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# Protein Metabolism in Rice (*Oryza sativa* L.) Seedlings
# IV. Peptides in the Guttation Liquid

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## Introduction

During germination the shoots of young seedlings grow by utilizing the degradation products of storage proteins in the seeds as the nitrogen source. As previously reported on the hydrolysis of storage proteins in rice endosperms by rice seed protease[2], peptides with high molecular weights were liberated as degradation products in the initial step, followed by peptides with low molecular weights, amino acids and amides. An uptake of di- and oligopeptides by germinating barley embryos has been observed[1] and it was suggested that peptides liberated by hydrolysis of the reserve proteins of endosperms might be hydrolysed to free amino acids in the scutella before "long distance" transport to the growing tissues of the seedlings[10]. On the other hand, Ozaki and Tai found three peptides in the guttation liquid of rice seedlings grown without nitrogen supply and considered that peptides might be the predominant forms of nitrogen transport in the xylem of rice seedling. In the present paper, the separation and identification of nitrogen constituents in the guttation liquid of rice seedlings are described. The relationship between the constituents of the guttation liquid and the degradation products of storage proteins in the endosperm is also discussed.

## Materials and Methods

### 1. Materials

Rice seeds (*Oryza sativa* L. cv. Norin 29) were germinated in tap water and seedlings were grown in plastic pots at 30°C in a dark incubator or under greenhouse conditions. The guttation liquid was collected with suction through a glass nozzle into a flask (Fig. 1) early in the morning. Sephadex G-10 was the product of Pharmacia Co. Ltd., Uppsala.

### 2. Gel permeation chromatography

Gel permeation chromatography was carried out at 4°C. The column of Sephadex G-10 (2.0×65 cm) was equilibrated with distilled water. The guttation liquid was lyophilized and dissolved in a small volume of water. Two ml of the sample was applied to the column, which was eluted with distilled water (flow rate 25 ml $h^{-1}$). The eluate was collected in 2.5 ml fracions.

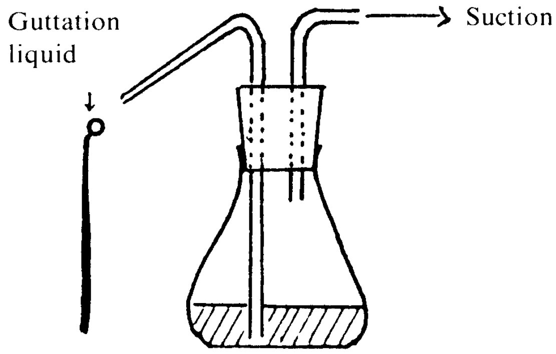


Fig. 1. Method for collecting the guttation liquid. The guttation liquid was collected with suction through a glass nozzle into a flask.

### 3. Paper chromatography

Samples were spotted with a capillary on a filter paper (Toyo No. 51) which was developed by acending chromatography using acetic acid : n-butanol : $H_2O$ (4 : 1 : 1 v/v) as solvent. The paper was then dried and sprayed with a 0.2 % (w/v) solution of ninhydrin in ethanol and heated at 100°C for 5 min.

### 4. Determination of Cu-Folin positive and ninhydrin positive substances

Cu-Folin and ninhydrin positive substances were determined according to Lowry *et al.*[5] and to Moore and Stein[7], respectively, with tyrosine as a standard.

### 5. Densitometry

Densitometric analysis of ninhydrin positive substances on the paper chromatograms was carried out at 500 nm using a densitometer (Aska Kogyo Co. OZ-82).

## Results

### 1. Separation of Cu-Folin and ninhydrin positive substances by gel permeation chromatography on Sephadex G-10

Both Cu-Folin and ninhydrin positive substances were found in the guttation liquid of rice seedlings and separated by gel permeation chromatography on Sephadex G-10 (Fig. 2). Most of the Cu-Folin positive substances passed through the Sephadex G-10 column without retardation (peak A), while most of the ninhydrin positive substances were retarded. The substances in peak A were much more sensitive to the Cu-Folin reagent than to ninhydrin reagent. One of the peaks of ninhydrin positive substances (peak B) appeared slightly later than peak A, followed by another large peak of ninhydrin positive substances (peak C).

