SDSによるイネ粗抽出物中のリブロース1・5ビスリン酸カルボキシラーゼ/オキシゲナーゼの蛋白分解誘導について

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Evidence for in vitro Induction of Proteolytic Degradation of Ribulose Bisphosphate Carboxylase/Oxygenase in a Rice Leaf Extract by Sodium Dodecylsulfate

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Synopsis

In vitro proteolytic degradation of ribulose bisphosphate carboxylase/oxygenase (RubisCO) in a leaf extract of rice, *Oryza sativa* L. was investigated. An endoproteinase was partially purified and characterized. Sodium dodecylsulfate (SDS) was found to be a key factor for the activation of this endoproteinase in the degradation of RubisCO. Endopeptidic cleavage of polypeptides by the SDS-activated proteinase resulted in the appearance and disappearance of new and existing polypeptides on SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

Key words : Rice, RubisCO degradation, Activation by SDS, Enzyme purification, Thiol-endoproteinase.

Introduction

It is well-known that RubisCO, the key enyzme of photosynthesis, undergoes little or no degradation prior to leaf senescence, and rapidness of its degradation during senescence differs among species (Huffaker and Miller 1978). However, the mechanism of induction of the RubisCO degradation in a leaf tissue remains to be solved.

In an attempt to identify specific antibody against RubisCO by Western blotting, I unexpectedly observed many degradation products of RubisCO large subunit (RubisCO Ls) on SDS-PAGE gels, as if it were a peptide mapping after a proteinase digestion. This degradation was strictly dependent on the presence of SDS in the homogenizing buffer. Since SDS releases proteins from the membrane, it is of interest to see whether SDS activates a latent proteolytic enzyme in the leaf extract containing cell organelles.

This report presents the existance of the endoproteinase activated by SDS in the leaf extract, the degradation products of RubisCO by the enzyme and partial purification and properties of the enzyme.

Materials and Methods

Plant materials and enzyme preparation. Rice plants (*Oryza sativa* L. cv. Nipponbare) were grown in a growth chamber under natural light conditions at 25°C. Sample leaves were taken from the plants at 30 days after sowing. Leaf segments (500 mg fresh weight) were homogenized in a mortar and pestle with 8 ml of 50 mM Tris/HCl (pH 6.8) containing 140 mM 2-mercaptoethanol (2-ME) and 0.02 % NaN_3. The homogenate was centrifuged at 12,000×g for 20 min at 40°C. The supernatant fraction was used as the leaf extract.

Proteinase assay. Proteolytic degradation of endogenous substrates (RubisCO etc.) in the leaf extract was assayed as reported by Ragaster and Chrispeels (1981). In brief, reaction was started by adding SDS to the extract. The reaction mixture was incubated at 25°C for 2 hr and the reaction was stopped by boiling for 3 min after adding 1/2 volume of a dissociation buffer (125 mM Tris/HCl [pH 6.8], 8 mM urea, 5 % 2-ME, 2.5 % SDS, 0.01 % bromophenol blue and pyronin). The reaction products (autodigested proteins) were immediately analyzed on the SDS-PAGE (12.5 % gels) as described by Laemmli (1970). After staining with Coomassie Brilliant Blue, the gels was destained, and scanned with dual-wavelength TLC Scanner (Shimadzu, Tokyo, Japan). The areas under the peaks corresponding to Ls of RubisCO on the gel were determined by weighing the papers and converting weight to area. Proteolytic activity was expressed as percent of the loss of Ls of RubisCO as compared to that of non-SDS treated control or that obtained without incubation.

When an exogenous substrate was used, the enzyme preparation was preincubated with 2-ME and SDS for 5-15 min at 25°C, and then the reaction was started by adding a known amount of RubisCO and allowed to proceed for specified period at 25°C. Analysis was carried out as mentioned above.

RubisCO purification. RubisCO in the rice leaf was purified by the method of Makino et al. (1983).

Electroanalysis of polypeptides from slab gels, electrotransfer to nitro-cellulose paper, and immunodetection. Ten or 20 μl of each sample per lane were run 3-4 hr on a slab gels (12×12 cm, 1 mm thickness) at 20-25 mA per gel. One gel was stained with Coomassie Brilliant Blue, while another was used for immunoblotting: the proteins in the gel was transferred electrophoretically to nitrocellulose sheets and detected immunologically as described by Towbin et al. (1979), using a peroxidase-conjugated immunoglobulin which is specific to RubisCO Ls.

Protein was determined either by Bio-Rad Protein Assay, using bovine serum albumin as standard, or spectrophotometrically at 280 nm for RubisCO as described by Makino et al. (1983).

