STUDIES ON THE LEVELS OF INDOLE-3-ACETIC ACID (IAA) AND INDOLE-3-ACETYL-L-ASPARATE (IAAsp) IN RELATION TO SOMATIC EMBRYOGENESIS OF CALLI DERIVED FROM GINSENG (*PANAX GINSENG* C.A. MEYER) ROOTS

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ABSTRACT

Ion-pair reverse phase HPLC techniques were used to compare the contents of IAA and IAAsp in the embryogenic and non-embryogenic calli derived from ginseng (*Panax ginseng* C.A. Meyer) root tissues. The contents of IAA and IAAsp of the embryogenic callus were much higher (7 to 18 X respectively) than those of non-embryogenic callus. There is a distinct fluorescent peak of an unknown component in the HPLC profile of the extract for indolic compounds from non-embryogenic callus. This distinct difference may be employed as a promising parameter to screen the culture pieces for obtaining the calli with high potential for embryoid formation.

INTRODUCTION

Somatic embryogenesis can be readily induced in cell suspension cultures by transferring the cultured tissues and cells in media containing auxin to auxin-free media. Exogenously supplied auxins inhibit induction of somatic embryogenesis (Halperin, 1966), and promotive effects of antiauxins have been demonstrated (NewComb and Wertherell, 1970; Smith and Street, 1974). Since rapid cell proliferation and differentiation occur in the process of embryogenesis in auxin-free media, The roles of endogenous auxins and their metabolites would be interested to know because auxin is considered to be essential for cell proliferation and development. Although in a number of experimental systems the inhibition of exogenous auxins on somatic embryogensis has been demonstrated, the role of endogenous auxins and their derivatives have not been exploited extensively. Only a few determination of the IAA content as related to somatic embryogenesis was documented. A poor embryoid-forming cell line of carrot accumulated more IAA than embryoid-forming cell lines (Sung, 1979). No difference of endogenous auxin content was demonstrated in carrot cell lines at three stages of the somatic embryogenesis (Fujimura and Komanine, 1979). The roles of endogeneous auxins on somatic embryogenesis in tissue cultures remained unsettled. This is associated with the fact that the usually employed physicochemical methods involving bioassays are nonspecific and do not ensure quantitative determination of endogenous auxins. Recent development of high performance liquid chromatography will benefit the assay of auxins in small samples.

We have previously established an experimental system of somatic embryogenesis in

ginseng (*Panax ginseng* C. A. Meyer) callus cultures (Chang and Hsing, 1980a, 1980b). We now report that the contents of IAA and IAA-aspartic acid in embryogenic tissues are higher than that of non-embryogenic tissues. The involvement of endogenous auxin in somatic embryogenesis will be discussed.

MATERIALS AND METHODS

a) Plant materials and culture methods

The callus culture used in these experiments was derived from pith tissues of a ginseng root and subcultured every two months for three years in a basal medium containing Murashige and Skoog (1962) salts with (in mg/ml): myo-inositol, 100; nicotinic acid, 0.5; pyridoxin, 1000; sucrose, 30000; Difco-Bacto agar, 10000; 2,4-D, 0.001, as described previously (Chang and Hsing, 1978a, 1978b). Poor embryoid forming and easy embryoid forming callus lines were isolated and subcultured separately at 27C in darkness. These embryonic and nonembryonic calli were sampled for IAA and IAA-aspartic acid analysis.

b) Synthesis of IAAsp

Indole-3-acetyl-D, L-aspartate was prepared by the method of Mollan *et al* (1972). The product was further purified on a Hibar semipreparative HPLC C-18 column (E. Merck Darmstadt. F.R. Germany) using water-methanol-acetate mixture (66.5-33-0.5) as eluent. A 15-19ml fraction was collected, and dried *in vacuo*. Identification was made using UV and IR. All spectra were in agreement with the report by Mollan *et al* (1972).

