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Biochemical Properties of Sweet Potato RNases

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ABSTRACT: Six groups of ribonucleases (RNases) activities from Sweet potato (*Ipomoea batatas* Lam. Cv. Tainong 57) leaves were identified and characterized by an RNA-cast polyacrylamide gel. Among these ribonucleases, five were major isoforms, and were designated to A-E groups, ranged in size from 14 to 47 kilodalton. They were consistently expressed in root, stem and leaf. In addition, a novel RNase isoform, designated F group, with a molecular mass about 6000 dalton, expressed specifically in the aging leaf. In this paper the biochemical properties of six groups RNase isoforms were characterized with catalytic ions, pH value, denaturing reagent of SDS and 2-mercaptoethanol.

KEYWORDS: Sweet potato, RNase, *Ipomoea batatas,* s-RNase.

INTRODUCTION

Ribonucleases (RNases) play a classical function in the processing of targeted RNA molecules (Green, 1994). In plant, more recently data showed that ribonuclease activity is associated with many phases of plant development. Among them, senescence is the most prominent process that influence the RNase content. In wheat leaves, there are three RNases, WHA, WHB and WHC increasing during the senescent state (Blank and Mckeon, 19911). Studies of the expression of the gene for RNS2 (a member of RNase group in *A. thaliana*) also showed that the S-RNase homolog proteins with sequence homologous to those of S-RNase group in *Arabidopsis thaliana* is a senescence-induced RNase (Taylor and Green, 1991). Accumulated data implied that some RNase activities may closely correlate with the physiology of plant senescent state. Our experiments showed that six groups RNase isoforms are present in sweet potato tissue. All of them are variable in qualitative and quantitative level, while the plant developments are under various environmental conditions. Especially in leaf senescent state, only some specific RNases appeared. In this paper, we present the biochemical properties of these RNases isoforms performed by substrate-based gel assay method(Blank, et al., 1982). This work is the first step to further purify RNase proteins.

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MATERIALS AND METHODS

Plant Materials

Sweet potato (*Ipomoea batatas* Lam. cv. Tainong 57) was used in this study. The tubers were soaked in water for sprouting within two weeks. The sprouts with 5 cm height were subsequently excised and grown in modified Hoagland solution in growth chambers under conditions of 8 hr light /16 hr dark and 70% RH at 28℃. The plants with abundant root system were subjected for protein extraction.

To survey the RNase pattern changed in developing leaves, samples were collected from budding to old leaf in a growing plant.

To induce senescence, three well-rooted sprouts were grown in continuous darkness chamber 28℃ for 3 days. The natural senescent yellow leaves were picked up for protein sample extraction from wild plants.

Preparation of Protein Extracts

Leaves were cut from the sweet potato sprouts, instantly frozen in liquid nitrogen, and stored at -70℃ for protein extraction. All homogenization steps of sweet potato tissue were performed at 4℃. Tissues were disrupted with a polytron homogenizer in extraction buffer (20mM Tris-HCl pH 7.4, 5% Glycerol, 0.1mM EDTA, 1mM DTT, and 0.05M KCl). The homogenate was centrifuged at 10000 rpm for 15 min. (Sorval, RC5C, SS-34), and the supernatant fraction were used as the source of enzyme for assay. The supernatant solutions were stored at -70℃, until the activity assay was performed.

Electrophoresis and Activity Staining

The RNase activity was measured following the method of Blank *et al.*, (1982). Briefly described, the crude protein extracts of tissue were electrophoresed by 15% RNA-cast polyacrylamide gel. After electrophoresis, the gel was washed with 25% isopropanol and 10mM Tris-HCl, pH 7.4, to remove SDS and renatured proteins. The RNase activities appeared while incubating the gel at 55℃ for 120 min. in 10mM Tris-HCl, pH 7.4, followed by toluidine blue O (0.2%) staining and destaining with 10mM Tris-HCl at room temperature. The micro-, macroelements, SDS, 2-mercaptoethanol, and varied pH buffers were added to the preincubation and incubation buffer in some experiments to assay their effects respectively and performed as same as the procedures mentioned above. All the enzyme activities were repeatedly performed for at least three times.

