Effects of Cycloheximide and Canavanine on the Increase of Microbodial Enzyme Activities of the Cucumber (*Cucumis sativus* L.) Cotyledons during Early Germination.

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發芽初期의 오이 子葉에서 Microbody 酵素活性增加에 미치는 Cycloheximide와 Canavanine의 影響

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—Summary—

In order to clarify whether the increase of enzyme activities in the cotyledons during early germination of cucumber (*Cucumis sativus* L.) is due to *de novo* protein synthesis or not, the effects of cycloheximide and canavanine were investigated.

Cycloheximide inhibited light-induced glycolate oxidase as well as isocitrate lyase, catalase and glycolate oxidase, and then the increase of the enzyme activities seems to be due to at least *de novo* protein synthesis.

Canavanine inhibited only two enzymes, isocitrate lyase and catalase, but the' effect seems to be due to inhibition of the protein synthesis or of the cotyledon development.

Introduction

The increase of enzyme activities observed during early germination could be due to (a) reactivation of enzymes reversibly inactivated during maturation ; (b) *de novo* synthesis depending on preexisting mRNA's ; (c) *de novo* synthesis of both mRNA's and enzyme proteins (Lado *et al*, 1968).

And cycloheximide, an inhibiter of RNA synthesis, inhibits the protein synthesis (Siegel and Sisler, 1963 ; Yang and Scandalios, 1977), and canavanine, a structural analogue of arginine (Fig. 1), also inhibits the growth of organisms and DNA, RNA and protein synthesis in several organisms (Bonner, 1949 ; Weaks and Hunt : 1973 a ; Weaks and Hunt, 1973 b ; Srivastava, 1975 ; Thinh and Griffiths, 1979).

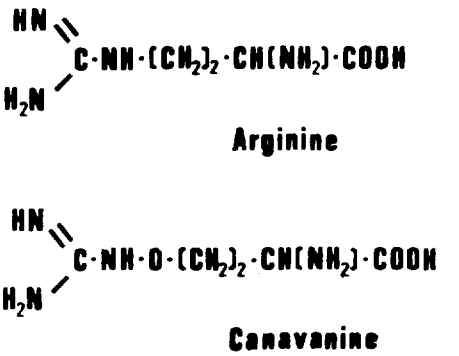

Fig 1. The formulas of arginine and canavanine.

In this paper, the author investigated the effects of cycloheximide on the increase of the microbodial enzyme actibities in order to clarify whether it is due to *de novo* protein synthesis or not and what effects on these enzymes are obtained by the treatment of canavanine. And, for seeking for the treatment days and methods of cycloheximide and canavaine, the developmental changes of microbodial enzyme activities in cotyledons of light or dark-grown cucmber seedlings were also investigated.

Materials and Methods

1. Plant meterial and growth conditions.

Seeds of cucumber (*Cucumis sativus* L.) were cold-imbibed (24 hrat 4°C) in distilled water, and then planted at the dishes containing about 1% agar medium. The dishes were either kept in continuous dark or illuminated for 12 hr/day with a fluorescent lamps at approximate intensity of 2,000 lux. The temperature was in both cases maintained at 30°C.

2. Preparations of Enzyme solution.

Five pairs of cotyledons were homogenated for 10 minutes in homogenizer with 5㎖ of 0.02 M HEPES buffer (pH 8.0) for determining of isocitrate lyase and catalase, and with 5㎖ of 0.02 M HEPES buffer (pH 8.0) consisting of 15mM glycolate for glycolate oxidase. The homogenate was centrifuged at 1,500*g* for 10 minutes and the resultant supernatant was centrifuged at 25,000*g* for 30 minutes to yield enzyme solution (Kwon *et al*, 1979).

3. Enzyme Assays

Isocitrate lyase and catalase were respectively determined by the methods of , Copper and Beevers (1969) and Chance and Maehly(1955). Glycolate oxidase was assayed by horseradish peroxidase(HPO) method (De Jong, 1973 ; Kwon *et al*, 1979) and the peroxidation of O-dianisdine was measured spectrophotometrically at 460nm (Worthington Biochemcal Co., 1977).

4. Treatment of Cycloheximide and Canavanine

The cotyledons were excised when each enzyme activity increased rapidly. The excised cotyledons were treated for 21hr or 48hr in 62mM Na-phosphate buffer (pH 6.2) plus cycloheximide or canavanine, and the other in buffer only, as a control (Tsui *et al*, 1980).