c) Extraction and pruification of indolic compounds

Ten gram (fresh wt.) newly transferred embryogenic and non-embryogenic calli of *Panax ginseng* was homogenized in 100ml precooled (-10C) methanol-water (80-20) mixture which contained 200mg/l sodium diethyldithiolcarbamate as antioxidant. The homogenized tissues were stirred for 8 hr. at 4C, filtered, and further concentrated *in vacuo* to dryness. The residue was purified according to Durley *et al* (1982) was dissolved in 1ml methanol-diethyl ether (1:1), then the concentrated ammonium hydroxide solution was added dropwisw shaking until the ammonium was excess (8-10 drops). The solution was evaporated *in vacuo*. Polyvinylpyrrolidone (PVP) powder was mixed throughly with distilled water and the fines decanted after setting for 5 min. When five decantations were completed, columns (10 x 1-2cm) were prepared in distilled water. The ammonium salt samples prepared were dissolved in 1ml water and chromatographed on the PVP columns with distilled water as eluent (flow rate 0.5ml/min). The 7-17th fractions (one ml each) of eluent were collected, adjusted to pH 3.0 (using 1M HCl). A column XAD-7 (Sigma, U.S.A.) was employed for further purification of extracts. The resin was purified according to Andersson (1982) and further packing into a 3ml measuring pipette with glass wool using distilled wat, and pre-eluted with 30ml of 0.1M citrate-phosphate buffer at pH 3.0. The elute from PVP column was applied to XAD-7 column with a flow rate of 1.9ml/min. The column was further washed with 4ml distilled water, followed by 5ml methanol. The methanol elute was collected and concentrated *in vacuo* and subjected to further HPLC analysis.

d) HPLC Analysis

Reverse phase ion-pair HPLC analysis of partial purifed extracts was conducted on an ODS analytical column (Laboratory Data Control, USA. 5um, 25cm), using MeOH/phosphate buffer (24/76) (0.05M, pH 7.0, with 0.1M tetrabutylammonium sulfate as pair ion) as mobile phase. Detections were by UV 254 detector (Model 1203, Laboratory Data Control, USA) and fluorescence detector (Fluorescence Spectrophotometer Model 204-A, Hitachi, Japan). The peaks at 6.45 min and 12.35min (Fig. 1) were collected separately and concentrated *in vacuo* to dryness. Further analysis was

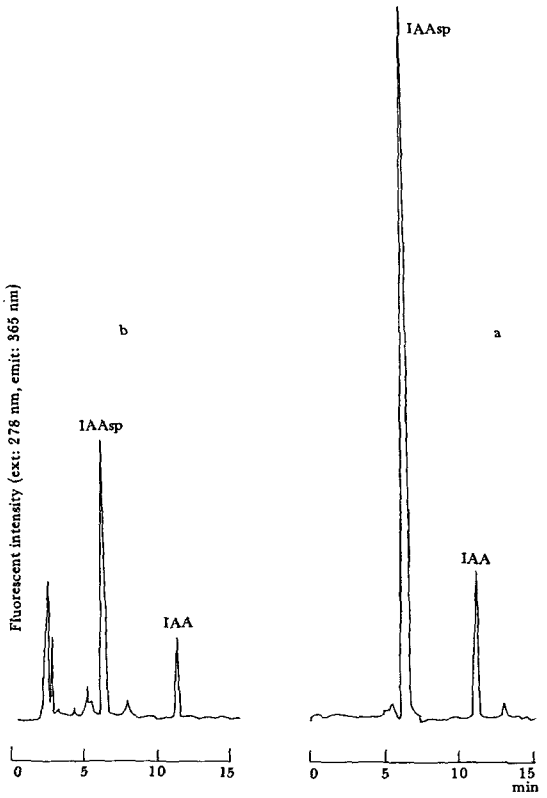

Fig. 1. HPLC. chromatograms of (a) 100ng authentic IAA and IAAsp, (b) extract from 5g embryogenic callus on C-188 ODS column. Eluent: MeOH/H_2O/acetic acid (33/65.5/0.5).

made on a reverse-phase ODS column (10um, 25cm) with MeOH/H_2O/acetic acid (33/65.5/0.5) as mobile phase. The qualitative detection and quantitative measurement were made by using a HP-1040A High Speed Spectrophotometric Detector (Hewlett Packard, USA).