RESULTS

Normal profile of sweet potato ribonuclease

To determine whether RNases are the tissue-specific, we established the RNases profile from different tissue of sweet potato. The protein samples extracted from leaf, stem, and root were loaded with equal amount and analyzed in a 15% RNA-cast polyacrylamide gel. As shown in Figure 1, five groups of RNase activity with apparent molecular masses

ranged from 14 KD to 47 KD were obtained. In general, five groups of RNases designated groups A to E were consistently observed from activity staining of SDS-PAGE.

The estimated molecular masses are 47, 27.5, 25, 20 and 14 KD respectively.

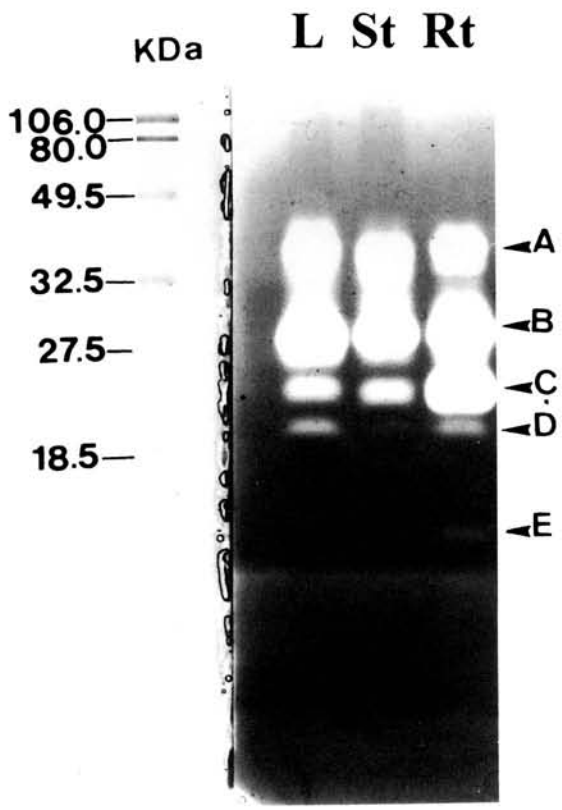

Fig.1. RNase profile of sweet potato leaf, stem, and root. 10μg of RNase protein was extracted from the tissue and analyzed on 15% SDS-PAGE, then treated as described in "Material and Methods". L, leaf; S, stem; R, root. The RNase isoforms were indicated at the right hand.

The variation of RNase activities profile from bud to senescent leaves

During the process of leaves development, leaves differentiate from the meristem and expand into mature leaves and finally enter the process of senescent state. During the leaves growth cycle, there are many kinds of metabolism occurred. The different mRNA isoforms would be produced alternatively and turn over in the processes. The RNases would play an important role in RNA degradation and RNA metabolism. It raises our curiosity to know RNase changement in qualitative and quantitative level. As data shown in Figure 2, in general, the A, B, C, and D groups RNase always expressed constantly in every state of growth, but the A group came to an abrupt disappearance on the yellow wilting leave. Morever, a specific RNase, above the A group with approximate 48 KD MW, increases gradually by the process of growing old leaves. It was named as A' RNase. The A' RNase reaches the maximal amount at the last green leave which will soon turn to yellow, then vanish in the yellow wilting leaves. More interestingly, a particular RNase group, RNase F, was specifically found in yellow leaves. It seems closely related with senescent leave stage.

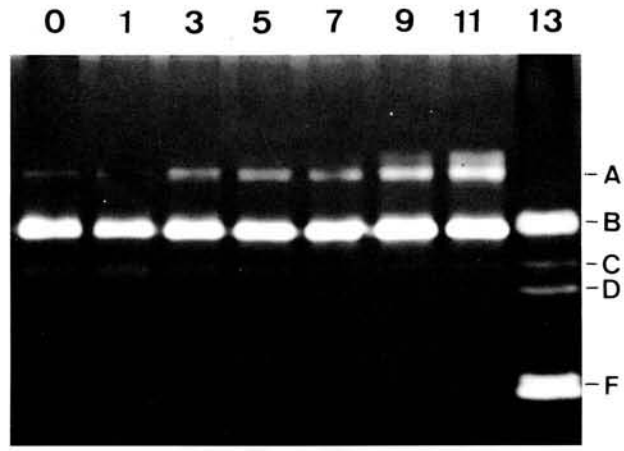

Fig. 2. The RNase profile varied with developing leaves. 10μg of protein extract each from bud leaf to yellow wilting leaf was analyzed on 15% RNA-cast polyacrylamide gel and assayed the activity as described in "Material and method".