Antibody preparation. Antibody against RubisCO was prepared as follows: two rabbits were injected subcutaneously with Freund's incomplete adjuvant containing 12

mg of RubisCO purified as described above. At 4 and the following every weeks they were intravenously injected with 3 mg of the protein for four times and then sacrificed. On the agar immunodiffusion test, the antiserum thus prepared formed a visible precipitin line under an immunoviewer after dilution to 1/128. Furthermore, the antiserum was immunologically specific to the large subunit of RubisCO, and was least reactive to the small subunit (Ss) after Western blotting.

Purification of endoproteinase. Forty-five gm of the rice leaves were homogenized in a Waring blendor with 250 ml of 100 mM Tris/HCl buffer (pH 7.5 at 4°C) containing 10 mM $MgCl_2$, 1 mM EDTA-Na, 20 mM 2-ME, and 5 % PVP. The crude homogenate was filtrated through eight layers of cheesecloth and the filtrate was centrifuged at 18,000×g for 30 min. The resulting supernatant was applied to a Sephadex G-25 column (4.5×45 cm) equilibrated with 25 mM Tris/HCl buffer (pH 7.5 at 4°C) containing 0.1 mM EDTA-Na and 20 mM 2-ME.

The protein fraction obtained (280 ml) were applied to a DEAE Toyopearlpak 650s column (Toyosoda, Japan, 2.2×20 cm) equilibrated with the same buffer. After the column was washed with the buffer, the endoproteinase was eluted with the same buffer increasing concentration of NaCl from 0.13 M to 0.3 M at a rate of 3 ml/min, and 8 ml-fractions were collected by FPLC (Fast Protein Liquid Chromatography, Pharmacia, Sweden). The active fractions were pooled (32 ml), concentrated to 0.65 ml by ultrafiltration (Millipore, USA) and applied to a Superose 12™ (Pharmacia, Sweden) column (1.0×40 cm) equilibrated with the above buffer containing 0.2 M NaCl. The endoproteinase was eluted with the same buffer at a flow rate of 0.5 ml/min and 0.5 ml-fractions were collected.

Results

1. Activation of protein degradation in rice leaf extract by SDS.

Proteolysis of RubisCO in a crude leaf extract could be monitored and quantified by separating the degradation products on SDS-PAGE gels and calculating the peak area of RubisCO Ls. When incubation was carried out at 25°C for 2 hr in the absence of SDS, no visible degradation of RubisCO Ls took place (Fig. 1A, Non SDS). When SDS was added to the reaction mixture, proteolysis was immediately provoked, and new polypeptides with molecular mass smaller than Ls appeared. The amounts of original (Ls) and new bands (A, B, C, in Fig. 1A, SDS) on SDS-PAGE gels changed with time; some (A, B, C) of them increased and others (Ls, D) decreased during incubation. Polypeptide bands A and C appeared within 15 min of incubation, and their amounts increased roughly in proportion to the loss of the RubisCO Ls for 1 hr. This suggests that an endopeptidic cleavage of the RubisCO Ls directly produced A and C polypeptides (Fig. 1B). The band B increased linealy in a similar fission to the band A, while the band D, as was also observed in the non-SDS control, remained to be minor during 2 hr-incubation time.

