Three RNases in Senescent and Nonsenescent Wheat Leaves¹

Characterization by Activity Staining in Sodium Dodecyl Sulfate-Polyacrylamide Gels

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ABSTRACT

We have described three RNases in wheat leaves (Triticum aestivum L. cv Chinese Spring) and developed assays for measuring each RNase individually in crude leaf extracts. We initially used activity staining in sodium dodecyl sulfate-polyacrylamide gels to characterize RNases in extracts of primary and flag leaves. We thus identified acid RNase (EC 3.1.27.1, here designated RNase WLA), and two apparently novel enzymes, designated RNasea WLA and WLC. RNase WLA activity displays a distinctive isozyme pattern, a molecular mass of 26 kilodaltons (major species), a broad pH range with an optimum near neutrality, insensitivity to EDTA, and stimulation by moderate concentrations of KCl and by MgCl₂. RNase WLC activity exhibits a molecular mass of 27 kilodaltons, a neutral pH optimum, insensitivity to EDTA, and inhibition by KCl, MgCl₂, and tri-(hydroxymethyl)aminomethane. Based on this catalytic properties established in gels, we designed conventional solution assays for selective quantitation of each RNase activity. We used the assays to monitor the individual RNases after gel filtration chromatography and native gel electrophoresis of extracts. In accompanying work, we used the assays to monitor RNases WLA, WLC, and WLD, which are present in senescent and nonsenescent leaves, during the course of leaf senescence.

A net loss of RNA, encompassing differential turnover of particular RNA species, is associated with leaf senescence (7, 9). However, the enzymology of this process is not defined and the mechanisms that regulate nucleic acid turnover during senescence are not known. We recently observed that the activity of SSP-nuclease ³ increases markedly during senescence of flag leaves in wheat, and during dark-induced senescence of wheat seedlings (3). In the present work, we describe RNases in wheat leaves that may also mediate RNA turnover during senescence.

Our experimental approach exploited activity staining in SDS-polyacrylamide gels (1) to separate and characterize each of the individual RNase species found in wheat leaves. By initially using gels to determine the catalytic properties of each RNase, we were able to characterize two apparently novel RNases in addition to the ubiquitous acid RNase and to establish solution assays that measure each enzyme individually in leaf extracts. This approach circumvented the longstanding problems posed by the multiplicity of RNases found in plant tissues (7, 13).

MATERIALS AND METHODS

Plant Material

Wheat seedlings (Triticum aestivum L. cv Chinese Spring) were grown in vermiculite in 7.5 cm pots at 25°C in a growth chamber under continuous light (300 µE, cool white fluorescent and incandescent). Plants were given modified Johnson's solution (8) on alternate days beginning on the fifth day [2 mM (NH₄)₂HPO₄, 6 mM KNO₃, 4 mM Ca(NO₃)₂, 1 mM MgSO₄, 0.2 mM ferric sodium EDTA, 50 mM KCl, 25 mM H₂BO₃, 5 µM MnSO₄, 2 µM ZnCl₂, 0.5 µM CuSO₄, 0.1 µM (NH₄)₆Mo₇O₄₂₄, and water on all other days. To induce senescence, 8-d-old seedlings (average length of primary leaves about 21 cm) were placed in continuous darkness at 25°C (14); some of these seedlings were then returned to continuous light to examine the reversibility of senescence. In other experiments on natural senescence, plants were grown to maturity in a greenhouse, given water twice weekly and quarter-strength Johnson's solution once weekly. Flag leaves were sampled at various times before and after anthesis.

