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On the Sulfur-incorporation into amino acids in germinating Soy Bean seeds using Sulfate-S^{35}

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大豆發芽種子에 있어서 아미노酸에의 硫黃結合에 關하여

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ABSTRACT

The sulfur-incorporation into amino acids in germinating Soy Bean seeds was studied with the aid of Sulfate-S^{35}. Water imbibition and isotope absorption were almostly pararelled. On the radioautogram S^{35} was detected in cysteic acids at 90 minutes. Cysteine, cystine and cysteic acid were appeared with radioactivity at 240 minutes after the infiltration of sulfate-S^{35}. Methione was not detected on the radioautogram in the range of 300 minutes. The radioactivity of S^{35} was detected highly in free amino acids than hydrolyzates.

INTRODUCTION

The metabolism of sulfur containing organics in lower and higher plants has been investigated with considerable attention in recent year.(1.3.5.6.10)

Protein synthesis and enzyme activation are in many cases as ociated with "SH" group and sulfur containing amino acids. In excised leaves of Mung Bean, Asahi(8) confromed that S^{35} was detected in cysteine and cystine, and in germinating Lettuce seeds Harber et al(1) had same results as above.

This experiment was intended to study the sulfurincorporation rate and order into amino acids in germinating Soy Bean seeds using sulfate-S^{35}. Also the imbibition of water and absorption time of isotope were studied.

MATERIAL & METHODS

5g of sterilized Soy Bean seeds, by 0.001% $HgCl_2$ for 5 minutes, were germinated in Hoagland's solution which contained isotope of sulfur-35 30μC per ml. The seeds used in this experiments contained 12% of water. This experiment was carried out at 26°-27°C with white light, and shaked at often times.

Sulfate-S^{35} was obtained from AERI of Republic of Korea as carrier-free isotopic compounds($H_2S^{35}O_4$) in dilute HCl. Isotopic compounds were titrated with 0.01N NaOH using neutralred as indicator.

All counting for radioactive were carried out with Carbon Counter, TGC-9 of Tracer Lab.

After the time ranged from 60 minutes to 300 minutes, the germinating seeds were fixed with 80% boiling ethanol.

Free amino acids were extracted with 80% ethanol and the residue was hydrolyzed with 6N HCl for 6 hrs. at 108°C. These extracted solution and hydrolyzate were passed through Merk Cation No. 1 and eluted with 2N NH_4OH. These elutions were concentrated on the water bath. These concentrated amino acids were spotted on the filter paper, Whatman No. 1, for developing with phenol satruated with water. After development the paperchromatography was reacted with 0.2% Ninhydrin. The identification of the chromatogram reacted with ninhydrin was made by co-chromatography obtained with original standards. The reacted paperchromatogram were attached to Curix X-ray film for three months.

RESULTS & DISCUSSION

The water imbition results and absorption of isotope are shown Fig. 1.

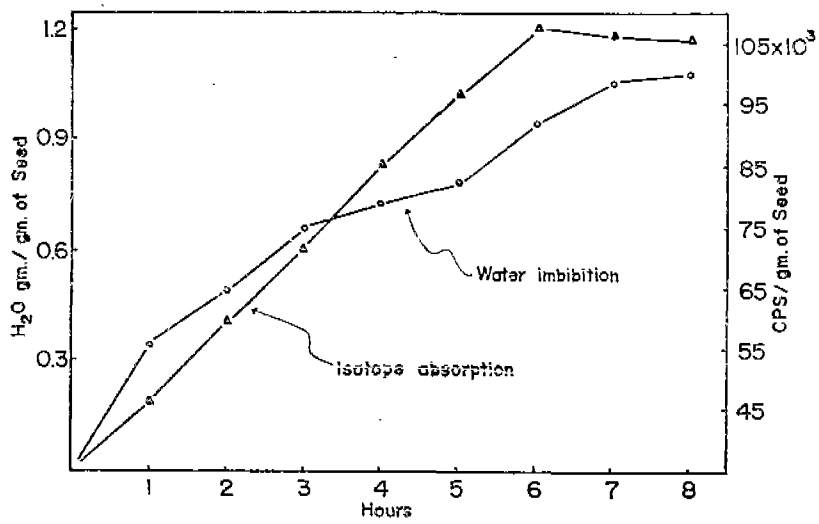

Fig.1. Water imbibition (water g per g of seeds) and Isotope absorption (cps/g of seeds)

Water imbibition is ranged maximum about 6 hrs. after the seeds being soaked in water, and after 7 hrs. the isotope absorption curve is decreased a little. It is assumed that the cause of absorption decrease after 7 hrs. is due to the physiological ion balance.

The attached X-ray film to the paperchromatogram was developed and compared with the paperchromatogram. Fig. II is the result of radioautogram.

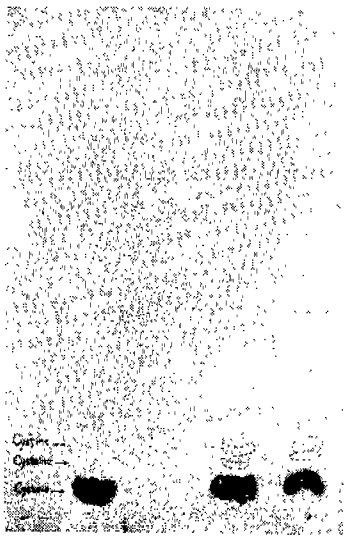

Fig. II. Radioautogram; left, 90 min.; middle, 240 min.; right, 300 min.

Cysteic acid was detected at 90 minutes, while cysteic, cysteine and cystine at 240 minutes. But methionine was not detected at any ranged time. One suggestion put forward by this experiment was that formation of methionine does not occur as a result of methylation of cysteine in germinating soy bean seeds. Also it is interested that S^{35} was detected highly in free amino acids than hydrolyzate. The reason of more activity of free amino acids is seemed that germinating soy bean seeds is under the condition of producing catabolic intermediates.

In the metabolism of sulfur-containing amino acids, cysteine was known as a center material and has come from serine added "-SH" group.

Van Halteren and Consden R., et al (2) demonstrated that cysteine can be oxidized to cysteic acid in the process of paperchromatography. But in this experiment only cysteic acid was detected at 90 minutes, and that cysteic acid, cystine and Cysteine were detected at 240 minutes. These evidences suggest that cysteine oxidized quickly to cysteic acid by some enzymatic ystem. There is support for this experiment come from enzymatic study in Yeast oxidative enzyme, cysteine sulfinic acid dehydrogenase (4, 7).

It is hoped that this enzymatic system will be studied in the future studies.

摘　　要

大豆發芽種子에서 아미노酸에 硫黃이 結合되어 들어가는 時間과 順序에 關하여 放射性同位元素 S^{35}를 使用하여 硏究하였다.

水分膨潤과 放射性同位元素의 吸收率과는 거의 一致하는 現象을 나타낸다. Radioautogram 한 結果는 放射性流黃은 Cystei acid가 處理後 90分에서 感光되었다. Cysteine, Cystine과 Cysteic acid의 三個의 아미노酸은 S^{35} 處理後 240分에서 感光되었으며 Methionine은 300分에서도 나타나지 않았다. 또 S^{35}는 加水分解해서 얻은것에서 보다 遊離아미노酸에 훨씬 더 많은 結合을 나타낸다.

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