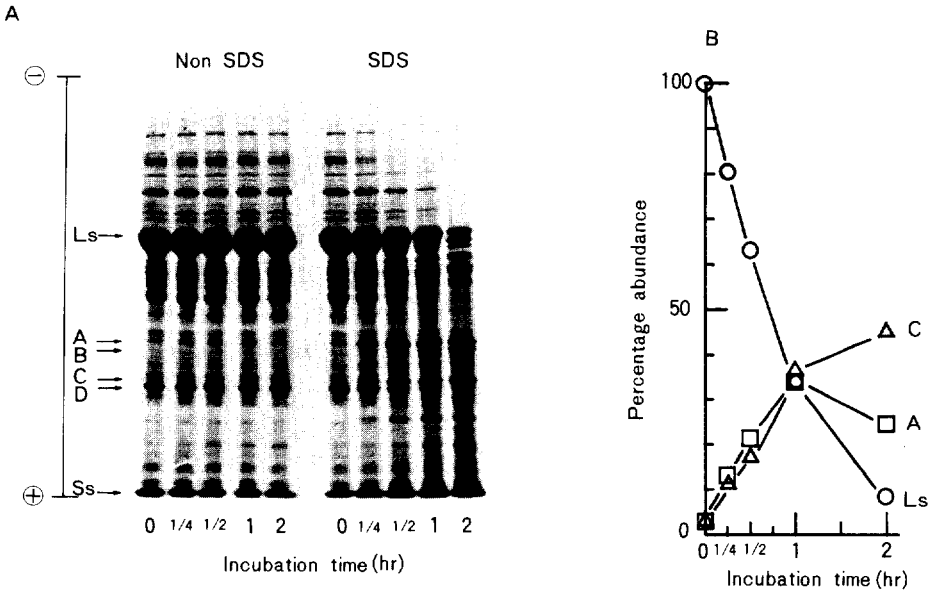


Fig. 1. Degradation of leaf proteins during autodigestion in leaf extract.
A : Electrophoretic patterns of leaf proteins after autodigestion in leaf extract with or without SDS.
Rice leaf extracts were autodigested at 25°C for the times indicated in figure after adding SDS (final concentration, 0.5 %) or distilled water (Non-SDS control) and subjected to electrophoresis in 12.5 % (w/v) acrylamide SDS gel. Amounts of protein applied to PAGE were 25.2 μg/each lanes. Ls and Ss indicate the large and small subunits of RubisCO, and alphabet shows the typical polypeptide bands giving visible changes of staining intensities after autodigestion.
B : Changes in the relative amounts of degradative products in the leaf extract during autodigestion.
Relative intensites of polypeptides, RubisCO Ls, polypeptide A and C on the SDS-PAGE gel in Fig. 1A were measured densitometrically after Coomassie-staining as described in the Materials and Methods, and were expressed as a percent of RubisCO Ls at 0-time incubation (=100). Ls, A and C in figure are the same as in Fig. 1A.

Effects of proteinase inhibitors on the proteolysis of RubisCO in the extract induced by SDS were investigated. Pretreatment with leupeptin (2 μg/ml) before SDS addition completely inhibited proteolysis in the extracts, showing neither loss of the RubisCO Ls, nor appearance of any degradation products on the SDS gels. However, pretreatment with PMSF (2 mM), or 4 M urea could not inhibit the proteolysis (data not shown).

Optimal concentrations of SDS for the activation of the proteolysis were found to be 1 or 0.5 %. Any concentration higher than these values inhibited reversely the

proteolysis, and lower concentration was insufficient for the activation (Fig. 2). Fig. 2 also indicates that the half life of RubisCO Ls at 25°C under optimum SDS concentration is about 45 min.

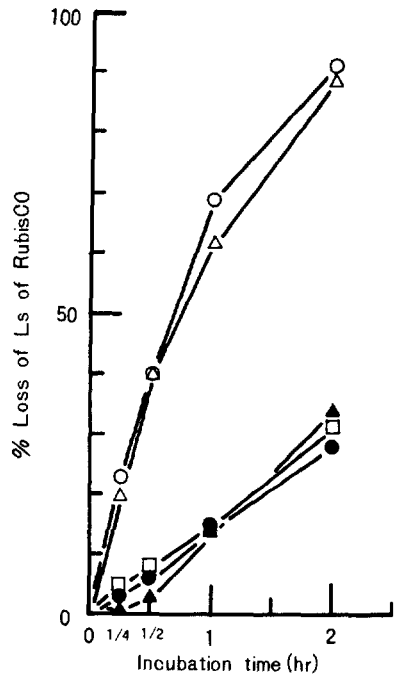


Fig. 2. Effect of SDS concentration on proteolysis in a leaf extract. A rice leaf extract was incubated at 25°C for several times after adding 0.01-5 % SDS (final concentration), and after SDS -PAGE percent loss of Ls of RubisCO was calculated as described in the Materials and Methods. Amount of protein applied to electrophoresis was 28.9 μg/each lane on the gel. Symbols are; ● 5 %, △ 1 %, ○ 0.5 %, □ 0.1 %, ▲ 0.01 %.

The activation of the proteolysis required 2-ME in addition to SDS. In the absence of SDS, addition of 2-ME up to 700 mM to the incubation medium produced no activation. When 2-ME was absent from the medium, activation occurred upon SDS addition, although it was lower than that obtained in the simultaneous presence of SDS and 2-ME. The other detergent, Triton X 100 (1 %) was ineffective to the activation (data not shown).

2. Effect of pH on degradation of RubisCO by endoproteinase.

An experiment was done to see whether more than one endoproteinase, which differ from each other in pH optimum and in peptide cleaving manner, were involved

in the breakdown of RubisCO. The enzyme preparation used here was made free from other proteins including RubisCO as follows; the leaf extract was autodigested for 3 hr at 25°C in the presence of 0.5 % SDS, and concentrated by an immersible CX-10™ ultrafiltration unit (Millipore, USA). It was diluted with 50 mM Tris-HCl buffer (pH 6.8) containing 140 mM 2-ME, and thus both SDS and most of the digested products were removed. This preparation had high endoproteinase activity, though it contained lots of undigested small peptides as revealed by very intense Coomassie-stained smares with broad ranges in the front part of the gel (Plate 1. lane E). The purified RubisCO was stable even when incubated with SDS (Plate 1. lane S). This indicates that the RubisCO preparation was essentially free of endoproteinase.