Sampling and Extraction

Sections of primary leaves (8 cm long), cut 2 cm from the tip, were frozen in liquid nitrogen and stored at −70°C until used. Leaf sections (0.40 g) were ground in liquid nitrogen with a mortar and pestle, and the powder was carefully transferred to a chilled plastic tube. Ice-cold buffer was added (0.05 M Tris-HCl [pH 7.5], 0.15 M NaCl, 1 mM N-ethylmaleimide, 10 ml/g leaf) and the suspensions were homogenized in an ice bath for approximately 15 s by using a Brinkmann Polytron. Aliquots (1 ml) of homogenates were centrifuged for 8 min at top speed (approximately 14,000g) in an Eppen-

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³ Abbreviations: SSP-nuclease, single-strand-prefering nuclease; WL, wheat leaf; Vₑ/Vₒ, relative elution volume.
dorph microcentrifuge at 6°C. The supernatant solutions were frozen in small aliquots on dry ice and stored at −70°C. Entire flag leaves were harvested at intervals after anthesis, and the leaves were frozen at −70°C. Individual leaves (0.35–0.89 g) were ground in liquid nitrogen and extracted as described above.

Electrophoresis

Activity staining in 12.5% SDS polyacrylamide gels was as reported earlier (1) with minor modifications. Separating gels included 0.1 mg/mL bovine fibrinogen (2) as well as 0.3 mg/mL wheat germ rRNA. Samples were heated for 2 min at 100°C in buffer containing 2% SDS. After electrophoresis at room temperature, SDS was removed from gels and enzymes were renatured as described (1, 3). Gels were then incubated at room temperature in various buffers for 15 h to permit in situ degradation of RNA by resolved and renatured RNases. Undigested RNA remaining in the gel was stained with toluidine blue. Activity staining in native gels was the same, with SDS and fibrinogen omitted from the protocol.

Enzyme Assays

RNase activity was assayed by measuring the release of acid-soluble material from wheat germ rRNA. Assay mixtures (300 μL) containing 5.2 A260 units/mL RNA, 0.1% BSA, buffer as indicated in the text, and 2.5 to 50 μL of sample were incubated for both 6 and 66 min at 31°C. After addition of 1 mL 3.4% perchloric acid, suspensions were held on ice for 10 min and then centrifuged for 5 min at top speed (approximately 14,000g) in an Eppendorf microcentrifuge at 6°C. Absorbance of the supernatant solutions was read at 260 nm, and the difference in A260 between 66 and 6 min was determined. One unit is that amount of enzyme yielding an absorbance change of 1.0 min⁻¹ mL⁻¹ of incubation mixture.

Phosphodiesterase activity was measured in assay mixtures (0.5 mL) containing 0.5 mM 5′-p-nitrophenylthymidylate (Sigma), 0.1 M Tris-Cl (pH 7.5), and 0.1% BSA. Mixtures were incubated at 31°C for 2.5 h and then treated with 0.5 mL 0.2 N NaOH on ice. Absorbance was read at 410 nm. Inclusion of 10 mM MgCl₂ in assay mixtures did not affect activity measured in leaf extracts or column fractions.

RESULTS

Characterization of RNases WLₐ, WLₑ, and WLᶜ in SDS Activity Gels

The activity gels in Figure 1 establish that wheat leaf extracts contain three different RNases with distinctive electrophoretic and catalytic properties. These enzymes are acid RNase (EC 3.1.27.1, here designated RNase WLₐ) and two apparently novel RNases we call RNases WLₑ and WLᶜ.⁴ The gels in

Figure 1. Characterization of RNases WLₐ, WLₑ, and WLᶜ in SDS-activity gels. Wheat leaf extracts were subjected to electrophoresis and activity staining as described in "Materials and Methods." Buffers used for in situ degradation of embedded RNA by electrophoretically resolved RNases were: (A) 0.05 M Na acetate, pH 5.7; (B) same as (A) +10 mM MgCl₂; (C) same as (A) +0.2 M KCl; (D) 0.1 M imidazole-Cl, pH 6.0; (E) same as (D) +1 mM EDTA; (F) 0.1 M imidazole-Cl, pH 8.0; (G) same as (F) +5 mM MgCl₂; (H) same as (F) +0.15 M KCl; (I) same as (F) +0.25 M KCl; (J) 0.1 M Tris-Cl, pH 7.4; (K) same as (F); (L) same as (I). Gels were loaded with crude extracts representing the specified wet weight of leaves: (1) primary leaves of 13-d-old seedlings grown in light, 375 μg; (2) senescent primary leaves of 13-d-old seedlings grown in light 8 d and held in dark 5 d, 375 μg; (3) flag leaf, 7 d preanthesis, 75 μg; (4) senescent flag leaf, 27 d postanthesis, 75 μg; (5) primary leaves of 13-d-old plants grown in light, 375 μg; (6) senescent primary leaves of plants grown in light 7 d and held in dark 6 d, 375 μg.