Results and Discussion

1. The Developmental Changes of Enzymes

After 1 to 6 days of germination, total enzyme activities in cucumber colyledons were determined (Fig. 2).

When the dark-grown seedlings were exposed to light, isocitrate lyase and catalase were effected irregularly whenever exposed (not showed), but glycolate oxidase developed rapidly. This accords well with NADH hydroxypyruvate reductase and glycolate oxidase in sunflower cotyledons (Schnarrenberger *et al*, 1977), glycolate oxidase, NADP trios phosphate reductase and NADH hydroxypyruvate reductase in watermelon cotyledons (Kagawa *et al*, 1973 ; Kagawa and Beevers, 1975). Like these enzymes, glycolate oxidase in cucumber cotyledons seems to depend upon light for attainment of full activity, but not for its initial appearence.

2. The Effects of Cycloheximide and Canavanine

Cycloheximide, when supplied to exised cotyledons

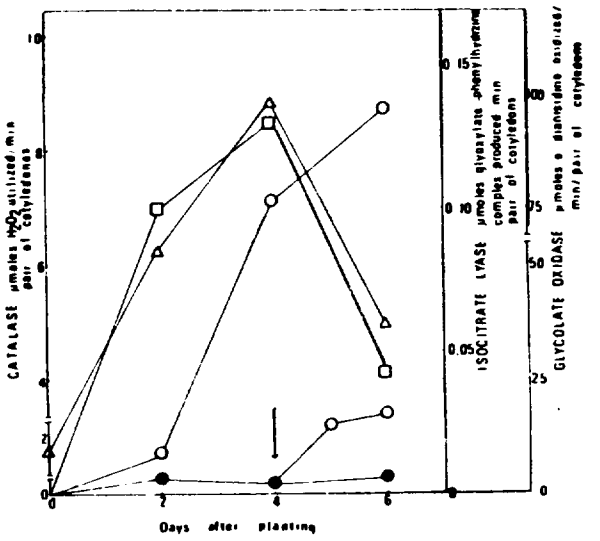

Fig 2. The developmental changes of microbodial enzymes in the cotyledons of light-grown(open symbols) and dark-grown(closed symbols) cucumber seedlings(□ : isocitrate lyase, △ : catalase, ○ : glycolate oxidase). The arrow indicates that seedlings were transfered to light condition at 4 days.

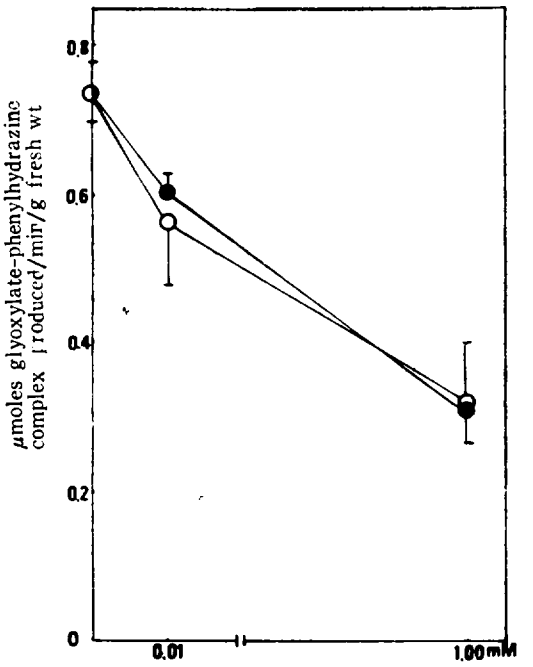

Fig 3. Effects of cycloheximide(●) and canavanine (○) on the isocitrate lyase when treated for 48 hrs in light on the cotyledons excised from the seeds imbibed for 24 hrs.