RESULTS AND DISCUSSION

Batch of 10g fresh weight of calli was sufficient for measurement of both free and conjugated IAA(Fig 1, 2). The HPLC procedures of IAA and IAAsp reported herein are rapid, effieient and sensitive although two purification steps are required there are relatively simple. Thus large numbers of samples could be processed.

Both embryogenic and non-embryogenic calli contain considerable amounts of IAA and IAAsp (Fig. 2 and Tab. 1). The content of IAA in embryogenic calli is about 18 times of that of non-embryogenic calli. The content of IAAsp of the embryogenic calli is also considerably higher than that of the non-embryogenic calli. Moreover, there is an unknown peak appeared right after the IAAsp in the HPLC-fluorescence profile of the extract of non-embryogenic calli extract (Fig. 2). Spiking of the extract with authentic IAA and IAAsp clearly demonstrated that the unknown component in the extract is not IAAsp (Fig. 2). The nature of this fluorescent component remained to be exploited.

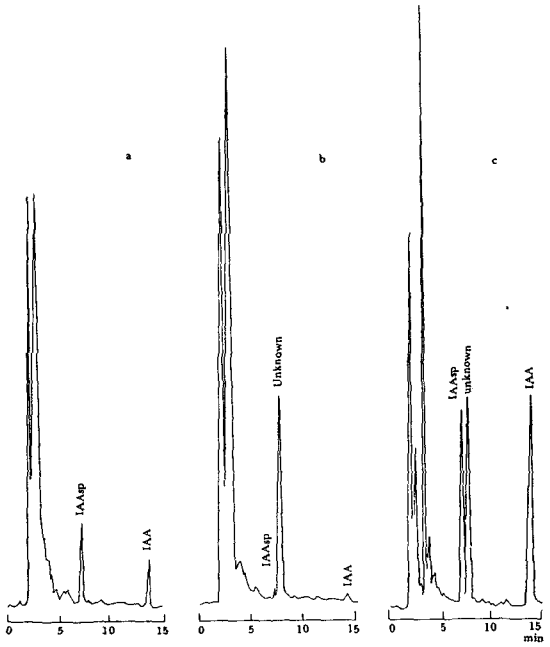

Fig. 2. HPLC cromatograms of (a) extract from 5g embryogenic callus, (b) extract from 5g non-embryogenic callus, (c) extract from 5g non-embryogenic callus spiking with 100ng of IAA and IAAsp.

Above mentioned results only represent some qualitative and quantitative differences in the partial purified extracts for analysis of indolic compounds. The relationship between these differences and the process of somatic embryogenesis needs further investigation. However, the differences between the HPLC-fluorescence profiles of embryogenic and non-embryogenic calli are quite intense, particularly the unknown peak shown in the profile of the non-embryogenic calli. The remarkable difference in HPLC profiles may be employed as a promising way to screen the culture pieces of calli in order to obtain the potent embryogenic calli.

Table 1. Quantitative analysis of indolic compounds in calli derived from ginseng roots using reverse phase-ion pair HPLC-fluorescence techniques.

	IAA	IAAsp	IAA/IIAsp
embryogenic	845 ng/g.f.w.	98	8.6
non-embryogenic	47	14	3.3
embryogenic/non-embryogenic	18	7	

인삼근 캘루스의 체세포 배아 발생과 관련한 IAA 및 IAAsp의 수준에 관한 연구

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인삼근에서 유래된 캘루스중 배분화 유조직과 배분화가 되지 않는 유조직에 있어서 Indol-3-acetic acid (IAA) 및 Indole-3-acetyl-L-aspartate (IAAsp) 함량을 Ion-pair reverse phase HPLC 기법을 이용하여 분석 비교하였다.

배분화 유조직의 IAA 및 IAAsp 함량은 배분화가 불가능한 유조직에 비해 각각 7 및 18배 함량이 높았다.

배분화가 되지 않은 캘루스로부터 얻은 추출물의 HPLC 프로파일상의 인돌 화합물 피크 부근에 특이한 미확인 피크가 나타났다. 이와 같은 분명한 차이는 배상체 발생 잠재력이 높은 유조직을 얻기 위한 배양조직을 선별하기 위한 확실한 파라메타로 이용될 수 있을 것이다.

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