⁴ For convenience, we designated wheat leaf RNases in order of decreasing electrophoretic mobility in SDS activity gels, with RNase WLₐ having highest mobility and RNase WLᶜ lowest. These designations are provisional, pending adoption of a systematic nomenclature for plant RNases.
Figure 1 were cast with rRNA in the matrix and, after electrophoresis, were incubated under varying conditions to permit degradation of embedded substrate by the resolved enzymes. Use of differing incubation conditions allows in situ determination of the properties of individual, catalytically active polypeptides and identification of isozymes. Properties of RNases WLₐ, WLₐ, and WL_c revealed in Figure 1 are summarized in Table I.

Hallmarks of plant acid RNases are pH optima near 5 or 6, inhibition by metal ions and by moderate ionic strength, insensitivity to EDTA, and molecular mass of about 20 kD (7, 13). The activity we call RNase WLₐ has these characteristics. RNase WLₐ is the fastest band in the gels of Figure 1A to C, which were incubated in acetate buffer at pH 5.7 for enzymatic degradation of RNA. RNase WLₐ is also active in imidazole buffer at pH 6.0 (Fig. 1D, E), but is inactive at higher pH values (Fig. 1F-L). Gel sections incubated in 0.1 M K acetate/KCl buffers of varying pH and constant ionic strength (I = 0.15) reveal greatest activity at pH approximately 5.0; in 0.1 M Na acetate buffers, without compensation for varying ionic strength, the apparent optimum is closer to pH 5.5 (gels not shown). RNase WLₐ is insensitive to EDTA (Fig. 1D) and is inhibited by MgCl₂ and by KCl (Fig. 1A–C). The molecular mass of RNase WLₐ is estimated to be 20 kD by comparing its mobility with that of protein standard run in the same gel (3, 12) under either nonreducing conditions (Fig. 2) or reducing conditions (data not shown). No band corresponding to RNase WLₐ appears in gels cast with denatured DNA. We conclude that RNase WLₐ corresponds to the acid RNase of wheat leaves (5).

Hallmarks of RNase WLₐ, observed in gels cast with rRNA, include a distinctive isozyme pattern, a molecular mass of 26 kD (major species), a broad pH range with an optimum near neutrality, insensitivity to EDTA, and stimulation by moderate concentrations of KCl or NaCl (e.g. 0.15 M) and by MgCl₂. RNase WLₐ can be seen in Figure 1A to C as a group of bands migrating above RNase WLₐ. A diagnostic isozyme pattern (e.g. Fig. 1C, lane 2) consists of an intense, broad central band with a discrete narrow band above and a fine doublet below. RNase WLₐ has a wide pH range, showing strong activity at pH 5.7 (Fig. 1B, C) and at pH 8.0 (Fig. 1H, I). Gel sections incubated in 0.1 M Tris-HCl/KCl buffers of varying pH and constant ionic strength (I = 0.15) (not shown) reveal strong activity between pH values 6.8 and 8.4 with a broad optimum near pH 7.6. A significant property of RNase WLₐ is its stimulation by moderate concentrations of KCl or NaCl, displayed at low pH in Fig. 1A and C. Stimulation is also observed in imidazole buffer at higher pH, under conditions in which RNase WL_c is also active. (Because RNase WLₐ migrates only fractionally slower than the central RNase WLₐ band, the distinctive isozyme pattern of RNase WLₐ is useful in distinguishing the two enzymes.) Inspection of Figure 1F, H, and I reveals that RNase WLₐ is active in 0.1 M imidazole buffer at pH 8.0 if either 0.25 M KCl (II) or 0.15 M KCl (I) is added, but is not active without added salt (I). (The activity seen in buffer alone [Fig. 1F] is RNase WL_c and, as Figure II and H show, RNase WL_c is inhibited by added salt.) RNase WLₐ can likewise be stimulated by MgCl₂ both at low pH (Fig. 1A, C) and at high pH (Fig. 1F, G). Importantly, however, Mg²⁺ is not required for activity, because addition of up to 10 mM EDTA is not inhibitory (e.g. Fig. 1D, E). As shown in Figure 2, molecular masses of 28,

