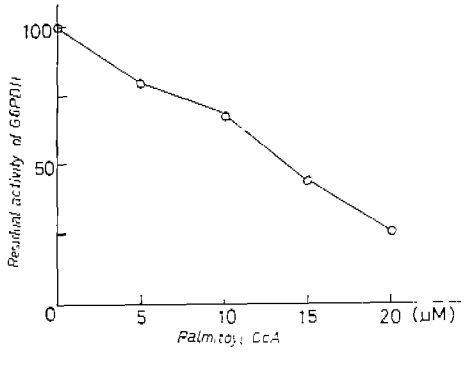

Fig. 1. Dependence of corn embryo axis glucose-6-phosphate dehydrogenase by palmitoyl CoA.

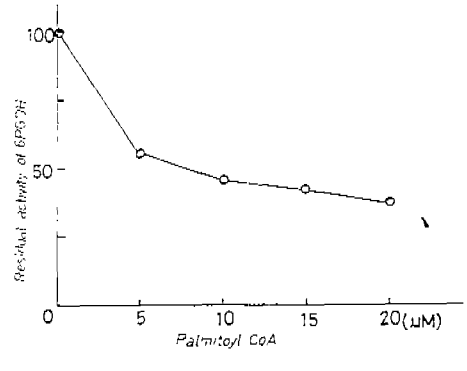

Fig. 2. Inhibition of corn eembryo axis 6-phosphoglucolate dehydrognase by palmitoyl CoA.

mixture minus $NADP^+$ in the absence of palmitoyl CoA. The same inhibition was also found in the preincubation minus both glucose-6-phosphate and $NADP^+$. without prein-

cubation palmitoyl CoA was found to exert any inhibition. The same result has been demonstrated in sea urchin eggs (Mita and Yasumasu, 1980), and yeast (Taketa and Pagell, 1966). Fifty percent inhibition of the enzyme in corn was obtained by palmitoyl CoA at concentration of 12.5 μM for glucose-6- phosphate dehydrogenase. The value is higher than that of urchinegg (Mita and Yasumasu, 1980). Palmitoyl CoA hydrolase activity was not checked and it was not sure if there was any change in palmitoyl CoA level during incubation period. However, the inhibition at higher concentration of palmitoyl CoA did not seem to be the presence of palmitoyl CoA hydrolase since inhibition of 6-phosphogluconate dehydrogenase by palmitoyl CoA was observed at 5 μm in Fig. 2, which was also demonstrated in sea urchin (Nita and Yasumasu, 1980). 6-phosphogluconate dehydrogenase was reported to be not stable in the dilution (Mita and Yasumasu, 1980). However, such trend could not be observed in corn. Adding polyamines, putrescine, spermidine and spermine after the start of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase resulted in inhibition of both enzyme as shown in Table 1. The degree of inhibition was found to be dependent on polyamine

Table 1. Dependence of corn embryo axis glucose-6-phosphogluconate dehydrogenase inhibitions on polyamine concentration

Polyamine concentration	Residual activity of glucose-6-phosphate dehydrogenase*	Residual activity of 6-phosphogluconate dehydrogenase*
putrescine (40 mM)	80	47
spermidine (40 mM)	63	55
spermine (40 mM)	29	31
putrescine (20 mM)	90	55
spermidine (20 mM)	80	69
spermine (20 mM)	80	37

* The control is arbitrarily given 100 and calculated on the basis of unit/mg protein.

Table 2. Effect of polyamine on inhibition of corn embryo axis glucose-6-phosphate dehydrogenase by palmitoyl CoA.

Polyamine concentration	Reversal activity of glucose-6-phosphate dehydrogenase*	Reversal activity of 6-phosphogluconate dehydrogenase*
putrescine (40 mM)	101	107
spermidine (40 mM)	127	105
spermine (40 mM)	139	133

* The control is arbitrarily given 100 and calculated on the basis of unit/mg protein.

Table 3. Radioactivity of casein and bovine serum albumin after corn embryo axis protein kinase incubated with [γ-^{32}P]ATP

[γ-^{32}P]— Acceptor	Protein kinase activity([γ-^{32}P]—incorporated)
Casein	152500 cpm
Bovine serum albumin	47300 cpm

concentration. Spermine was found to be the most effective inhibitor for both enzymes. Polyamines have been found to have reversal effect on inhibition of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase by palmitoyl CoA (Mita and Yasumasu, 1980). Polyamines are found to be so inhibitory that inhibition of both enzyme by palmitoyl CoA is expected to be higher in the presence of polyamines in corn. Unexpectedly, there was reversal palmitoyl CoA inhibition of both enzyme by polyamine, especially spermine as shown in Table2. These polyamine themselve were also reported to reduce enzyme activity in the presence of palmitoyl CoA (Mita and Yasumasu, 1980). If palmitoyl CoA level is so sufficient that it inhibits glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, these enzyme activities are probably reactivated by polyamines. As shown in Table 2, spermine is the most effective in the reversal of the enzyme activity. Therefore, the enzyme activity is responsible for formation including reverse enzyme system (Jänne et al., 1978; Seiler *et al.*, 1978; Seiler *et al.*, 1981). Furthermore, the concentration of $NADP^+$ was reported to depend on activation of NAD kinase. This finding suggests that increase of $NADP^+$ concentration also releases dehydrogenases from palmitoyl CoA coused inhibition since

