RESEARCH NOTE
Bot. Bull. Academia Sinica **13**: 121-124 (1972)

ISOLATION OF A PEPTIDE FROM PLANT TISSUES[1][2]

CHI-CHENG CHANG[3]

There have been few studies on the isolation of plant peptides. In the course of isolation of a crown-gall tumor growth factor from Pinto bean leaves (Lippincott *et al.*, 1972), I encountered a solouble peptide of some length. To my knowledge, existence of such a peptide in plant tissues has not been reported. Its isolation from other plants was therefore carried out in order to know more about the peptide pool (Waley, 1966) in cells about which little is understood.

Two weeks old seedlings of *Phaseolus vulgaris* L. cv. "stripe-seeded" and one month old tomato seedlings were cut into portions of leaves, stems and roots and extracted separatedly. Crown-gall tumors induced on stems of tomato seedlings and on leaves of *Bryophyllum sp.* were also examined for this peptide.

Cold 90% methanol was added to the tissues at a ratio of 100:15 (v/w) and the mixture blended in a Waring blender for 3 minutes. The homogenate was centrifuged at 10,000×g for 15 minutes at 4°C The filtered supernatant was then evaporated to small volume in a rotary evaporator at 35°C and lyophilized. The dried residue was resuspended in water (one tenth of tissue fresh weight) and extracted five times with an equal volume of ethyl acetate. The thick water phase was passed through a Sephadex SP (Na^+), C-25 column at pH 3. Almost the same thick and colored solution was obtained as effluent. The column was washed thoroughly with water before the adsorbed basic materials were eluted with 0.5 N NH_4OH. The eluate was lyophilized and taken up in a few ml of water. It was then fractionated by gradient elution on a 170×1 cm column of Sephadex SP, C-25. The column was prepared in 0.1 M sodium citrate, pH 3.2. The gradient was obtained by placing 450 ml of 0.1 M sodium citrate, pH 3.2 in the mixing flask and 450 ml of 0.1 M sodium citrate, pH 5.0 in the reservoir flask. Samples were applied at pH 3. Effluent

(1) This investigation was supported by the National Science Council, Republic of China.
(2) Paper No. 125 of the Scientific Journal Series, Institute of Botany, Academia Sinica.
(3) Associate Research Fellow, Institute of Botany, Academia Sinica.

fractions of 4.4 ml were scanned individually for their UV absorption spectra by a Perkin-Elmer spectrophotometer. All the plant materials examined gave rise to the same general elution pattern of UV absorbing materials. Figure 1 shows the sequence of eluted UV absorbing materials before histidine from tomato stem sample.

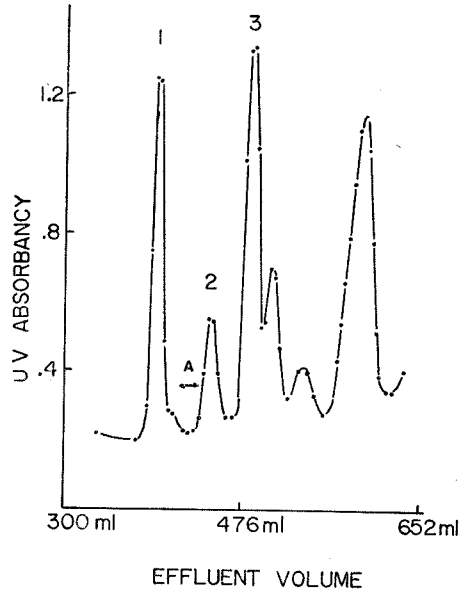

Figure 1. Elution sequence of portion of UV absorbing materials from tomato stem from a 170×1 cm column of Sephadex SP in 0.1 M sodium citrate by pH gradient. Absorption maxima: peak 1, 245-265 mμ; peak 2, 282 mμ; peak 3, 245-270 mμ. A, fractions containing the unknown amino acid.