As shown in Plate 1, optimal pH of the endoproteinase was about 6 and active in a broad range of pH (5-7). However, at alkaline side pH (9-10), the activity was strongly inhibited. There was no difference in the peptide cleavage patterns of the large subunit between neutral and acid pH. Possibly, only one of the proteinases in leaf extracts was preferentially activated by SDS and broken down RubisCO Ls.

Plate 1 also indicated that the relative proportion of various polypeptides, the degradation products, varied during proteolysis. The products are classified into two groups: one with slow mobility (50-40 kd polypeptide group) and other with fast mobility (30-20 kd polypeptide group, Bands A, B and C in Plate 1). No degradation products with molecular weight between 40 kd and 30 kd polypeptides were seen during a short incubation. Judging from the molecular weight of degradation products, polypeptide group of 50-40 kd might be produced from the RubisCO Ls by a sequential 2 kd peptide deletion, and the polypeptide group of 30-20 kd by a single cleavage at near the midpoint of the Ls, producing almost identical two polypeptides.

Fig. 3 shows the densitometrical analysis of degradation products after Coomassie- staining and immunoblotting. Since the antibody used was specific to the Ls of RubisCO, the degradation products of RubisCO Ls were easily detected. However, some breakdown products lost antigenicity. Such fragments similar in molecular size to the RubisCO Ls (50-40 kd polypeptides) gradually lost immunoreactivity as their molecular size decreased by the sequential deletion of 2 kd peptide during incubation. Perhaps, the antigenic site(s) may be on the surface of Ls molecule, and therefore, the highly immunoreactive polypeptide band A was formed directly from the RubisCO Ls with least changes in molecular form.

Fig. 4 shows a densitometric pattern of degradation products of RubisCO Ls after SDS-PAGE and Coomassie-staining. In this experiment, relatively large amount of purified RubisCO (about 1.7 times as much as used in routine assay) was used as the substrate so that the degradative products after SDS-PAGE could be detected. The analysis revealed at least 28 polypeptides derived from Ls. Their molecular weight as larger than Ss and the typical peptides A, B, and C bands, behaved as those in the experiment as shown in Fig. 1.

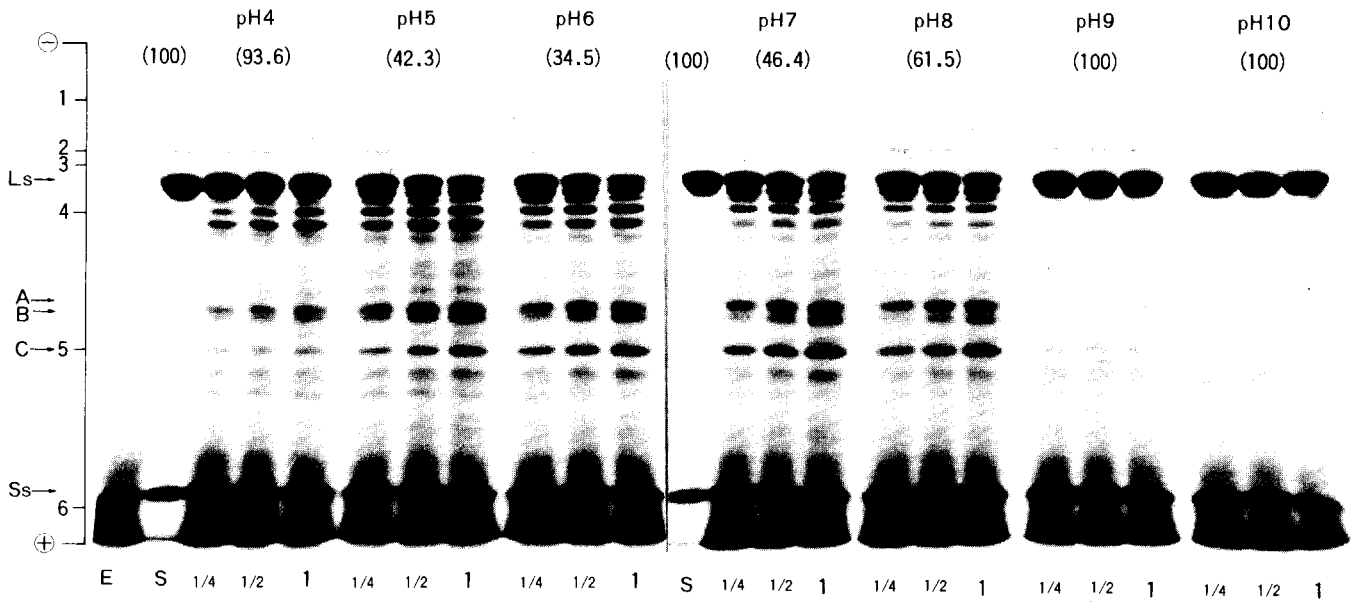


Plate 1. RubisCO degradation as a function of pH.