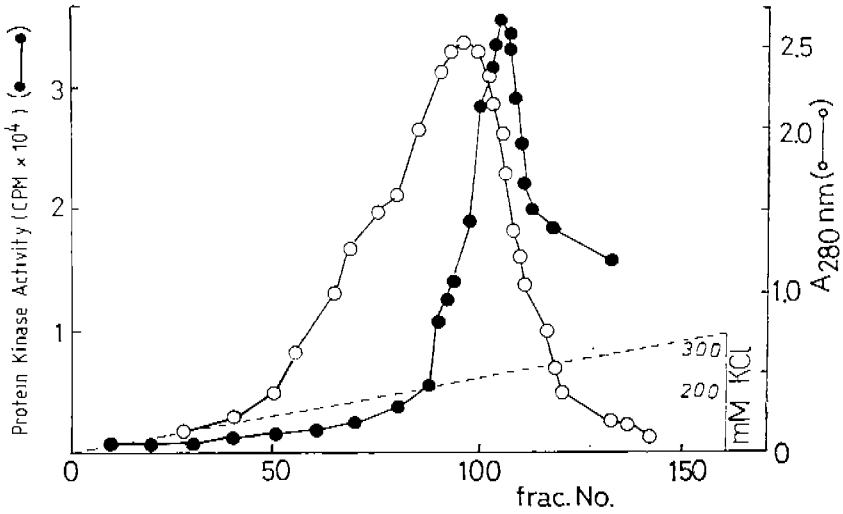

Fig. 3. Purification of corn embryo axis protein kinase by DEAE-cellulose colum chromatography. Each fraction was 4.6 ml

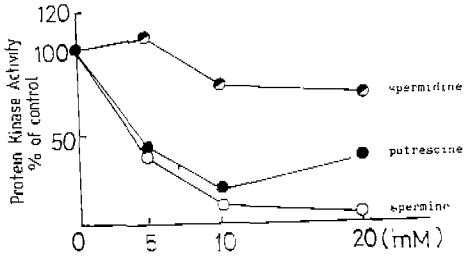

Fig. 4. Effect of polyamines on corn embryo axis protein kinase

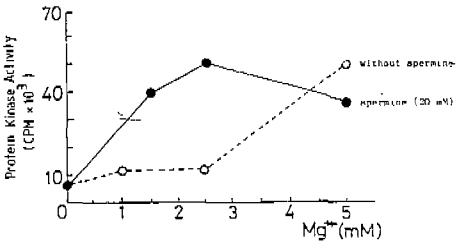

Fig. 5. Activation of corn embryo axis protein kinase

the type of inhibition is competive with respect to $NADP^+$ (Mita and Yasumasu, 1980). If such mechanism is in corn, NAD kinase will be a critical enzyme. The corn embryo asis protein kinase was partially purified and fraction number 100 was found to be the most active Figure 3. In this study, fraction from 95 to 105 was utilized. The enzyme catalyzes the phosphorylation of casein significantly but not of bovine serum albumin as shown in Table1. Accordingly, the enzyme might belong to casein kinase referred to in wheat germ (Davies and Polya, 1983). The activity of the protein kinase is known to be strongly inhibited by spermine and be slightly inhibited by spermidine (Yan and Tao 1982). And putrescine is also known to inhibit the protein kinase. Such trends (Figure 4) are in a good agreement to revious results (Yan and Tao 1982). Spermine was observed to be the most effective inhibitor. $MgCl_2$ was found to enhance the enzyme activity at higher than 2.5 mM of $MgCl_2$ and reverse the inhibition of the enzyme by spermine as shown in Figure 5. Such trend was also demonstrated in wheat germ (Davies and Polya, 1983). The protein kinase activity was found to increase after germination as shown in Figure 6. It suggests that the protein kinase should get involved in phosphorylation of proteins including enzymes.

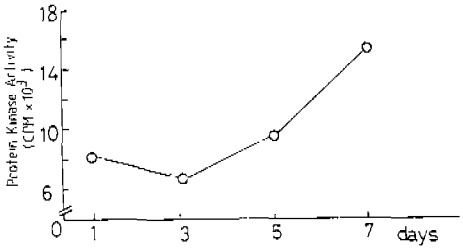

Fig. 6. Dependence of corn embryo axis protein kinase activity on the time course in corn

Polyamines themselves inhibit glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase responsible for NADPH formation, and reversed inhibition of both enzyme by palmitoyl CoA. Such results suggest that long chain fatty acid biosynthesis will not be interupted as long as the adequate concentration of polyamines is kept in corn. If protein kinase is responsible for lipase phosphorylation which is active in plant like animals, accumulation of fatty acid would be in plant and utilized for grown cells in connection with structural function. Increase in activity the protein kinase would be

made by light in spinach (Alfonzo *et al.*, 1980). Still little is know about protein kinase in plant tissue and the role of the protein kinase should have been throughly done.

摘 要

Palmitoyl CoA는 옥수수 배 glucose-6-phosphate dehydrogenase와 6-phosphogluconate dehydrogenase를 저해함을 찾았다. 또한 두 효소는 polyamine에 의하여 저해된다. 그러나 palmitoyl CoA에 의한 두 효소의 저해를 spermine에 의하여 억제할 수 있음을 알았다. 옥수수 배 protein kinase의 활성도는 발아후 점차 증가함을 보였다. Protein kinase의 활성화 및 저해는 $MgCl_2$와 모든 polyamine에 의하여 각각 이루워졌다. 이러한 결과는 지방산 합성 및 지질문제가 옥수수 배에서 polyamine에 의하여 어느 정도 조절될 수 있음을 암시한다.

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