In the isolation of the peptide from Pinto bean leaves, the effluent from the column was monitored by a refractive index monitor. The peptide was found in a very small peak that immediately followed a big peak representing an unknown non-protein amino acid. The position of this amino acid, detected by paper chromatography, is indicated in figure 1 by A. Peak 2 fractions, therefore, should contain the peptide (if any), since it follows the unknown amino acid.

The pooled peak 2 fractions were then desalted on a Sephadex SP (H^+), C-25 column and eluted with 0.5 N NH_4OH. The eluate was lyophilized and portion was hydrolyzed in 6 N HCl at 110°C for 24 hrs. The hydrolyzed products were chromatographed on silica gel TLC sheets with *n*-propanol-34% ammonia (7:3) as solvent. All the plant samples examined gave rise to the same group of seven ninhydrin positive spots as represented by that obtained

from bean leaf and *Bryophyllum* leaf tumor sample in Figure 2-B. Cochrmatographies with authentic amino acids identified the spot with the lowest Rf value as lysine.

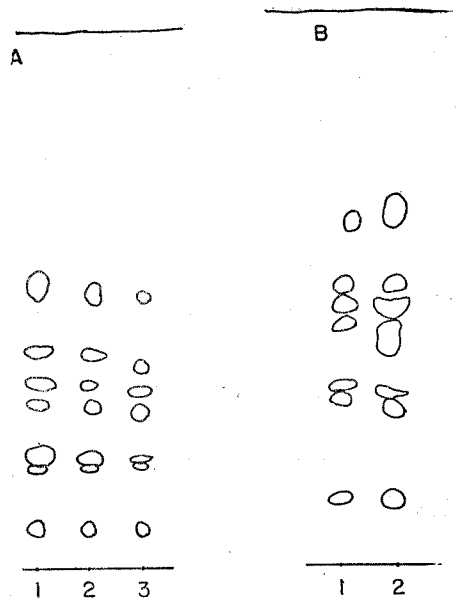

Figure 2. A: Silica Gel TLC chromatography of amino acids produced by hydrolyses of the peptide fractions that have been purified on Sephadex G-10 columns. 1, Tomato root; 2, tomato leaf; 3, *Bryophyllum sp.* stem tumor. B: Silica gel TLC chromatography of amino acids produced by hydrolyses of desalted peak 2 compounds. 1, Bean leaf; 2, *Bryophyllum sp.* stem tumor. Solvents: *n*-propanol-34% ammonia.

Desalted peak 2 fractions from tomato leaf, tomato root and bean leaf were further fractionated on 170×1 cm Sephadex G-10 columns using 0.02 M NH_4HCO_3 as eluant. When lyophilized void volume fractions were hydrolyzed, the same group of ninhydrin positive spots was again obtained (figure 2-A). This indicated that peptide with molecular weight of more than 700 is involved. Two dimentional chromatography on silica gel TLC sheet with 1. butanol-acetic-water (4:1:1), 2. *n*-propanol-34% ammonia (7:3) did not reveal any more ninhydrin positive spot. The UV absorbing material, however, was this time separated from peptide and came out much later from the column.

Portion of the unhydrolyzed peptide fraction from Sephadex G-10 column was chromatographed on silica gel TLC sheet with solvent *n*-propanol-34% ammonia, no definite color spot could be observed after ninhydrin spraying. Possibly a cyclic peptide is involved.

Acknowledgmeut

This investigation was carried out with the assistance of Miss Bi-Yu Lin to whom the author wishes to express his appreciation.

Literatnre Cited

LIPPINCOTT, JAMES A., BARBARA B. LIPPINCOTT, and CHI-CHENG CHANG. 1972. Promotion of crown-gall tumor growth by lysopine, octopine, nopaline and carnosine. Plant Physiology **49**: 131-137.

WALEY, S. G. 1966. Naturally occurring peptides. Advances in Protein Chemistry **21**:1-112.

一 種 植 物 胜 的 分 離

張 啓 正

作者在分離一種瘤腫生長因子的過程中，發現一種含有七種不同氨基酸的胜。這種胜在幾種被檢查的植物器官中都存在着。