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Studies on the Mechanisms of Gibberellic Acid Action

Ⅱ. Regulation of Protein Biosynthesis and Phosphorylation by GA_3 in the Presence of Actinomycin D

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Gibberellic Acid의 作用 機作에 관한 硏究

Ⅱ. Actinomycin D 처리시 GA_3에 의한 蛋白質의 生合成 및 燐酸化反應의 調節

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ABSTRACT

As a part of the studies on the regulatory mechanism of gene expression by gibberellic acid, the effects of GA_3 on the protein biosynthesis and phosphorylation in maize seedlings were investigated in the presence of actinomycin D. The activities of protein biosynthesis and phosphorylation in germinating seeds treated with GA_3 were greater than those of the control at the 3-day point after germination. It is assumed that the enhancement of protein biosynthesis by GA_3 in the presence of actinomycin D is due to the effects of GA_3 on the translational processes in which protein is produced from the mRNA synthesized previously.

INTRODUCTION

It is well known that gibberellic acid regulates the growth and differentiation of plants. In connection with the mechanisms of GA_3 action, it has been demonstrated that GA_3 enhanced the synthesis of enzymes (Chrispeels and Varner, 1967; Jacobsen and Varner, 1967) and that GA_3-induced synthesis of various enzymes was suppressed in

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the presence of actinomycin D (Varner and Chandra, 1964). Chen and Osborne (1970) reported GA_3 is closely connected with the transcription of mRNA from the DNA template and the translation of mRNA in the process of protein synthesis. In the previous communication (Sim and Roh, 1979), we reported that protein biosynthesis was stimulated by the addition of exogenous GA_3. The present report describes the effects of GA_3 on the protein biosynthesis and phosphorylation in the presence of actinomycin D. This investigation was carried out in order to study whether GA_3 facilitates the protein biosynthesis at the transcriptional level or at the translational level.

MATERIALS AND METHODS

Materials. Maize seeds (*Zea mays* L. cv. Golden growthbandam) were purchased from Sakata seed Co. Gibberellic acid was a Sigma product. Actinomycin D was supplied by P-L Biochemicals, Inc. (U.S.A.). ^{14}C-labeled phenylalanine (specific activity. 513 mCi/m mol) was obtained from the Radiochemical Centre, Amersham (England).

Seed germination. The weight of maize seeds varied from 0.1 to 0.23 g and hence seeds of a similar weight (200±5 mg) were selected. Maize seeds were sterilized in 20% sodium hypochlorite solution for 15 min and washed 2 times with sterilized distilled water. For the experiment in protein biosynthesis, sterilized material was soaked in the Nitsch medium for 5 hr, and then 0.125 μCi ^{14}C-phenylanine was injected into each seed. For the test on protein phosphorylation, 11.5 μCi ^{32}P was added to 5 ml Nitsch medium. In case of necessity, 40 μM actinomycin D was added to Nitsch medium, and the endosperm material was carefully removed from the shoots and embryonic axis and weighed. The seeds were germinated at 30°C.

Preparation of crude proteins. Crude proteins were prepared from maize seedlings as described previously (Sim and Roh, 1979). All operations were carried out at 2~4°C. Maize seedlings taken at the selected time intervals after imbibition were weighed, homogenized in a mortar with sand, and extracted with 0.2 M phosphate buffer (pH 7.5) containing 0.01 M EDTA, 0.01 M KCl and 0.001 M $MgCl_2$.

Homogenates or crude extracts were filtered through cheesecloth and centrifuged at 4,500 g for 20 min. The pellet was discarded and 10% TCA was added to the supernatant to a final concentration of 5% TCA. The suspension was left, with occasional shaking, at 0°C for 15 min and centrifuged at 10,000 g for 10 min. The pellet was resuspended in cold 5% TCA and recentrifuged at 10,000 g for 10 min. The pellet was washed twice with cold 5% TCA. After washing, the resultant pellet was designated as crude soluble protein fraction.

Purification of proteins. According to the method of Schneider (1945), proteins were purified from crude protein.

(1) Crude soluble proteins were extracted with cold 10% TCA (3 times for 30 min,

15 min and 15 min), and (Ⅱ) the residue was extracted twice successively with 95% ethanol. (Ⅲ) The residue thus obtained was treated 3 times with ethanol-ether (3 : 1) for 3 min in a boiling water to remove lipid. (Ⅳ) The residue obtained from procedure (Ⅲ) was suspended in cold 10% TCA. (Ⅴ) The residue collected by centrifugation was resuspended in 5% TCA and heated for 15 min at 90°C. (Ⅵ) The resulting residue was dissolved by boiling with 2% NaOH for 10 min, which is referred to as a purified protein fraction.