<table>
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<tr>
<th>Property</th>
<th>RNase WLₐ</th>
<th>RNase WLₐ</th>
<th>RNase WL_c</th>
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<tr>
<td>Molecular mass</td>
<td>20 kD (A,D)</td>
<td>26 kD (major)</td>
<td>27 kD (F,K)</td>
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<td></td>
<td>28 kD (minor)</td>
<td>28 kD (minor)</td>
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<td></td>
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<td>24 kD doublet (minor) (C,L)</td>
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<tr>
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<td>None (D vs. E)</td>
<td>None (D vs. E)</td>
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<tr>
<td>Effect of KCl</td>
<td>Inhibitory (A vs. C)</td>
<td>Stimulatory (A vs. C, F vs. H/I, K vs. L)</td>
<td>Inhibitory (F vs. H/I, K vs. L)</td>
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<tr>
<td>Effect of MgCl₂</td>
<td>Inhibitory (A vs. B)</td>
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<tr>
<td>Other distinguishing</td>
<td>Inactive pH &gt; 6 (F–L)</td>
<td>Active at pH values 5.7–8 in presence of KCl, NaCl or MgCl₂ (B, C, G–J, L)</td>
<td>Can be inhibited by Tris (I vs. K) and OAc (D vs. A)</td>
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Table I. Properties of Wheat Leaf RNases Shown in Figures 1 and 2

The molecular masses listed are from the experiment of Figure 2. The catalytic properties and isozyme patterns are depicted in the gels of Figure 1. The letters following each property refer to individual gel sections in Figure 1. With respect to the last entry under RNase WL_c, the samples applied to sections A and D differ, but section F shows RNase WL_c in sample 1.
pH values from 6

ID

gels

The band migrating just before RNase WLA, WLB, and WLC as well as SSP-nuclease (see Fig. 1). The $R_f$ values of activity bands (indicated by arrows) were used to estimate the mol wts of individual RNases (3, 12). Protein standards (filled circles) and molecular masses used were: BSA, 66 kD; ovalbumin, 43 kD; rabbit muscle glyceraldehyde-3-phosphate dehydrogenase, 36 kD; carbonic anhydrase, 29 kD; trypsinogen, 24 kD; trypsin inhibitor, 20.1 kD; bovine alpha-lactalbumin, 14.2 kD.

26, and 24 kD are estimated for the upper isozyme of RNase WLa, the broad central band, and the lower doublet, respectively.