The system was composed of enzyme (135.6 μg protein) as shown in the text, purified RubisCO (192.5 μg), and 50 mM citrate-phosphate buffer (pH 4-6) or 50 mM Tris-HCl buffer (pH 7-10). Total volume was 200 μl. At the time indicated at the bottom of the figure, each reaction medium (50 μl) was withdrawn, and the polypeptides were separated by SDS-PAGE as described in the Materials and Methods. Numbers in parentheses denoted the relative amounts of RubisCO Ls after 30 min of incubation at each pH levels. 1: Phosphorylase a (mol. wt. 94 kd), 2: Bovine serum albumin (mol. wt. 66 kd), 3: Catalase (mol. wt. 60 kd), 4: Ovalbumin (mol. wt. 45 kd), 5: Chymotrypsinogen (mol. wt. 25 kd), 6: Cytochrome c (mol. wt. 12.5 kd). Symbols are as for Fig. 1A. E: Enzyme only, S: Substrate only.

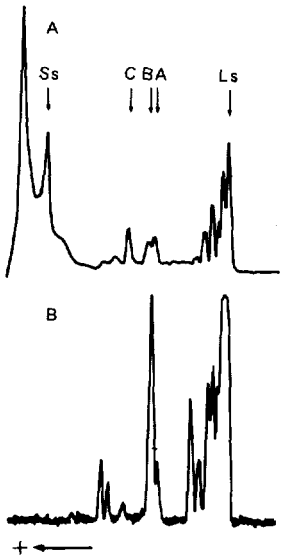


Fig. 3. Densitometric analysis of RubisCO Ls and its breakdown products on SDS-PAGE gel by western blotting.
Materials, enzyme assay (pH 6, 1/2 hr-incubation) and analysis are as for Plate 1.
A: Coommassie-stained gel, B: Western blotting.

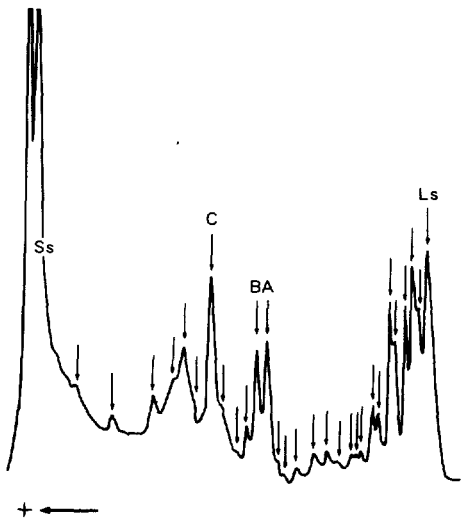


Fig. 4. Densitomeric patterns of polypeptides in the degradation products.
The system was the same as shown in the legend of Plate 1 except for the substrate (RubisCO) concentration (154 μg/100 μl of total volume). The incubation was at pH 6.0 for 1 hr. Arrows indicate degradation products of RubisCO Ls. Symbols are the same as in Fig. 1A.

3. Re-activation of endoproteinase.

The endoproteinase was strongly inactivated when it was dialyzed against a buffer devoid of the SDS and 2-ME. This inactivated enzyme could be reactivated to some extent by adding only SDS (Fig. 5). By increasing the concentration of 2-ME (1.4 -140 mM) in the presence of 0.5 % SDS, the activation was complete and even exceeded 100 %. This unexpected result may be due to a possible autolysis of the active enzyme used as control during storage at 4°C.

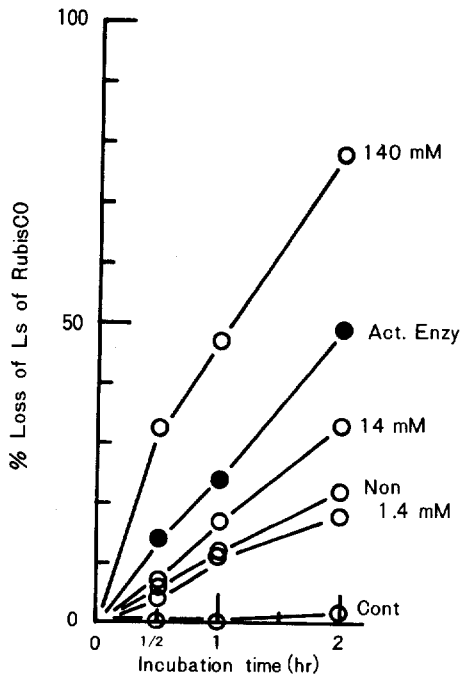


Fig. 5. Effect of 2-ME concentration on the re-activation of an inactivated enzyme.

