行政院國家科學委員會專題研究計畫成果報告

（計畫編號：NSC 90-2313-B-038-001）
執行期限：90 年 8 月 1 日至 91 年 7 月 31 日

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一、中文摘要

多元胺 (polyamine)，普遍存在於動物、植物與微生物中。常見可以包括以下四種:Putrescine (Put)，Spermidine (Spd)，Spermine (Spm) 及 Cadaverine (Cad)。在生理環境下，多元胺爲 polycations, 可以與細胞內的大分子產生交互作用，包括細胞膜的磷脂質 (phospholipid), 細胞壁中的果膠分子, 核酸與蛋白質。根據最近研究結果--篩選阿拉伯芥缺乏多元胺生合成的變異株, 與大量表現多元胺的轉殖植物--顯示多元胺與植物的生長發育有非常密切的關連性。利用甘藷(*Ipomoea batatas* [L.] Lam cv. Tainong 57) 不同部位組織, 包括休眠塊根, 發芽塊根與芽爲材料, 檢測三種不同鍵結型式的多元胺的變化, 包括游離型多元胺(free polyamine), 多元胺共價結合於小分子 (例如:酚類化合物), 與多元胺共價結合於大分子 (例如:蛋白質) 。初步的研究結果顯示個別多元胺的不同型式的含量在不同的甘藷組織中有不同的分布,但是以多元胺總量而言 (nmole/g FW 表示), 芽 > 發芽塊根 > 休眠塊根。其中發現, 不同組織的多元胺共價結合於大分子的含量約佔總多元胺含量的三分之一。事實上, 胰蛋白胺抑制因子爲甘藷塊根含量最高的儲藏性蛋白質。因此, 本計畫的目的將以甘藷不同組織爲實驗材料，純化其儲藏性蛋白質----胰蛋白胺抑制因子，測試甘藷胰蛋白胺抑制因子是否共價結合多元胺，並推測其可能扮演的生理意義與角色。

關鍵詞：多元胺;甘藷；儲藏性蛋白質；胰蛋白胺抑制因子；生理意義

Abstract

Trypsin inhibitors (TIs) were purified from storage roots, sprouted roots and sprouts of sweet potato variety Tainong 57 (T57) by ammonium sulfate precipitation and Sephadex G-75 chromatography. Active fractions were further purified by affinity chromatography on trypsin-Sepharose 4B. Activity staining of TIs on a 15% SDS-PAGE gel revealed TI bands (73, 38 and 22 kDa) in storage roots, sprouted roots and sprouts. TIs, purified by the affinity column, were hydrolyzed by mixing with an equal volume of 12 N HCl at 110^{o}C for 16 h. The hydrolysates were benzoylated with benzoyl chloride in alkaline condition, and the polyamines (PAs) were identified by HPLC using 64% methanol as an eluent. Cad, Spd and Spm were found in all TI hydrolysates with different amounts in storage roots, sprouted roots and sprouts. TIs purified from the sprouts had higher PA titers, which were expressed as nanomole/μg protein, than those from sprouted roots or storage roots. The possible physiological roles of PA-bound TIs were discussed.

Keywords:
Polyamine; Trypsin inhibitor; Ipomoea batatas L.; Affinity column; Physiological role.

二、緣由與目的

The possible roles of polyamines (PAs) in growth and development processes of various plant systems have been reported. Non-growth tissues exhibit low levels of PAs, while actively growing tissues have high ones. PAs in plants may exist in free and/or conjugated forms. Slocum and Galston (1985) proposed that PA conjugates may serve as a storage form of PAs and nitrogen sources in tobacco plants. Torrigiani et al., (1987) proposed that free and conjugated forms of PAs seem to be involved in both the reproductive and vegetative phases of tobacco growth and developments. The

common PA-conjugates that belong to PCA-soluble or TCA-soluble were caffeoyl-PAs, ferulic-PAs and coumaric-PAs. These conjugated PAs were PAs conjugated to small molecules. However, there was another form of PA-conjugates in which PAs were conjugated to macromolecules (ex. RNA, proteins and cell wall materials etc.) and which belongs to PCA- or TCA-insoluble types. In animals, the posttranslational covalent linkages of PAs to proteins have been demonstrated and isolated, but the covalently bound PA-protein complexes are practically unknown in plants. Although some authors found that PAs conjugated to proteins in plants, they did not know what those proteins are. They thought that the physiological functions of conjugated PA-protein complexes must connect with protein's functions. In this report, we demonstrate for the first time that PAs bind covelently to TIs in SP's storage roots, sprouted roots and sprouts, and the possible physiological functions of PA-bound TIs will also be discussed.

三、結果

3.1. PA titers in storage roots, sprouted roots and sprouts of SP

The PA titers in different fractions of storage roots, sprouted roots and sprouts of SP after 5% PCA extraction and hydrolyzed by 12N HCl were shown in Table 1. This has been done twice and the results were similar. The non hydrolyzed PCA supernatant containing the free PAs (S), the hydrolyzed PCA supernatants (SH) , the hydrolyzed pellets (PH) containing PAs released from conjugates were benzoylated and analyzed by HPLC. Cad, Spd and Spm were all found in storage roots, sprouted roots and sprouts with different amounts. The total PA titers, which was expressed as nmole/g fresh weight, were higher in sprouts than in sprouted roots or storage roots. The results were similar to those of Kaur-Sawhney et al. (1982) [12] and Slocum et al.(1984) [13] in that the non-growth tissues exhibit low levels of PAs, while actively growing tissues have high ones. The PH fractions, in which the PAs were conjugated to macromolecules, ranged from 23% to 36% of total PA titers of different developmental stages of SP (Table 1) . In recent years, people have paid much attention to PAs bound to macromolecules, especially those bound to proteins [17,18,25-27]. They thought that the physiological functions of conjugated PA-protein complexes must connect with those protein's functions. Since the most abundant proteins in SP roots are TIs [9], we went further to purify TIs to examine whether PAs bind to TIs or not.