A third RNase, RNase WLC, was noted earlier as a broad band migrating just above the central RNase WLa species (Fig. 1F). The distinction in mobility and isozyme patterns between RNases WLC and WLa is precisely illustrated in gel section K/L, which contains three lanes loaded identically. The central lane was cut down the middle after electrophoresis, and the left half of the gel section was incubated under conditions appropriate for RNase WLC and the right half under conditions appropriate for RNase WLa. Although it is apparent that RNases WLC and WLa separate only narrowly in SDS gels, the two enzymes are markedly different catalytically. Distinguishing characteristics of RNase WLC, observed in gels cast with rRNA, include a molecular mass of 27 kD, neutral pH optimum in imidazole buffer, activity in the presence of EDTA, inhibition by moderate concentrations of KCl or NaCl (e.g., 0.15 M) and by MgCl₂, and inhibition by Tris-HCl and Na⁺ or K⁺ acetate buffers. As illustrated in Figure 1D and F, RNase WLC is active in imidazole buffer at pH values from 6 to 8. In 0.1 M imidazole-HCl/KCl buffers of varying pH and constant ionic strength (I = 0.15), an optimum between pH 7.0 and pH 7.5 was observed (gels not shown). Un Unexpectedly, both 0.1 M Na⁺ and K⁺ acetate buffers and 0.1 M Tris-HCl buffer are strongly inhibitory. Inhibition in acetate buffer is exemplified at pH 5.7 in Figure 1A to C, and was also observed in acetate at pH 6.0 (not shown), whereas imidazole buffer at pH 6.0 permits activity (Fig. 1D).

Inhibition in 0.1 M Tris-HCl buffers is exemplified in Figure 1J and was observed in RNA-cast gels at pH values from 7.0 to 9.0; the enzymes active in gel J are RNase WLa and SSP-nuclease (3). The inhibition of RNase WLC by Tris buffer can be alleviated, e.g., by addition of 1 to 5 mM EDTA at pH 7.4, and both the inhibition and its relief serve as useful diagnostic indicators of the enzyme. As noted earlier, RNase WLC is inhibited by addition of either KCl or MgCl₂ to imidazole buffer at pH 8.0 (Fig. 1F–I), whereas RNase WLa is stimulated. As shown in Figure 2, the major RNase WLC band exhibits a molecular mass of 27 kD.

Neither RNase WLC nor WLa appear in gels cast with denatured or native DNA instead of RNA, but both enzymes, as well as RNase WLa, are active in gels containing poly(U), poly(C), and poly(A) (not shown).

Selective Measurement of Individual Enzymes

To facilitate monitoring of individual wheat leaf RNases, we defined standard conditions for preferential detection of RNases WLa, WLB, and WLC, using rRNA as a substrate. We used these conditions to visualize each enzyme selectively in gels and to assay each enzyme preferentially in crude extracts and in chromatographic fractions. RNase WLa can be detected preferentially in 0.1 M sodium acetate, or 0.1 M sodium cacodylate, at pH 5.7. (It is significant that residual activity of RNase WLa is not completely excluded when present at high levels, as in extracts of leaves well into senescence [Fig. 1A, lanes 2 and 4]). The divergent responses of RNases WLa and WLB to added salt allows selective measurement of RNase WLa in 0.1 M imidazole-HCl/0.2 M KCl, pH 8.0 (Fig. 1H, I), whereas RNase WLC can be selectively measured in 0.1 M imidazole-HCl, pH 8.0 (Fig. 1F). SSP-nuclease activity can be selectively measured by using DNA as a substrate (3).

Activity Staining in Native Gels

Electrophoresis of crude enzyme preparations in nondenaturing gels can be complicated by self-aggregation of enzymes and by adventitious association of catalytically active polypeptides with other macromolecules, with low molecular mass impurities, and with the polyacrylamide matrix. When substrate is embedded in the gel, enzyme-substrate interactions can also occur. The resulting multiplicity of bands is difficult to interpret. Nonetheless, we subjected wheat leaf extracts to activity staining in native gels to observe the patterns of RNase activity under the selective conditions described above (Fig. 3). RNase activity at pH 5.7, due presumably to RNase WLa, migrates as a pair of sharply delineated bands with high

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1 These gel sections were loaded with extracts of nonsenescent primary leaves, which have a high ratio of RNase WLC to RNase WLa and are favorable for observing the response of both enzymes simultaneously.
mobility (Fig. 3A). This pattern is consistent with the finding that wheat leaf acid RNase elutes from DEAE-Sephadex in two peaks (5). Activity in 0.1 M imidazole-HCl/0.25 M KCl, pH 8.0 (Fig. 3B), presumably RNase WLb, migrates primarily as a pair of slow bands. Activity in imidazole buffer alone (Fig. 3C), indicative of RNase WLc, has a complex pattern including several bands that travel faster than RNase WLb and do not appear in buffer with added KCl (Fig. 3B). Thus, native gels reveal activity bands with pH and buffer preferences expected of RNases WLa, WLb, and WLc.