The leaf extract was prepared and autodigested as described in the legend of Fig. 1A. One part of the autodigested sample was extensively dialyzed against 25 mM phosphate buffer (pH 6.8) (=inactive enzyme), and the other was stored at 4℃ until use (=active enzyme). The inactivated enzyme (167 μg protein) was preincubated with 0.5 % SDS and various concentrations of 2-ME at 25℃ for 1 hr and assayed at pH 6.8. The active enzyme (199 μg protein) was assayed without preincubation with SDS and 2-ME. Proteolytic activity was measured by calculating percent loss of RubisCO Ls as described in the Materials and Methods. Cont and Non indicate the experiments in which either SDS or 2-ME was omitted from the preincubation medium, respectively. Figures are concentrations of 2-ME in the preincubation medium.

4. Purification and characterization of endoproteinase.

The SDS-activatable endoproteinase was partially purified and characterized. The crude leaf extract was essentially free of thylakoid membrane.

By DEAE column chromatography, the endoproteinase activity was eluted as single peak at 0.2 M NaCl between RubisCO and ferredoxin. The active fractions pooled after DEAE column chromatography were free from RubisCO, but still had many contaminating polypeptides upon SDS-PAGE examination.

Fig. 6A shows the profiles of Superose 12™ gel filtration chromatography of the endoproteinase preparation after DEAE chromatography. Endoproteinase activity was eluted as a single peak. The peak fractions completely hydrolyzed RubisCO Ls to smaller peptides (Fig. 6B), but only in the presence of SDS, indicating that the enzyme purified was in a latent form (see Table 1). The molecular weight of the endoproteinase was estimated to be about 150,000.

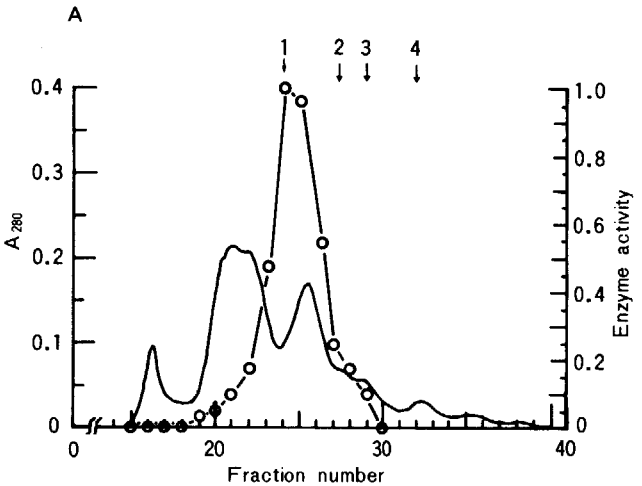
Table 1 shows the effects of some inhibitors on the endoproteinase activity. The endoproteinase was completely inhibited by leupeptin and antipain. pCMB and NEM also inhibited the activity considerably. The others (soybean trypsin inhibitor, pepstatin A, phosphoramidon, EPNP, EDTA, $HgCl_2$) were least inhibitory. PMSF, TPCK and IAA were inhibitory in the absense of 2-ME. Possibly, 2-ME, a sulfhydryl compound, competes with the SH group of endoproteinase for such proteinase inhibitors. In this experiment, no different patterns were seen in the degradation products on the SDS-PAGE gels as compared with that obtained in the experiment as seen in Plate 1. These results suggest that the purified enzyme preparation contained only one species of endoproteinase which is classified as thiol type.

Discussion

The purpose of this study was to investigate the in vitro induction of proteolysis in the endogenous substrate including mainly RubisCO in a rice mature leaf by chemicals and to identify such a proteinase on the proteolysis. The preliminary experiment on the proteolysis in the leaf extracts after homogenizing in a SDS-urea dissociation buffer is to reveal that SDS is the major effector to induce proteolysis among the buffer reagents, Tris, SDS, urea, and 2-ME by the examination of SDS-PAGE.

The present results reveal that SDS accelerates proteolysis to a rapid degree to degrade soluble proteins into smaller in mol wt peptides, and furthermore, there is one endoproteinase activated by SDS in the 12,000×g supernatant of leaf homogenates.