Effect of the pH on RNase activities

The effect of pH on the RNases activities was studied in details.On the SDS-PAGE, RNase profiles of normal leaves, dark-growth leaves and natural senescent leaves (yellow wilting leaves) was used in this study. As shown in Figure 3, the A group of RNase displayed activity from pH 7 to pH 9. The best pH optimum was pH 8. The activity of B group located between pH 4 to pH 8, while the C group was between pH 5 to 8, and D group was between pH 4 and 7. In general , the B, C,and D group reached the maximum activity at pH 6, pH 6 and pH 5 respectively. E group was inconsistent in the leaves , therefore, the pH optima of E group was relatively uncertain. Finally, the activity of F group was with broad pH range from 4 to 9, and the optimum pH for its activity seemed ranging from 5 to 7.

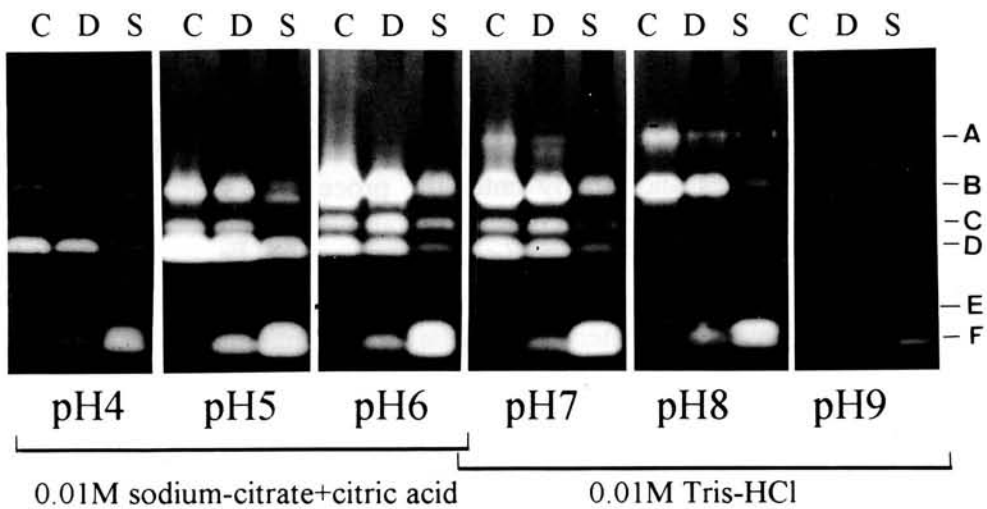

Fig. 3. Effect of the pH on RNase activities. 10μg of sweet potato leaf protein extracts was first analyzed on 15% SDS-PAGE. The gels were subsequently washed, incubated, stained and destained in the buffers at the pH values indicated below the gel. (C) indicates protein extracted from normal growth seedling leaf. (D) leaf from dark growth seedlingfor 3 days . (S) yellow wilting leaf from senescent seedlings.

Cation effect on the RNase activity

Several macro- and microelements were tested for their effect on the sweet potato RNase activities when they were added to the incubation buffers. The macro elements, such as Ca^{+2}, K^{+}, and Mg^{+2} were performed at the concentrations from 0 to 20 mM. As Figure 4 showed, K+ ion could enhance all the activities of sweet potato RNases by the concentration near 5 mM. However, the high concentration, with 20 mM, evidently decreased the RNases activities.

On the other hand, both of the divalent cations,Ca^{+2} and Mg^{+2} ,even at the small concentration of 2 mM could weak the RNases activities. At the 20 mM concentration , all the sweet potato RNases activities were considerably inhibited. (Fig. 4.A and C)

With regard to the effect of microelements, those of Mn^{+2}, Zn^{+2}, and Fe^{+2} were performed to assay from 2 μM to 200 μM respectively. The results showed that the tested microelements were not only nonessential for all the activities, but also clearly repressed at low concentration of 2 μM (Fig.4 D, E and F). To test whether the activities were sensitive to chelation of divalent cations by EDTA. The gel with control, dark-induced and senescent RNases were incubated in 1 mM and 10 mM EDTA respectively. As shown in Figure 5 , the isoform A was completely inhibited by the presence of 1 or 10 mM EDTA in incubation buffer. On the contrary, the isoform B was strongly enhanced by 10 mM EDTA and C was partially inhibited. Interestingly, The F RNase seemed unaffected in this condition.