**Gel Filtration of Extracts**

Wheat leaf extracts were chromatographed on a Sephadex G-75 column to obtain further information concerning their RNase activities and to correlate chromatographic profiles with electrophoretic data. Individual column fractions were assayed, and also activity stained in SDS gels, under the selective conditions defined earlier. Figure 4 shows the results obtained with an extract of senescent flag leaves. Acid RNase activity, measured in test tube assays, elutes as a symmetrical peak (Fig. 4, top). Activity observed in gels (Fig. 4, bottom) parallels in intensity that measured in assays and has the mobility of RNase WLa. RNase activity assayed in 0.1 M imidazole-HCl/0.2 M KCl, pH 8.0, elutes broadly and heterogeneously. Activity in gels has the isozyme pattern and mobility of RNase WLa and corresponds in intensity to activity measured in solution. The upper RNase WLa band, the broad central band, and the lower doublet elute from the column with progressively higher Vf/Vo, consistent with their increasing mobility in SDS gels. Considerable trailing of RNase WLb is evident, despite inclusion of detergent in the elution buffer; if detergent is omitted, recovery is very poor, indicative of yet stronger adsorption to the gel matrix. Activity assayed in 0.1 M imidazole-HCl, pH 8.0, elutes heterogeneously and displays the pattern and mobility of RNase WLc in gels. Notably, the elution profile differs quantitatively from that of RNase WLb. For example, the ratio of RNase WLc to RNase WLb assayed at Vf/Vo = 1.60 is double that at Vf/Vo = 2.10. Inclusion of detergent in the elution buffer is not required for recovery of RNase WLc activity, as is the case for RNase WLb, SSP-nuclease activity chromatographed in two peaks, one in the void volume and another preceding the RNase activities. The second peak is heterogeneous and contains the major electrophoretic species observed in gels. The peak in the void volume contains electrophoretic species of high mobility, possibly partially degraded molecules generated by proteolysis in senescent extracts. These species may aggregate or bind avidly to nucleic acid or other material, resulting in early elution from the column; they are evident in SDS-activity gels of unfractionated extracts of senescent leaves (3). Phosphodiesterase activity that hydrolyzes thymidine 5'-monophosphate p-nitrophenyl ester elutes between the nuclease peaks.

**DISCUSSION**

RNase activity is ubiquitous in plant tissues. Moreover, changes in RNase activity are associated with developmental processes such as senescence and with physiological responses to injury, environmental stress, and disease (7, 13). Yet, we lack basic information concerning the number and properties of individual RNases in various plant tissues and are virtually ignorant of the functions of individual enzymes in the maintenance and turnover of nucleic acid populations. The voluminous work on RNases and nucleases in plant tissues has established that acid RNase (EC 3.1.27.1), SSP-nuclease (EC 3.1.30.1), and phosphodiesterase (EC 3.1.4.1 or 3.1.15.1) occur broadly. In addition to these relatively well characterized enzymes, a multitude of additional RNase activities has been reported. However, as noted by Farkas (7), it has not been possible to meaningfully compare these other activities or to catalog their occurrence among different species, tissues, and organelles. Obstacles to systematic integration of available data include the unknown purity of almost all the RNase preparations, and differences in purification procedures, assay conditions, and properties analyzed.

