

The Modification of Glycolate Oxidase Activities in the Cucumber Cotyledons by Glycolate

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Glycolate에 의한 오이 子葉에서의 glycolate oxidase 活性의 調節

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Summary

Glycolate oxidase was extracted from cucumber(*Cucumis sativus L.*) cotyledons and assayed by HPO method. The addition of glycolate into homogenizing medium stimulated the glycolate oxidase activity and showed the complete developmental patterns of the glycolate oxidase.

Introduction

Glycolate oxidase assay has been carried out with etiolated tissues and green leaves by many researchers(Tolbert and Cohan, 1953; Kuczmak and Tolbert, 1962; De Jong, 1973). The effects of FMN, chlorogenic acid and other polyphenols on the glycolate oxidase have been investigated by Kuczmak and Tolbert(1962) and De Jong(1973), and the effects of glycolate, particularly, by Tolbert and Cohan(1953).

In this paper, the activation and the developmental changes of glycolate oxidase in the homogenates of the cotyledons from dark- and light-grown seedlings were reinvestigated. And it was also investigated that the enzyme developmental patterns in the light-grown seedlings accorded with the developmental profile of chlorophyll.

Material and Methods

The material(*Cucumis sativus L.*), the growing

conditions and the selection of cotyledons from the germinating seedlings was described previously (Koh, 1981). The detached cotyledons were, by modification of the method of De Jong(1973), ground in a precooled homogenizer with 0.02M HEPES buffer(pH 8.0) or 0.02M HEPES buffer(pH 8.0) consisting of 15mM glycolate, and enzyme assays were also previously mentioned(Koh, 1981). Chlorophyll was determined by the method of Arnon(1949).

Results and discussion

When the glycolate was added to the homogenizing medium, the rate of enzyme reaction increased greatly(Fig. 1).

This seems to be that the preincubated substrate-enzyme solution mixture effects on the inactivated enzyme in the dark-grown seedlings. This phenomenon was explained that the substrate activated the enzyme and glycolate was not present in the etiolated tissues(Tolbert and Cohan, 1953). Although a very active glycolate oxidase was found in the extracts from the cotyledons of light-grown seedlings,

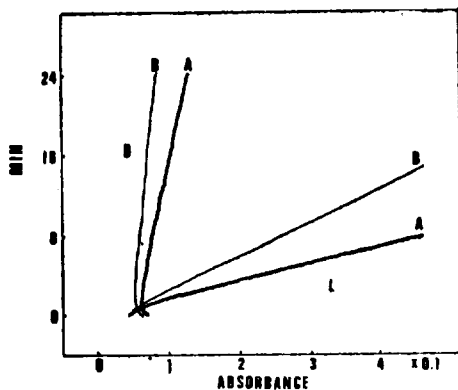


Fig. 1. The absorbance of glycolate oxidase extracted with(A) or without glycolate(B) in the 5 pairs of cotyledons of the seedlings grown dark(D) and light(L) for 6 days.

the activity was also increased greatly by addition of glycolate. And then this need to be studied by addition of FMN.

The developmental changes of glycolate oxidase activities and chlorophyll content in cucumber cotyledons were showed(Fig.2).

Glycolate oxidase of dark grown-seedlings was too low to be detected by the method of De Jong(1973), but easily detected by addition of glycolate to the homogenizing medium, and the developmental patterns, when extracted with glycolate, accord well with the reports of Becker et al.(1978) and Köller and Kindl(1978). This also suggested that glycolate oxidase was activated

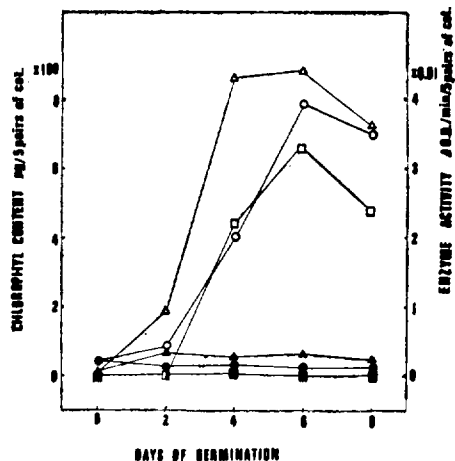


Fig. 2. The developmental changes in glycolate oxidase and chlorophyll in the cotyledons of dark-(closed symbols) and light-grown(open symbols) cucumber seedlings; Glycolate oxidase extracted with glycolate(Δ) and without glycolate(\square), and chlorophyll(\circ).

by glycolate, but regardless of addition of glycolate, the overall patterns of glycolate oxidase, grown in light, rised dramatically from low levels at 2 day to a maximum at 6 day. This pattern was also showed by chlorophyll contents. But glycolate oxidase increased in the cotyledons of dark-and light-grown seedlings by addition of glycolate and then the further investigations are required.

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국문초록

Glycolate에 의한 오이 子葉에서의 glycolate oxidase 活性的 調節

發芽中인 오이(*Cucumis sativus* L.)의 子葉에서 酵素溶液을 抽出하여, HPO法에 의하여 活性을 測定 結果, 한 homogenizing溶液에 glycolate의 添加는 暗處에서나 光下에서 glycolate oxidase의 活性을 增加시켰으며, 이 酵素의 完全한 活性化를 위해서는 빛과 glycolate 외에도 다른 要素가 作用하리라는 것을 追則할 수 있었다.