The most interesting observation is the fact that there are endopeptidic cleavages of RubisCO Ls by the endoproteinase with two proteolytic steps, of which one is likely to hydrolyse with 2 kd peptide delation, and one other being to split with almost equal cleavage to produce a almost identical two polypeptides. One can easily explain, therefore, that RubisCO degrade rapidly to smaller in mol wt peptides with various



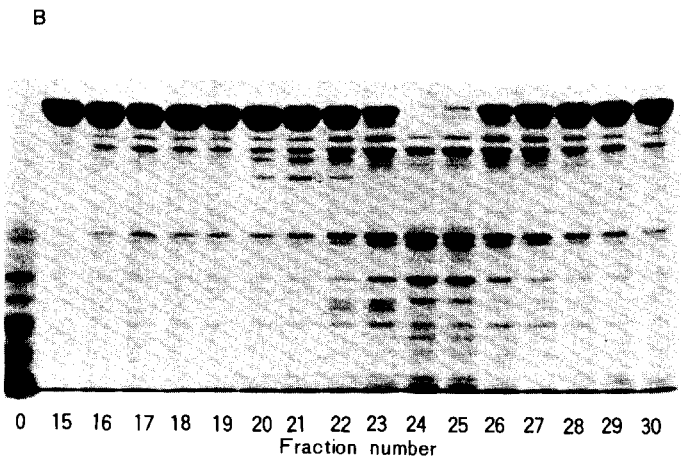


Fig. 6. Purification of an endoproteinase from rice leaves.

A : Separation of endoproteinase by gel filtration.
The active enzyme fraction (0.52 mg protein/200 μl) from DEAE-column was applied to a Superose 12™ column and eluted as described in Materials and Methods. Enzyme activity was measured as described in Fig. 6B and expressed as a relative value of the loss of RubisCO Ls to that obtained in the complete digestion (=1.0).
Solid line: Protein concentration, Open circle: Enzyme activity.
1: Rabbit muscle aldolase (mol. wt. 160 kd) , 2: Bovine serum albumin (mol. wt. 67 kd), 3: Egg albumin (mol. wt. 45 kd), 4: Chymotrypsinogen (mol. wt. 25 kd).

B : Degradation of RubisCO Ls by Superose 12™ elutes.
Enzyme assay (40 μl/each fractions, pH 6.8, 25 min-incubation at 25°C) was the same as in Fig. 3 except that the pre-incubation (5 min at 25°C) of each fraction with SDS (0.5 %) and 2-ME (140 mM) was as described in Materials and Methods. Lane 0 indicates the enzyme assay with 20 μl (52 μg protein) of the active enzyme fraction after a DEAE column. Numbers correspond to the fraction numbers in Fig. 6A.

Table 1. Effects of inhibitors on endoproteinase activity.

Treatment	Final concentration	Enzyme activity
Non-SDS		0
SDS	0.5 %	100
Soybean trypsin inhibitor	10 μg/ml	100
PMSF[1)]	1 mM	80 (81)
TPCK[1)]	2 mM	62 (26)
Leupeptin	20 μg/ml	0
Antipain	20 μg/ml	10
Pepstatin A	20 μg/ml	98
pCMB[2)]	0.5 mM	36 (0)
NEM[1)]	2 mM	74 (2)
IAA[1)]	2 mM	96 (52)
Phosphoramidon	20 μg/ml	100
EPNP[1)]	2 mM	96
EDTA-Na	2 mM	99
$HgCl_2$	1 mM	99

Enzyme (16.8 μg protein) purified as described in text was pre-incubated with each inhibitors in 50 μl of reaction medium for 15 min at 28℃ and pH 6.8, and then activated by adding SDS (0.5 %) and 2-ME (140 mM) to the medium. Endoproteinase activity assay was started by adding RubisCO (163.5 μg) and buffer (50 mM, pH 5.5) and allowed for 30 min at 28℃. Final volume was 100 μl. Enzyme activity was expressed as percent loss of RubisCO Ls to Non-SDS control. The values in parentheses are those for the incubation without 2-ME.

Abbreviations are: SDS, sodium dodecylsulfate; PMSF, phenylmethane sulfonyl fluoride; TPCK, N-tosyl-L-phenylalanine chloromethyl ketone; pCMB, p-chloromercuribenzoate; NEM, N-ethylmaleimide; IAA, iodoacetamide; EPNP, 1, 2-epoxy-3 (p-nitrophenoxy) propane; EDTA-Na, ethylenediaminetetraacetic acid disodium salt.

[1)]dissolved in ethanol, [2)]dissolved in 0.1N NaOH.

molecular masses. In this case, the differences in numbers and mol wt of the degradation products arise as compared with those in some reports as described by Shurtz-Swirski and Gepstein (1985), Colas des Frances et al. (1985) and Paech and Dybing (1986). This reason is due to the differences in the experimental conditions and plant materials, of which extraction has been argued identification of the products.