Measurement of radioactivity. The radioactivities of crude protein and purified protein fractions were determined in Liquid-Scintillation-Spectrometer (Beckman LS-100) for measurements of protein biosynthesis and phosphorylation.

RESULTS

Effect of concentration of actinomycin D. For the purpose of determining the concentration of actinomycin D which inhibits the protein biosynthesis by 50%, the effect of

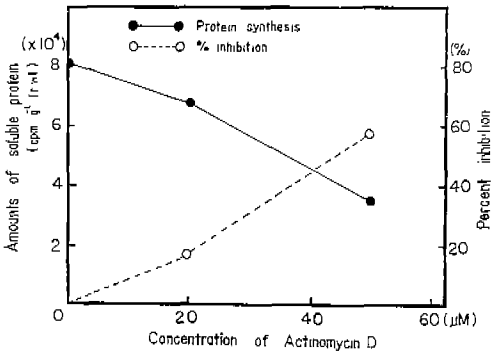

Fig. 1. Effect of actinomycin D on the protein biosynthesis. Maize seeds injected with ^{14}C-phenylalanine (0.125 μCi/2.5 μl/seed) were germinated in Nitsch medium at 30°C for 2 days. The amounts of soluble proteins were measured by counting the radioactivities in the crude protein extracts.

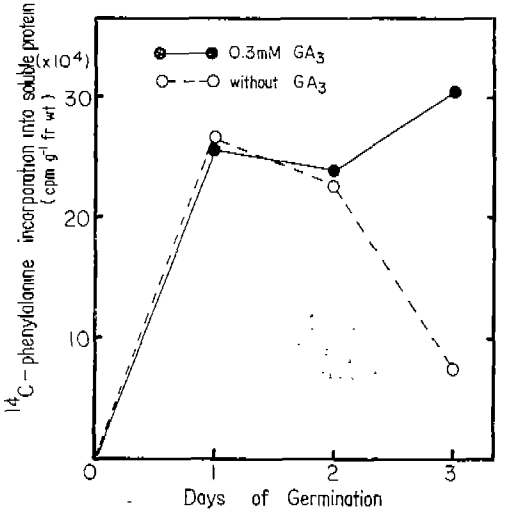

Fig. 2. Effect of GA_3 on the protein biosynthesis in germinating maize seeds in the presence of actinomycin D. Maize seeds injected with ^{14}C-phenylalanine (0.125 μCi/2.5 μl/seed) were germinated in Nitsch medium containing actinomycin D. The amounts of soluble proteins were measured by counting the radioactivities in the crude protein extracts.

actinomycin D on the protein biosynthesis in the germinating maize seed was examined. As shown in Fig. 1, protein biosynthesis was reduced with increasing the concentration of actinomycin D, and 40 μM actinomycin D showed inhibition of about 45%.

In view of the above result, we have used 40 μM actinomycin D as an inhibitor of transcription in all experiments.

The amount of proteins. The effect of GA_3 on the amount of proteins in maize seedlings during the germination was investigated in the presence of 40 μM actinomycin D. As shown in Fig. 2, in both experiments with and without GA_3 the amounts of proteins was increased by the first day of germination. After 24 hr germination, the amount of protein from seedlings soaked in GA_3 remained nearly unchanged until the third day of germination, while that from the untreated seedlings declined strikingly at 3 day-point after germination.

These results suggest that GA_3 stimulates protein biosynthesis at the translational level. Fig. 3 showed the amount of proteins secreted into the endosperm. Those from seedlings both treated and untreated with GA_3 were increasing during the first 2 days of germination. However, the maximum value was observed after 3 days incubation in the experiment with GA_3, whereas the amounts of proteins rather decreased following the second day of germination in the absence of exogenous GA_3.