### 2. Paper chromatography of the gel permeation chromatography fractions

Figure 3 shows the separation by paper chromatography of materials in fractions A, B, and C shown in Fig. 2, respectively. The material of fraction A remained at the starting point, while the material of fraction B was separated into three ninhydrin positive spots.

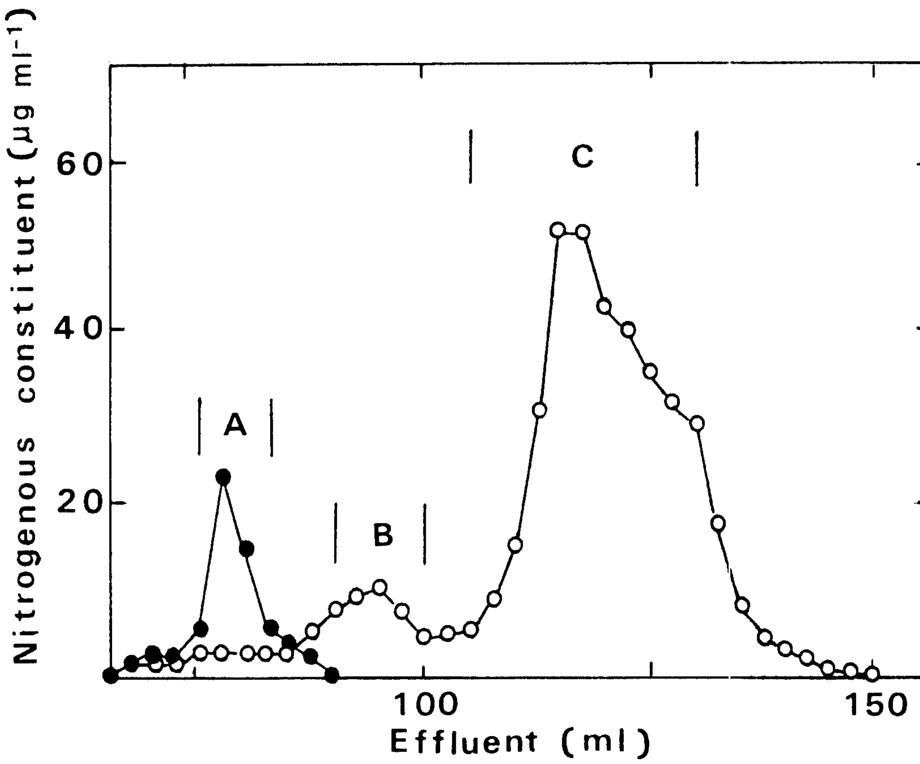


Fig. 2. Gel chromatography on Sephadex G-10 of guttation liquid. Rice seedlings were grown in tap water in an incubator at 30°C in the dark for 10 days. Amounts of Cu-Folin (●—●) and ninhydrin (○—○) positive substances were determined as described in **Materials and Methods**.

The spot of low $R_f$ value on chromatogram B was hydrolysed in 6 *M* HCl, followed by rechromatography. The hydrolysate yielded several spots (chromatogram B′). Fraction C was also separated into several spots by paper chromatography.

### 3. Changes in the concentration of Cu-Folin and ninhydrin positive substances in the guttation liquid during germination by application of $NH_4^+$

The concentration of Cu-Folin positive substances in the guttation liquid of control seedlings increased during germination earlier than ninhydrin positive substances did (Fig. 4 A). Application of $NH_4^+$ to the culture medium suppressed the increase in Cu-Folin positive substances and brought an earlier appearance of ninhydrin positive substances in the guttation liquid (Fig. 4 B).

