山藥塊莖儲藏性蛋白質的研

行政院國家科學委員會專題研究計

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主持人：侯文琪 台北醫學大學生藥學研究所

一、中文摘要

我們發現山藥儲藏性蛋白質---dioscorin 具有的胰蛋白酶抑制因子（trypsin inhibitor）、dehydroascorbate reductase 及 monodehydroascorbate reductase 活性。

關鍵詞：山藥、儲藏性蛋白質、胰蛋白酶抑制因子

Abstract

We found that the purified dioscorin from yam tuber exhibited trypsin inhibitory activity and the dehydroascorbate reductase and monodehydroascorbate reductase activities.

Keywords: Yam, storage protein, trypsin inhibitor, dehydroascorbate reductase, monodehydroascorbate reductase

二、緣由與目的

山藥（*Dioscorea* spp.）是薯蕷屬中能形成地下肉質塊莖的栽培種。山藥塊莖蛋白質約佔其乾重的 1.5-4% (Baguar and Oke, 1976)。Harvey 和 Boulter (1983) 以八月薯［山藥的一種］(*Dioscorea rotundata*) 為材料，發現其粗抽萃取液中含有一主要水溶性蛋白質，約佔所有蛋白質的 85%。經過離子交換管柱層析分離，利用 150 mM NaCl 即可溶離出主要的儲藏性蛋白質。此蛋白質分子量約為 30 kD，依不同的層析條件（包括離子強度、蛋白質濃度、pH 值等），則可以形成分子間與分子內的雙硫鍵聚合物。Conlan 等人 (1995)，以開揚薯蕷 (*Dioscorea cayenensis* Lam.) 為材料，篩選其儲藏性蛋白質（命名為 dioscorin）的兩個 cDNA clones (clone A 和 clone B），推演其胺基酸序列並經由 gene expression 合成儲藏性蛋白質。以 Northern blotting 分析發現，dioscorin 祇有地下莖有表達，葉子、葉柄與莖則無。今農業生物技術國家型計畫與保健食品計畫正積極開發其省產山藥的利用，但是山藥塊莖儲藏性蛋白質的相關研究則少見於文獻中。我們以自日本進口山藥 (*Dioscorea batatas* Decne) 的塊莖為材料，依 Harvey 及 Boulter (1983) 方法加以修飾，所純化的 dioscorin，我們意外的發現其具有胰蛋白酶抑制因子的活性 (trypsin inhibitory activities)，同時和甘藷塊根儲藏性蛋白質---trypsin inhibitor 的抗血清有交互反應。這是除了甘藷外，在不同材料的儲藏性蛋白質中第一次發現具有胰蛋白酶抑制因子的活性。而甘藷的儲藏性蛋白質具有 monodehydroascorbate reductase 及 dehydroascorbate reductase 的酵素活性 (Hou and Lin, 1997)，皆和逆境環境中的植物本身的保護有關。Dioscorin 是否也具有此一酵素活性，是未來研究中的一個特點。

三、結果與討論

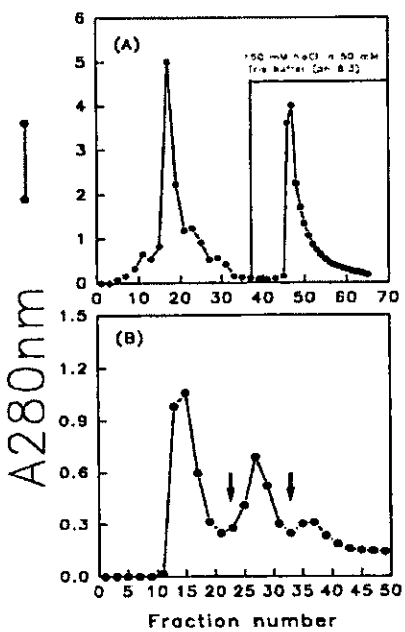

Figure 1. Chromatograms of dioscorin purified by (A) DE-52 ion exchange column and then by (B) Sephadex G-75 gel filtration after precipitation from 45-75% ammonium sulfate saturation. For DE-52 column (2.0 × 20 cm): washing buffer 50 mM Tris-HCl buffer (pH 8.3); eluting buffer, 150 mM NaCl in 50 mM Tris-HCl buffer (pH 8.3); flow rate, 50 ml/h; fraction size, 5 ml/tube. For Sephadex G-75 column (1.6 × 70 cm): eluting buffer, 100 mM Tris-HCl buffer (pH 7.9) containing 100 mM NaCl; fractions between two arrows (fraction number 23 to 33) were collected; flow rate, 27 ml/h; fraction size, 3.6 ml/tube.