In the present work, we exploited both the resolving power...
RNase $W_{LA}$ includes two electrophoretically distinct species separable in native gels. Thus, RNase $W_{LA}$ most likely corresponds to RNases I and II of Chevrier and Sarhan (5). RNase $W_{LA}$ appears to differ, however, from the acid RNase of wheat germ purified by Torti et al. (11). This wheat germ enzyme displays catalytic properties characteristic of plant acid RNases but exhibits a remarkably low molecular weight (9000) on Sephadex G-100. SDS activity gels of wheat germ extracts show the presence of two major RNases active in 0.1 M Na acetate buffer, pH 5.7. One activity has the mobility of RNase $W_{LA}$, whereas a second, not seen in wheat leaf extracts, migrates 1.3 times faster than RNase $W_{LA}$ (our unpublished data). It is this second, lower mol wt activity that Torti et al. appear to have purified. In support of this interpretation, RNase $W_{LA}$ exhibits a mol wt of 20,000 on Sephadex G-75 (our unpublished data). Moreover, Torti et al. (11) observed two chromatographic peaks of acid RNase activity in crude preparations and purified only one of them.

RNase $W_{LA}$ is unusual in its broad pH range and stimula-
tion by MgCl$_2$ and salt. The stimulation is probably due to increased ionic strength rather than to activation by specific ions, especially in view of the insensitivity to EDTA; possibly the enzyme has a preference for double-stranded regions in RNA that would be stabilized by addition of salt to the assay buffer. Our conclusion that the enzyme has not been described previously is supported by the fact that RNase $W_{LA}$ is a minor component of the total RNase activity of nonsenescent leaves. RNase $W_{LC}$ is unusual in its inhibition by Tris-HCl buffers, but shares with other RNases such commonly observed properties as inhibition by MgCl$_2$, salt, and acetate. It is not possible to ascertain the relationship of RNases $W_{LA}$ and $W_{LC}$ to the nuclear RNase activity reported by Chevrier and Sarhan (6). This activity displays behavior apparently unlike that of RNase $W_{LA}$ (e.g. inhibition by 0.15 M NaCl) and of RNase $W_{LC}$ (e.g. activity in the presence of Tris-cacodylate buffer and inactivity toward poly(C).) The RNase preparation of Chevrier and Sarhan (6), which was partially purified from chromatin in exceedingly small quantities, is a good candidate for activity gel analysis.

A major purpose of this study was to develop assays for independent measurement of each of the RNases in wheat leaf extracts. Initial characterization of RNases $W_{LA}$, $W_{LB}$, and $W_{LC}$ in SDS activity gels allowed us to design such assays, which were used in the accompanying study (4) to delineate differential changes in the activity of each RNase during senescence. The occurrence of RNases $W_{LA}$, $W_{LB}$, and $W_{LC}$ in other wheat organs (our unpublished data) suggests that the assays may have general utility in monitoring wheat RNases during other developmental processes and physiological responses.

In conclusion, we emphasize that wider use of SDS activity gels in characterizing plant RNase preparations would lend greater specificity and clarity to the findings of individual laboratories and expedite integration of data from different laboratories. The technique provides, with ease, speed, and sensitivity, information concerning the number, mol wts, and catalytic properties of individual RNase species in either crude or purified preparations. Because it permits characterization of RNases without any prior purification, the technique is ideally suited to direct comparison of the RNase complement of SDS-PAGE to separate the multiple RNases in wheat leaves and the versatility of activity staining to characterize them. In addition to SSP-nuclease, which is described elsewhere (3), we observed three catalytically distinct RNases, each of which includes two or more electrophoretic species. These three different RNases and their relationships to previously described wheat RNases (5, 6, 11) are discussed below.

The acid RNase of wheat leaves has been partially purified by Chevrier and Sarhan (5) and is comparable in pH optimum, insensitivity to EDTA, inhibition by MgCl$_2$, and moderate ionic strength to the acid RNase (RNase $W_{LA}$) we have observed in SDS gels. The acid RNase activity of Chevrier and Sarhan includes two chromatographically distinct species (RNases I and II) separable on DEAE-Sephadex, whereas
of different species, tissues, and organelles, and to monitoring changes in RNase patterns during development and physiological challenge. Indeed, the technique is applicable to numerous catalytic activities other than RNase (2, 10) and would serve well in studying a variety of plant enzymes.

LITERATURE CITED