### 4. Effects of $NH_4^+$ on the nitrogenous constituents of the guttation liquid

Chromatogram A in Fig. 5 shows a paper chromatography pattern of ninhydrin positive constituents of the guttation liquid of plants grown in tap water. The $R_f$ 0.15 spot was identified as glutamine by its exact correspondence with $R_f$ value of standard glutamine. The two spots of lower $R_f$ values were considered to be peptides according to the results of Fig. 3. The effect of $NH_4^+$ addition to the culture medium on the paper chromatography pattern is shown in Fig. 5 B. Ammonia caused a remarkable increase in the glutamine

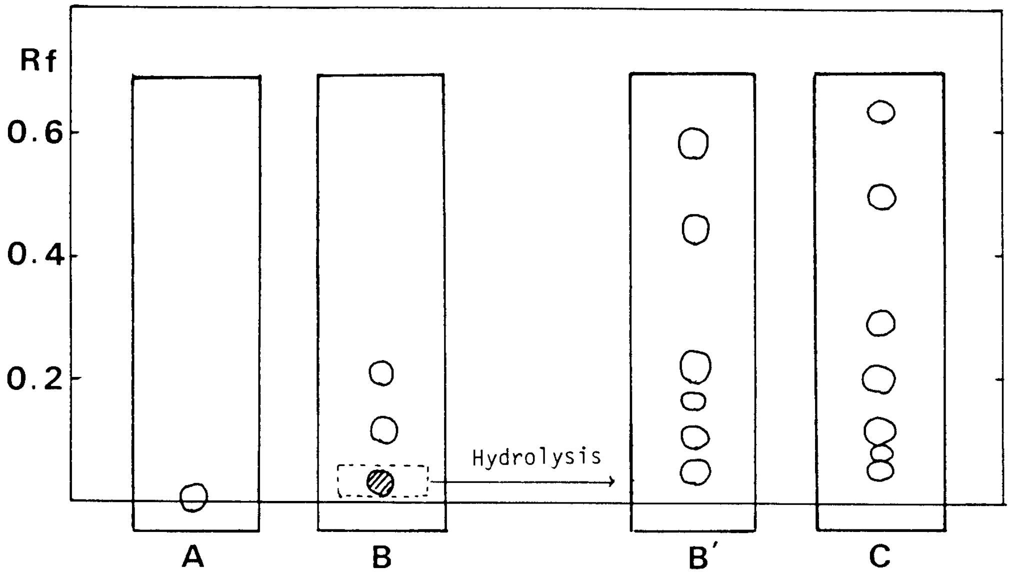


Fig. 3. Paper chromatography of the ninhydrin positive substances in the fractions of the guttation liquid separated by gel permeation chromatography. Fractions A, B and C in Fig. 2 were lyophilized and dissolved in a small volume of water, followed by paper chromatography as described in **Materials and Methods**. A, B and C represent the chromatograms for the fractions A, B and C, respectively. The lowest spot in chromatogram B was extracted and hydrolysed in 6 *M* HCl for at 110°C for 24 h before rechromatography (B′).

content in the guttation liquid.

## Discussion

Most of Cu-Folin positive substances in the guttation liquid of rice seedlings seem to be composed of peptides whose molecular sizes are larger than the pore size of Sephadex G-10 since they passed through the Sephadex column without retardation (Fig. 2). Most of the nihydrin positive substances seem to be composed of substances with molecular weights lower than those of Cu-Folin positive ones since they were retarded on the gel. Fraction B in Fig. 2 also appeared to contain peptides since it could be separated into several ninhydrin positive spots after acid hydrolysis and rechromatography. Ozaki and Tai[8] reported that the guttation liquid of rice seedlings grown without nitrogen supply contained neither amino acids nor amides but only three peptides. They considered that peptides might be the major form of nitrogen transport in rice seedlings. Horiguchi and Kitagishi[3] found peptidase activity in rice seed embryos but none in the starchy endosperms where the major storage protein exists. Higgins and Payne[1] suggested that di- and oligopeptides are absorbed intact by germinaing barley embryos and subsequently undergo hydrolysis. Sopanen *et al.*[10] reported that the scutella of germinating barley grains had the capacity for a rapid, active uptake of small peptides from the starchy endosperms, and concluded that peptide transport plays an important role in the moblization of reserve proteins during germination. As previously reported on the hydrolysis of the storage protein of rice endosperms by rice seed protease[2],