3.2. Purification of TIs from storage roots, sprouted roots and sprouts of T57

The Sephadex G-75 chromatograms of TIs from storage roots, sprouted roots and sprouts of T57 were shown in Fig. 1. The precipitates after ammonium sulfate fractionation were dissolved in small volumes of 100 mM phosphate buffer (pH 7.0) and loaded onto a Sephadex G-75 column. The TI activity in chromatograms of Fig. 1 was expressed as μg trypsin inhibited per 100 g fresh weight of roots or sprouts. TIs purified from sprouts had lower levels of trypsin inhibition activity than those from storage roots or sprouted roots, and the molecular size shifts of TIs were also found among storage roots, sprouted roots and sprouts. The active fractions after gel filtration were collected and concentrated by Centriprep-10 (molecular weight cutoff is 10 kD) concentrators. Fig. 2 shows the affinity chromatograms of TIs from storage roots on a self-prepared trypsin-Sepharose 4B affinity column. The absorbed TIs on the column were eluted with 0.2 M KCl buffer (pH 2.0). TIs purified by the affinity column from sprouted roots and sprouts had similar results (data not shown). After buffer exchange with 100 mM phosphate buffer (pH 7.0) on the PD-10 column, the purified TIs from affinity column were examined by both protein staining and activity staining on 15% SDS-PAGE gels (Fig. 3). There were all three same protein bands (73, 38 and 22 kD) corresponding to TI activity bands, respectively, in storage roots, sprouted roots and sprouts. The results

were similar to results of Chan and de Lumen (1982) [28] in which TI mixtures were obtained from affinity column and examined by SDS-PAGE. Thereafter, we used these affinity-purified TI mixtures as samples for PA analysis. 3.3. PA analysis

Fig. 4 shows the HPLC chromatograms of TI hydrolysates from storage roots, sprouted roots and sprouts. The benzoyl-hydrolysates were eluted with 64% methanol and monitored at 254 nm. Compared with standard mixtures of benzoyl-Put, -Cad, -Spd and -Spm (Fig. 4A), there were Cad, Spd, Spm and an unknown bound to TIs purified from storage roots, sprouted roots and sprouts (Fig. 4B-D). The use of recovery experiments by mixing the standard benzoyl-PAs mixtures with different TI hydrolysates also had similar results as Fig. 4B-D revealed. The control samples of commercial soybean TI (Fig. 4E) and bovine serum albumin (Fig. 4F) did not show any PAs. Different amounts of Cad, Spd and Spm expressed as nanomole per microgram protein were found among storage roots, sprouted roots and sprouts (Fig. 5). In Fig. 5, the purified TIs from sprouted roots and sprouts had much higher levels of bound PAs than those from storage roots.

四、討論

This is the first report concerning PAs bound to TIs. However, the real physiological functions of PAs-bound TIs in SP are still unknown. Although possible functions of PAs including free and conjugated forms in different plants have been suggested, few information is available about PAs conjugated to macromolecules such as proteins. Our present results may lead to further understanding of the possible physiological functions of both PAs and TIs in sweet potatoes.

One of TI's functions in SP is as storage proteins [10]. After sprouting, some proteases including aminopeptidases [32,33] were found in sprouts. In soybean, after sprouting, the protease G1, protease C1 and protease K1 can hydrolyze specifically the glycinin, β-conglycinin and TIs [34]. If some proteases participate the hydrolysis of TIs in SP during sprouting, the molecular size shifts of TIs (Fig. 1) may be explained as such. The released PAs-conjugated peptides or free PAs may be the nitrogen and carbon sources or the former may serve as a storage form of PAs [14]. In the regulating processes of plant development, the PAs-peptide conjugates have been found in apple pollen in vitro germination [35] or free PAs were involved in the break of dormancy of potato tuber [12], and free and conjugated PAs were involved in reproductive and vegetative phases of tobacco [16]. In Table 1, the free PAs (fraction S) increased dramatically from storage roots to sprouts. It is possible that the released PAs-conjugated peptides or free PAs may break the dormancy of SP and regulate the sprouting process.

The PAs conjugated to TIs did not seem to affect TI's activity drastically (Fig. 1-3). The transglutaminase-like activities have been found in plants [26,27]. They connect PAs to glutamine residue of peptides [22]. It is possible that PAs bound to TIs at such a place that was far away from TI's active site.

TIs from SP may respond to various stress conditions, including drought [5], heat, exogenous sugar, exogenous plant growth regulators [6], water-deficiency [7]. PA levels and PA synthetic enzyme activities of plant tissues were also reported to increase in response to various stress conditions [36-37]. In SP, the biosynthesis of both TIs and PAs in response to some stress conditions may be controlled cooperatively. This postulation needs further investigation.

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計畫類別：☒個別型計畫　　□整合型計畫

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計畫參與人員：

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執行單位：台北醫學大學生藥學研究所

中　華　民　國　　91　年　　8 月　1　日