Activity of protein phosphorylation. The effect of GA_3 on the protein phosphoryla-

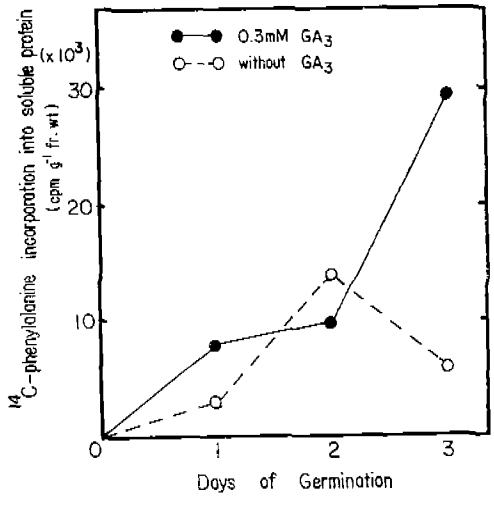

Fig. 3. The amounts of proteins secreted into endosperm. Maize seeds were germinated in the presence of actinomycin D. The amounts of ^{14}C-phenylalanine incorporated into soluble proteins were measured by the method as described in "materials and methods"

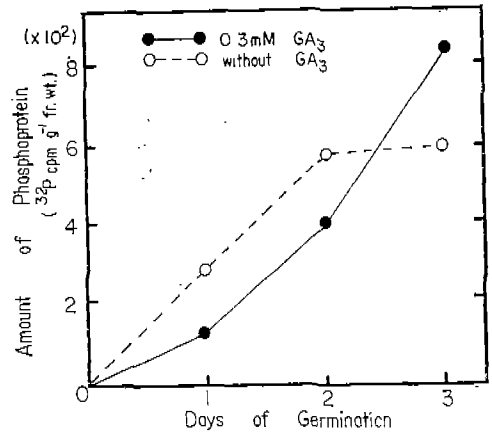

Fig. 4. Effect of GA_3 on the protein phosphorylation in the presence of actinomycin D. Soluble proteins were isolated from germinating maize seeds treated with actinomycin D and ^{32}P (11.5 μCi /4 ml medium containing 10 seeds). The radioactivities in the protein fractions were counted for the estimation of the amounts of phosphoprotein.

tion under the condition of transcriptional inhibition was investigated. As shown in Fig. 4, the activities of the protein phosphorylation were increased during the germination in both experiment with and without GA_3, and the amount of phosphorylated proteins from the seedlings soaked in GA_3 showed a gain of about 45 percent over the control at 3 day-point after germination.

DISCUSSION

In order to clarify the mechanisms of GA_3 partially, the activities of the protein biosynthesis and phosphorylation were investigated under the inhibitory condition of the transcription. It is known that actinomycin D inhibits DNA-dependent RNA synthesis by RNA polymerase(Hurwitz *et al.*, 1962; Gale, 1963; Key, 1964; Fujisawa, 1966; Yuyama, 1975) and leads to an inhibition of portein synthesis (Levinthal *et al.*, 1962; Haywood and Sinsheimer, 1963; Sacher *et al.*, 1975; Lin and Key, 1968). The effect of actinomycin D on the protein biosynthesis in germinating maize seeds (Fig. 1) is similar to that in soybean (Lin and Key, 1968).

GA_3 stimulated the biosynthesis of RNA and protein (Van Overbeek, 1966; Zolotov and Leshem, 1968; Higgins *et al.*, 1976), but the facilitation induced by GA_3 was suppressed in the presence of actinomycin D (Varner and Chandra, 1964; Chrispeels and Varner, 1967). By the way, Figs. 2 and 3 showed that the protein biosynthesis was stimulated by GA_3 at the 3 day-point after germination in the persence of actinomycin D. Judging from the above results, it appears that GA_3 enhances both transcription of mRNA from the DNA template and translation of mRNA formed previously. In order to study further the mechanisms of GA_3, the relationship between the activities of various factors involved in protein biosynthesis and GA_3 will be investigated.

The protein phosphorylation in the germinating seeds treated with actinomycin D was promoted by exogenous GA_3 after 3 days of germination (Fig. 4). This result is similar to that in the previous experiment using the germinating seeds untreated with actinomycin D (Sim and Roh, 1979).

摘 要

Gibberellic acid에 의한 遺傳子 發現의 調節機作을 究明하려는 硏究의 一部로서 actinomycin D 존재하에서 蛋白質의 生合成 및 燐酸化 反應에 미치는 GA_3의 效果를 硏究하였다.

GA_3로 처리된 發芽中인 種子 내에서 蛋白質의 生合成과 燐酸化 反應은 actinomycin D의 존재하에서 發芽 3일 후에 對照區에 비하여 현저히 촉진되었다. 發芽 3일 후에 蛋白質의 生合成이 촉진된 것은 GA_3가 transcription 과정에서 뿐만 아니라 이미 합성된 mRNA로부터 蛋白質이 生合成되는 translation 過程에도 영향을 주기 때문인 것으로 생각된다.

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