As we know, the SDS which was a kind of detergent could denature protein into a linear form and break the hydrogen bond of protein. In the SDS solution, the protein was with negative charge and would lose the enzyme activity. We incubated the RNases gel in 0.1% SDS buffer at 55℃ for 2 hr and stained by toluedine blue O. All the RNases lost their activity as the common ones. Especially, the F RNase still retained partial activity. It indicated that F RNase had some tolerance to SDS. On the assay of 2-mercaptoethanol, the result showed only the F group retained little activities.

DISCUSSION

The alterations of RNase activity in plant tissues are associated with developmental processes such as senescence (Green, 1994), environmental stress(Yang and Green ,1991), disease (Randles, 1968), and the change of hormones (Naito , et al.,1979). Though we have not enough evidence to identify the role which the individual RNase plays in sweet potato, it seems that their activities quite variable during the developmental stages. It implies that there would be corresponding physiological roles with these RNase groups. Moreover, the E group RNase was root specific one but it always occurred inconsistently. Further observation was necessary to investigate it's properties.

Another particular RNase group (RNase F) was specifically expressed in yellow wilting leaves. RNase F appeared primarily at stress condition. Therefore, the physiological function was possibly correlated with stress state.

Macro- and microelements requirement

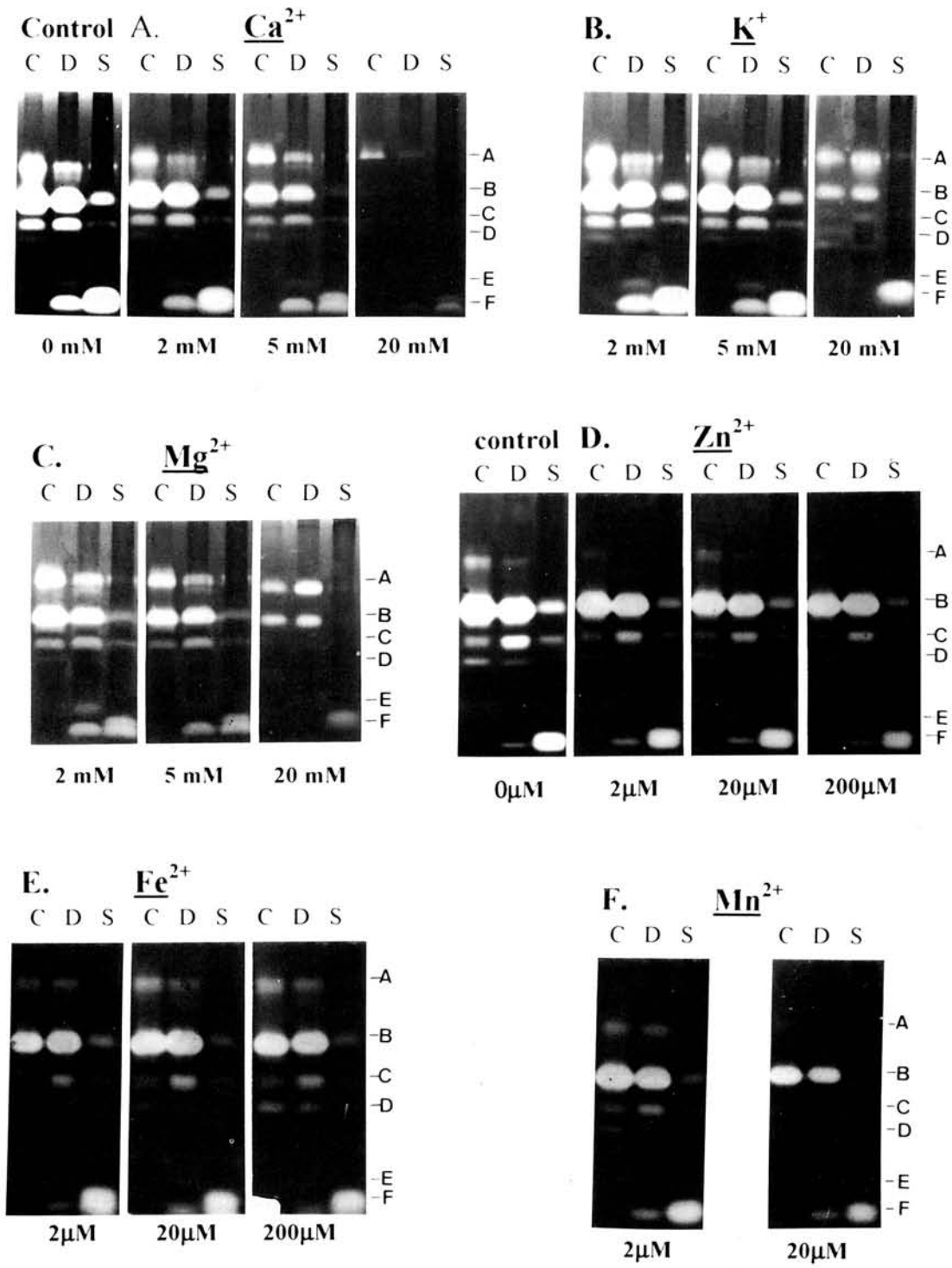