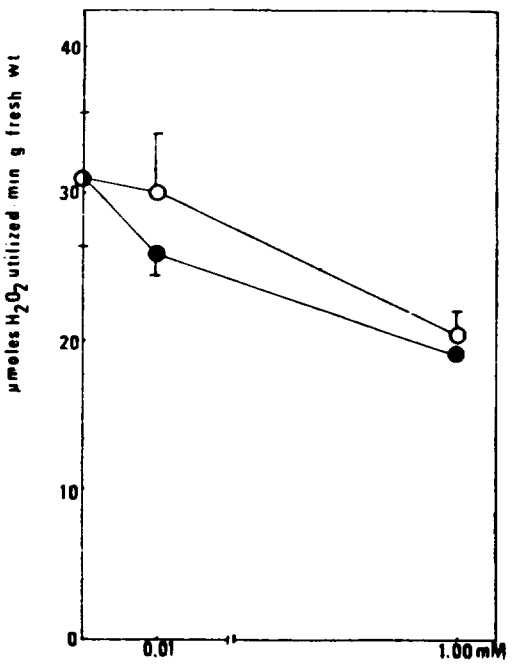

Fig 4. Effects of cycloheximide (●) and canavanine (○) on the catalase activity when treated for 24hrs in light on the cotyledons excised from seeds imbibed for 24hrs.

at the concentrations of 0.01mM and 1mM, inhibited isocitrate lyase, catalase, glycolate oxidase and light-induced glycolate oxidase (Fig. 3, 4and Table 1). This available evidence appears to favor the view that most, if not all of the increase of these enzymes in the cotyledons, depends on *de novo* RNA synthesis or at least *de novo* protein synthesis. The results like this were reported that the presence of cycloheximide or actinomycin D during water imbibition inhibited the increase of isocitrate lyase in the peanut cotyledons (Fientka-Rychter and Cherry, 1968), the presence of puromycin in the culture solution severely inhibited formation of isocitrate lyase in the squash cotyledons (Penner and Ashton, 1967), and actinomycin D almost completely inhibited the rise of isocitrate lyase as well as that of glucose-6-phosphate dehydrogenase, of phosphogluconate dehydrogenase and of isocitrate dehydrogenase in the castorbean. (Lado *et al*, 1968).

Table 1. Effects of cycloheximide and canavanine on the glycolate oxidase activity when treated in light for 24hrs on the cotyledons excised from the light-grown seedlings for 2 days (A) and dark-grown seedlings for 4 days (B).

Additions mM	umoles o-dianisidine oxidized/min/g fresh wt	
	A	B
None	347±66	117±31
0.01 Cycloheximide	307±11	55±18
0.01 Canavanine	336±7	115±27
1.00 Canavanine	394±39	113±26

The effects of canavanine on the activites of these enzyme were different according to the esenzym(Fig. 3.4 and table 1). On the isocitrate lyase and the catalase its effects were nearly similar to each other and accords similarly that canavanine inhibited the nitrate reductase induction in maize roots (Srivatava, 1975), but on the glycolate oxidase and the light-induced glycolate oxidase it stimulated development of these enzymes rather than inhibited. These effects of canavanine seems to be due to inhibition of cotyledon development during treatment or of *de novo* protein synthesis. The former were reported that canavaine inhibited the growth of maize root (Weaks and Hunt, 1973) and the increase of respiration induced by IAA in *avena* coleotile (Bonners, 1949). The latter were also reported that canavanine inhibited protein and nucleic acid synthesis in soybean(Weaks and Hunt, 1973 b) and the repressive effect of canavanine plus chloramphenical on the photosynthesis and the numbers of organisms was reversed more rapidly by arginine than that of canavainine only(Thinh and Griffiths, 1979).

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〈국문초록〉

發芽初期의 오이 子葉에서 Microbody 酵素活性 增加에 미치는 Cycloheximide와 Canavanine의 影響

發芽初期의 오이(*Cucumis Sativus* L.)의 子葉에서 isocitrate lyase, catalase와 glycolate oxidase의 活性이 增加하는 原因을 糾明하기 위하여 蛋百質 合成의 轉寫過程을 抑制하는 物質인 cycloheximide와 arginine의 構造 類似物인 canavanine의 效果를 調査하였다.

cycloheximide는 isocitrate lyase, catalase와 glycolate oxidase 뿐 아니라 빛에 의해 誘導되는 glycolate oxidase도 抑制하여 이들 酵素들의 活性 增加는蛋百質의 *de novo* 合成에 의한 것으로 思料되며 canavanine은 酵素에 따라 抑制效果가 다르며 이를 處理하는 동안 子葉의 生長이나 蛋百質 合成에 관여하는 것으로 思料된다.