The striking evidence will be that the endoproteinase is easily activated, inactivated and reactivated by the addition or removal of SDS and 2-ME in the reaction system. Of course, these chemicals are not present in intact cells of a mature leaf. As far as the present time natural substances substitutive for SDS and 2-ME are not discovered to activate the enzyme. Dahlmann et al. (1985) report that not only SDS, but fatty acids activate pronouncedly the multicatalytic proteinase from rat skeletal muscle, and potential activators such as fatty acids can probably induce in vivo the enzyme to participate in muscle protein breakdown. Therefore, a potential candidate for natural activators of the leaf endoproteinase may be fatty acids. This findings will, nevertheless, contribute to elucidate the mechanism that regulate the breakdown of RubisCO from intact plants during senescence.

Mae et al. (1985) found various kinds of proteinase in rice leaf extract. These activities, assayed by casein as substrate, as well as their elution profiles from a DEAE- cellulose column changed as rice leaves aged. These enzymes were characterized as metal- and serine proteinases from inhibitor studies. In contrast, the rice leaf proteinase purified in the present study was inhibited by various sulfhydryl reagents, but least by metal chelators. Therefore, the enzyme may be characterized as thiol-type, and is different from those reported by Mae et al. (1985).

The present enzyme is rather similar to the barley endopeptidase (EP_1) in several points, e.g. optimal pH, requirement of sulfhydryl reagent for stability, stability in SDS, and complete inhibition by leupeptin (Miller and Huffaker 1981), and comparison with the degradation patterns of RubisCO Ls incubated with EP_1 and lysed vacuoles of barley leaf (Thayer and Huffaker 1984). It can be speculated, therefore, that the endoproteinase will be localized in vacuole of a rice leaf even though further observations are necessary.

In addition, the present study indicates the potential danger of treating crude leaf extract directly with SDS and 2-ME prior SDS-PAGE. Special attention should be paid to avoid an unexpected cleavage of proteins through the activation of such endopeptidase(s).

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Summary

In vitro activation of a ribulose bisphosphate carboxylase/oxygenase (EC. 4. 1. 1. 39., RubisCO) degrading enzyme activity in a rice leaf extract was investigated. In measuring proteolytic degradation of endogenous substrates (RubisCO) in crude

extracts, SDS-polyacrylamide gel electrophoresis and Western blotting with specific antibody to RubisCO large subunit were used. The endogenous substrate in the extract was hardly degraded unless SDS was added at an appropriate concentration. Time required for degrading half the amount of RubisCO large subunit was estimated to be about 45 min at pH 6.8 and 25°C. The SDS-activated proteinase was strongly inactivated when SDS was removed by dialysis. Reactivation of the inactivated enzyme to the initial level required both 2-ME and SDS. Profiles of the RubisCO degradation products on SDS-PAGE gels indicated that the degradation took place through two manners: one splitting polypeptide from one end producing 2 kd peptide, and the other at the midpoint of RubisCO Ls polypeptide. Furthermore, as a result of proteolytic degradation of the Ls, 28 polypeptides with molecular weight larger than the small subunit (14 kd) were detected on the SDS-PAGE gels, and some of them were reactive to antibody against RubisCO Ls after Western blotting. The endoproteinase was partially purified from rice leaves by ion-exchange column chromatography and gel filtration. The apparent molecular weight of purified enzyme was estimated to be 150 kd by gel filtration. The enzyme was inhibited by several SH reagents.

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和文摘要

SDSによるイネ粗抽出物中のリブロース 1・5 ビスリン酸カルボキシラーゼ／オキシゲナーゼの蛋白分解誘導について

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イネ葉粗抽出物中のリブロース1・5ビスリン酸カルボキシラーゼ／オキシゲナーゼ(RubisCO)の分解に関与する蛋白分解酵素の試験管内活性化を試みた．この実験では，粗抽出物中に共存するRubisCO（基質）を分解する酵素の分解率の測定および酵素－基質反応系中分解される基質蛋白の定量にSDS－ポリアクリルアミド電気泳動法およびウェスタンブロッティング法を用いて行った．その結果，粗抽出中に共存する基質は，SDSが反応抽出物組成中に添加されない限り分解はしなかった．この分解の半減率を求めるとpH 6.8，25°Cで45分であった．

SDSによって活性化された酵素はSDSを透析して除去することにより失活したが，その再活性化はSDSと2-MEを必要とした．

蛋白分解酵素によるRubisCOの分解は，2段階により行われ，そのひとつは2 kdペプチドずつ分解される過程と，他は基質をほぼ等分割して相同のポリペプチドを生成する分解様式がある可能性が示された．その中で，RubisCOの大サブユニットに関連する分解物で，その小サブユニットより大きい分子量を持つ分解産物は28個あり，その中の幾つかは特異的な抗原性を示すことがわかった．

この蛋白分解酵素は，イネ葉粗抽出物よりイオン交換クロマトグラフィおよびゲルろ過法により不活性型として部分的に精製された．この酵素はゲルろ過法で分子量150 kdと算出された．また，この酵素は阻害実験によりチオール型蛋白分解酵素であることが明らかにされた．