Fig. 4. Effect of the ion on RNase activities. 10μg of sweet potato leaf protein extracts was analyzed and treated as described in "Materials and Methods". The gels were assayed with different ions as labeled beyond the photograph, and the ion concentraction was indicated below. Lane (C), RNase samples extracted from normal growth leaves; lane (D) from 3-day dark growth leaves , and Lane (S) from natural senescent leaves (wilting)

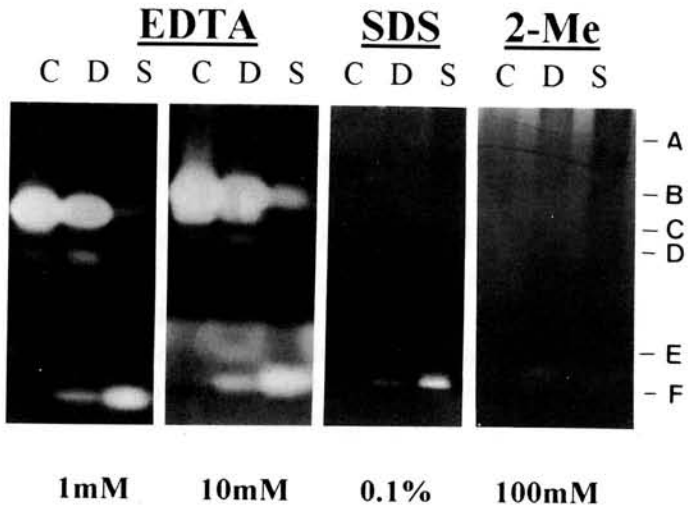

Fig. 5. Effect of EDTA chelation SDS, 2-mercaptoethanol denaturation on RNase activities. 10μg of sweet potato leaf protein extracts was analyzed and treated as described above.

In summary, our data reveal that at least six groups of RNase are present in sweet potato. The activities of optimal pH, ion dependence were characterized in details. Moreover, the occurrence and location in leaf growth development was preliminarily surveyed. It is interesting to note that some groups of RNase were induced under stress condition and varied coordinately with growth developmental stage. Based on this, one could imagine these enzymes in sweet potato might play different physiological roles. More important, further studies are indeed needed to preform in this regards.

ACKNOWLEDGEMENTS

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甘薯核醣核酸水解酵素生化性質的探討與分析

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摘　　要

我們利用核醣核酸電泳膠片技術 已經成功地分析出甘薯（台農 57 號）核醣核酸水解酵素的一些蛋白質特性及基本性質。在甘薯的根、莖、葉中有介於 14-47 kDa分子量的五群核醣核酸水解酵素（A-E）恆定表現。然而卻有一低分子量（約 9,000 kDa）核醣核酸水解酵素在甘薯老化的葉片裡大量合成並且表現出來，我們稱此特殊表現的蛋白質爲 F 群核醣核酸水解酵素。在此篇文章中，我們探討這六群核醣核酸水解酵素之生化活性與 pH 值、離子種類和濃度的關聯性，並利用 SDS 和 2-mercaptoethanol 變性劑觀察此類酵素的生化活性。

關鍵詞：甘薯，核醣核酸水解酵素，自我不親和性核醣核酸水解酵素。

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