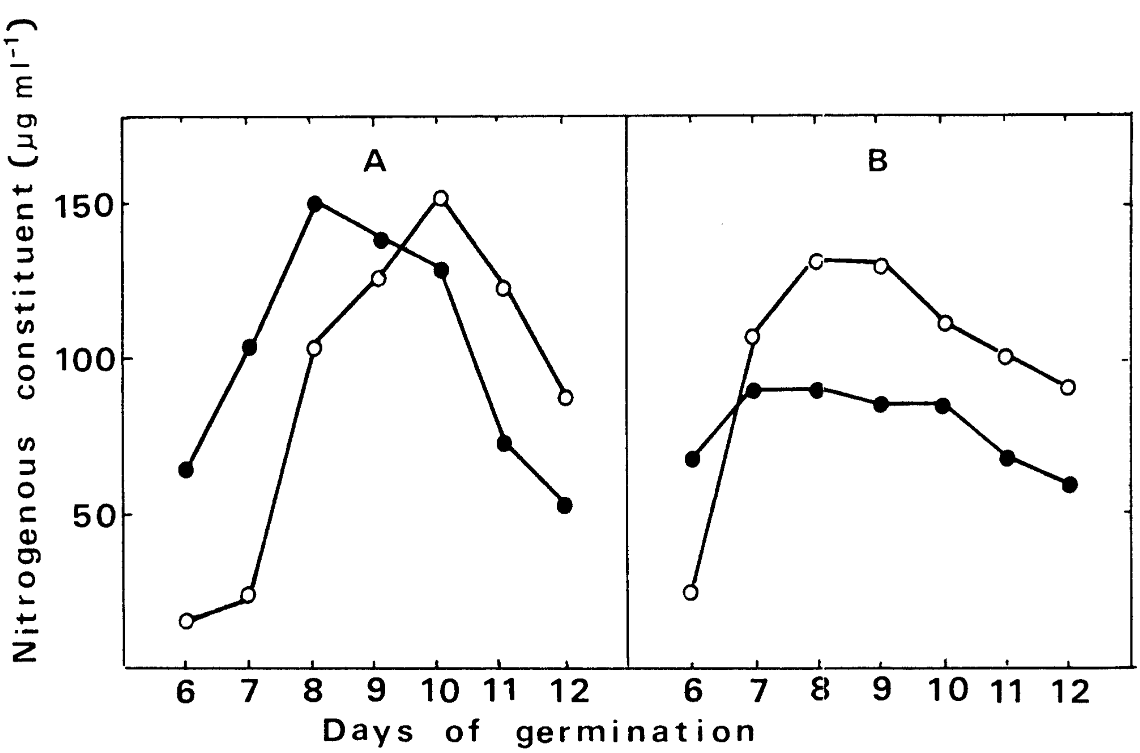


Fig. 4. Changes in the concentration of Cu-Folin positive and ninhydrin positive substances in the guttation liquid during germination by application of $NH_4^+$. Rice seedlings were grown at 30°C in the dark. Control plants (A) were grown in tap water, while plants (B) were grown in a solution containing 20 ppm N as $(NH_4)_2SO_4$. Changes in the amounts of Cu-Folin (●—●) and ninhydrin (○—○) positive substances were followed from day 4 to day 12 of germination.

peptides with high molecular weights were liberated in the initial step, followed by peptides with high molecular weights, amino acids and amides. Ammonia supply to the culture medium of rice seedlings suppressed the breakdown of the storage protein in the endosperms[4]. Changes in the nitrogenous constituents in the guttation liquid (Fig. 4) were compatible with those in the degradation products in the endosperms. This suggests that the nitrogenous constituents of the guttation liquid in rice seedlings are degradation products of the seeds. Appearance of glutamine in the guttation liquid of the plants grown in tap water (Fig. 4) indicates that some portions of the degradation products is converted into glutamine during germination. The remarkable increase in glutamine in the guttation liquid of plants supplied with $NH_4^+$ suggests that the $NH_4^+$ absorbed through the roots is mainly converted into glutamine and then transported.