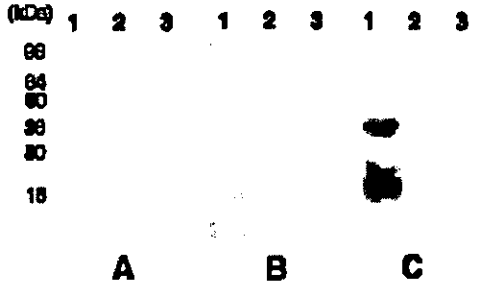

Figure 2. (A) protein staining, (B) trypsin inhibitor activity staining and (C) immuno staining of a polyclonal antibody against trypsin inhibitor from sweet potato on 15% SDS-PAGE gels and PVDF membrane, respectively, of dioscorin. Lane 1, purified trypsin inhibitors from sweet potato as positive controls (see Hou and Lin, 1997a); lane 2, purified dioscorin without 2-ME treatment; lane 3, purified dioscorin with 2-ME treatment. SeeBlue pre-stained electrophoretic markers were labeled. 8 μg protein was loaded on each well.

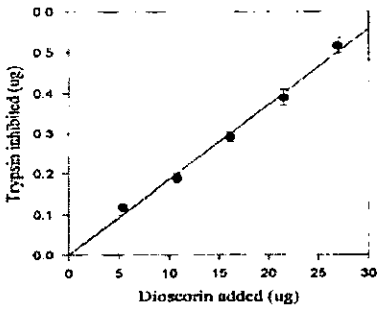

Figure 3. Determination of trypsin inhibition activity exhibited by dioscorin. Mean of three determinations was plotted against different amounts of dioscorin added.

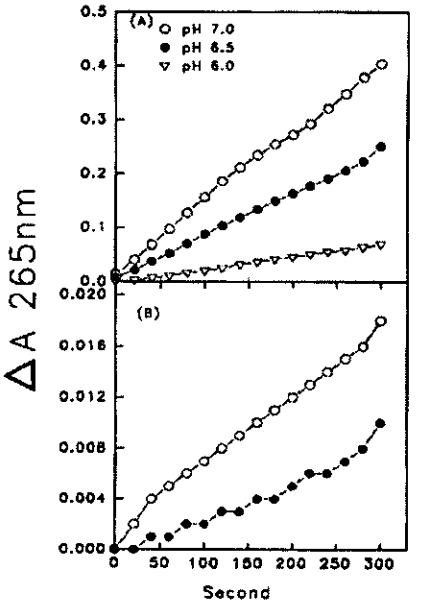

Figure 4. Effect of pH (6.0, 6.5 and 7.0) on dehydroascorbate reductase activity of dioscorins purified from yam tubers with (A) or without (B) 4mM glutathione added in the reaction mixtures.

Figure 5. Protein (A) and thiol-labeling (B) stainings of dioscorins in 15% SDS- PAGE gels. Before (lanes 1 and 5, in distilled water) and after reacting with dehydroascorbate for 30 min in phosphate buffer at pH 6.0 (lanes 2 and 6), pH 6.5 (lanes 3 and 7) and pH 7.0 (lanes 4 and 8) in the absence of glutathione. Samples in lane 1 to lane 4 were incubated in sample buffer without 2-mercaptoethanol; while in lane 5 to lane 8, samples were incubated in sample buffer with 2-mercaptoethanol in a final concentration of 14.4 mM. "M" represents the molecular weight marker and 10 μg protein was loaded in each well.

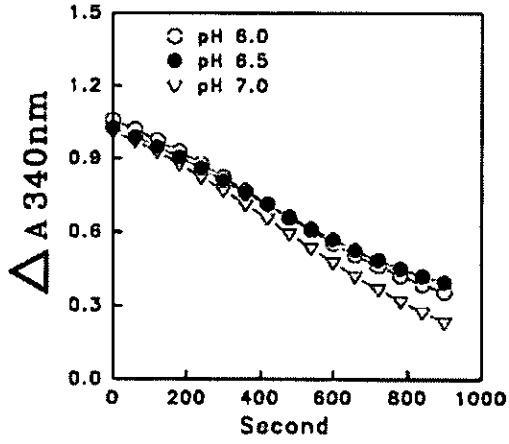

Figure 6. Effect of pH (6.0, 6.5 and 7.0) on monodehydroascorbate reductase activity of dioscorins purified from yam tubers.

Figure 7. Protein (A) and diaphorase activity (B) stainings in 15% SDS-PAGE gels for detection of monodehydroascorbate reductase activity of dioscorins purified from yam tubers. Samples in lane 1 were incubated in sample buffer without 2-mercaptoethanol; while in lane 2, samples were incubated in sample buffer with 2- mercaptoethanol in a final concentration of 14.4 mM. "M" represents the molecular weight marker and 10 μg protein was loaded in each well.