Some portions of the degradation products of storage proteins are likely to be transported as peptides through the xylem and excreted into the guttation liquid. A question is raised whether the cells of growing shoots have the ability to take up peptides from the ascending xylem stream. Sopanen[9] reported that the root tips of barley took up a peptide although the rate of uptake was less than that by the scutella. Mikola and Kolehmainen[6] found peptidase activities in the growing shoots and the roots of barley. Therefore, it seems possible that part of the hydrolysis products are absorbed as peptides and hydrolysed to amino acids

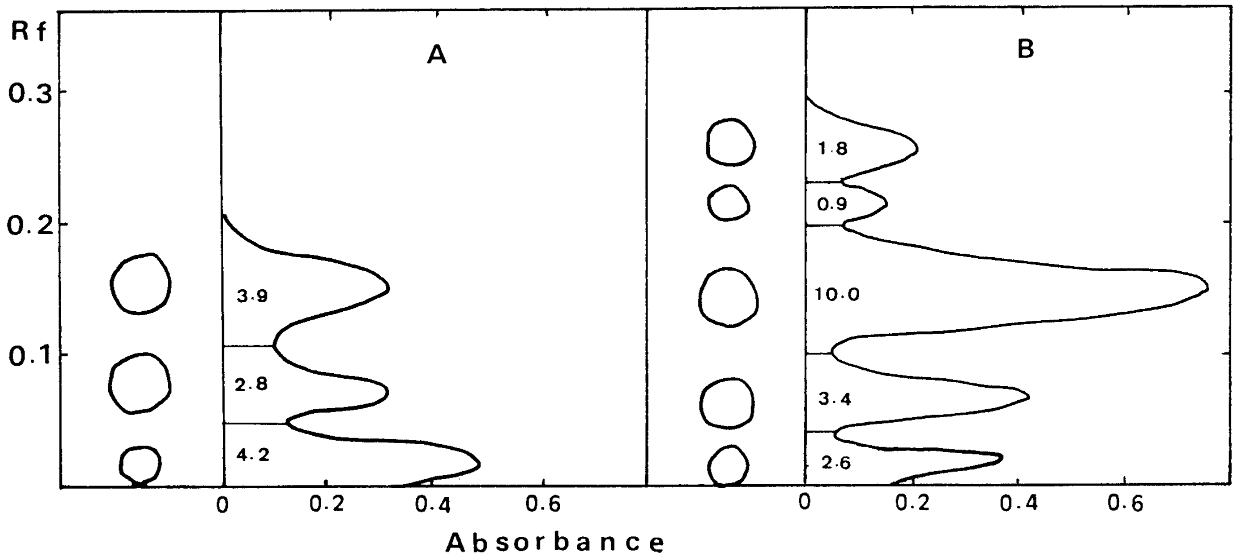


Fig. 5. Effect of $NH_4^+$ application on the nitrogenous constituents of the guttation liquid. Rice seedlings were grown for 10 days under greenhouse conditions. The culture solutions for the control plants (A) and $NH_4^+$ applied plants were the same in Fig. 4. Paper chromatography and densitometry were performed as described in **Materials and Methods**. The figures represent approximate relative quantities of the ninhydrin positive substances.

in the growing tissues. Elucidation of this question awaits further work.

## Summary

As an attempt to elucidata a picture of xylem transport of nitrogenous compounds in rice (*Oryza sativa* L. cv. Norin 29) seedlings, nitrogenous constituents of the guttation liquid were investigated. After the guttation liquid was collected with suction, the nitrogenous constituents were separated by means of gel permeation chromatography and paper chromatography, and subjected to colorimetric analysis with Cu-Folin and nihydrin reagents. Most of the Cu-Folin positive substances in the guttation liquid passed through the Sephadex G-10 column and appeared in the range of void volume, while most of the ninhydrin positive substances permeated the gel phase. The Cu-Folin positive substances were assumed to be peptides whose molecular size was larger than the pore size of Sephadex G-10. Part of the ninhydrin positive substances were demonstrated to be peptides by paper chromatographical separation of the hydrolysate. The Cu-Folin positive substances in the guttation liquid appeared in the early stage of germination, followed by the ninhydrin positive substances. Supply of $NH_4^+$ to the incubation medium of rice seedlings caused a decrease in Cu-Folin positive substances as well as the earlier appearence of the ninhydrin positive substances in the guttation liquid. In the guttation liquid of the plants grown in tap water, peptides and glutamine were the major constituents of ninhydrin positive substances. Addition of $NH_4^+$ to the medium caused a remarkable increase in glutamine in the guttaction liquid. It seems likely that some portions of the degradation products of reserve proteins in the endosperm are translocated in the form of peptides through the ascending xylem stream and then serve

as a source of nitrogen for the growing shoots.

## Acknowledgement

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