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Depurination of plant ribosomes by pokeweed antiviral protein

Blair E. Taylor* and James D. Irvin

Department of Chemistry, Southwest Texas State University, San Marcos, TX 78666, USA

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Mammalian ribosomes have been shown to be enzymatically modified by ribosomal inactivating protein (RIPs) via specific depurination of rRNA. Here we report that ribosomes isolated from wheat germ contain intact and undepurinated rRNA and are depurinated by pokeweed antiviral protein (PAP). Pokeweed ribosomes isolated under the same conditions are depurinated. Total RNA isolated from pokeweed in the presence of strong denaturants was found to pbe partially depurinated. We conclude that wheat germ ribosomes are resistant to the endogenous RIP, tritin, but are sensitive to PAP and that pokeweed ribosomes can be depurinated by the N-glycosidase activity of endogenous PAP during isolation.

Pokeweed antiviral protein; Ribosome inactivating protein; Depurination; Plant ribosome

1. INTRODUCTION

The class of proteins named ribosomal inactivating proteins (RIPs) has been shown to inactivate mammalian ribosomes by a specific depurination of adenine 4324 in the large (28 S) RNA of rat liver ribosomes. Deadenination at position 4324 in the 28 S RNA followed by aniline treatment releases a fragment containing approximately 400 nucleotides from the 3′ end of 28 S RNA [1-3]. There are two types of RIPs; dimeric toxins, such as ricin, which are extremely lethal to mammalian cells [4] and single chain proteins, such as pokeweed antiviral protein (PAP), which are considerably less toxic [5,6]. All of these proteins catalyze the same reaction on mammalian ribosomes but only some of these can inactivate plant and other lower eukaryotic ribosomes. Toxins like ricin do not efficiently inactivate plant or amoebic ribosomes in contrast to RIPs like PAP which readily inactivate ribosomes from these sources [7-9].

Ribosomes isolated from pokeweed have been reported to be resistant to the action of PAP based on in vitro assays with ribosomes possessing very little activity [7]. Ready et al. have proposed that pokeweed ribosomes should be sensitive to PAP to account for its localization outside the cell membrane for possible use in a suicide action if the cell membrane is damaged in some manner by an invading organism [10].

Correspondence address: J. Irvin, Department of Chemistry, Southwest Texas State University, San Marcos, TX 78666, USA

**Present address:* Department of Pharmacology, University of Texas Health Science Center, 7703 Floyd Curl Dr., San Antonio, TX78284, USA

Abbreviations: PAP, pokeweed antiviral protein; RIP, ribosomal inactivating protein

Here we report that plant ribosomes are depurinated by PAP and that the rRNA isolated from pokeweed leaves has been partially deadeninated by endogenous PAP, presumably after rupture of the cell membrane. Though wheat germ ribosomes are readily depurinated by PAP the endogenous RIP, tritin, does not damage these ribosomes.

2. MATERIALS AND METHODS

2.1. *Ribosomes*

Ribosomes were isolated from wheat germ and pokeweed employing the technique described by Walthall et al. [11] with the modification of adding 1 mg/ml of heparin to the extraction buffer. Aseptic techniques were observed at all times to reduce nuclease damage to the rRNA.

2.2. *PAP treatment of ribosomes*

A 22.5 mg sample of wheat germ ribosomes was incubated with 1 nmol of PAP, purified as previously described [12], for 10 min at 25°C. The sample was frozen in an ethanol/dry-ice slurry to terminate the reaction prior to phenol extraction of rRNA.

2.3. *RNA extraction*

Wheat germ and pokeweed rRNA were extracted using the SDS-proteinase K method [13]. Pokeweed RNA was also isolated directly from plant leaves by the guanidine hydrochloride method as outlined by Logemann et al. [14].

2.4. *Aniline modification of RNA*

A modification of the aniline acetate treatment of RNA described by Peattie [15] was used to induce chain scission at the depurination site in 25 S RNA.

2.5. *Electrophoresis of RNA*

Treated and control samples of pokeweed and wheat germ RNAs were analyzed by electrophoresis in composite gels of 2.5% (w/v) acrylamide/0.5% (w/v) agarose in a buffer containing 45 mM Tris, 45 mM boric acid, and 1 mM EDTA, pH 8 [16]. Denatured RNA was analyzed in 2% agarose gels containing 40 mM Hepes, pH 7, 10 mM sodium acetate, 1 mM EDTA, and 2.2 M formaldehyde [17]. Preliminary screening of the gels was performed using ethidium bromide; desirable gels were stained with Stains All and photographed.

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3. RESULTS AND DISCUSSION

Fig. 1 shows the electrophoretic analysis of combinations of PAP treatment of wheat germ ribosomes with aniline treatments of isolated rRNA. The figure shows that wheat germ rRNA is isolated completely intact and is not depurinated. Treatment of wheat germ ribosomes with PAP produces rRNA which releases a small RNA fragment upon aniline treatment indicating that specific depurination has occurred. Based upon the published sequence of rice 25 S RNA [18] we expect that this fragment results from the aniline catalyzed β-elimination at the depurinated site formally occupied by A_{3017}. The predicted recognition sequence for PAP in plant 25 S RNA is identical to that found in rat liver 28 S rRNA. The target is a loop near the 3′ terminus which contains the conserved sequence AGUACGA*GAGGAAC with the central A* removed by PAP.

Analysis of pokeweed rRNA isolated directly from the leaves using the guanidine hydrochloride method produces strong bands of intact 25 S and 18 S RNA with some degradation products (Fig. 2). Treatment of the RNA with aniline results in the release of a small RNA fragment with a mobility identical to the one produced from PAP treated wheat germ ribosomes. Also, as a result of the fragment release, the majority of the 25 S RNA now migrates more rapidly through the gel while a smaller amount is resistant to aniline treatment which indicates that it has not been depurinated.

Electrophoresis of rRNA obtained from isolated pokeweed ribosomes (Fig. 2) shows that it is extensively degraded by nuclease action even though precautions were taken to minimize such activity during the isolation. Wheat germ ribosomes isolated by the same method contain undegraded rRNA (Fig. 2). The wheat germ ribosomes were very active in a cell-free translation system but the pokeweed ribosomes had little if any activity (data not shown). Treatment of the rRNA from the pokeweed ribosomes with aniline produces even more extensive degradation suggesting that the RNA may contain multiple depurination sites. It has been previously shown that ricin in high concentrations, 10 μM and higher, causes depurination at certain GAGA containing loops in both naked *E. coli* and rat liver rRNA [19]. We estimate that extracts of pokeweed leaves contain approximately 10 μM PAP and thus could cause nonspecific depurination. It is also possible that nuclease damage may facilitate nonspecific depurination or that depurination may make the ribosomes more sensitive to nucleases.

Analysis of aniline treated pokeweed and wheat germ rRNA in denaturing formaldehyde agarose gels indicated that the size of the released fragment is approximately 330 bases (data not shown). On the basis of the structure of 25 S RNA from rice [18] the predicted size of the fragment is 365 bases for both wheat and pokeweed which is in reasonable agreement with the

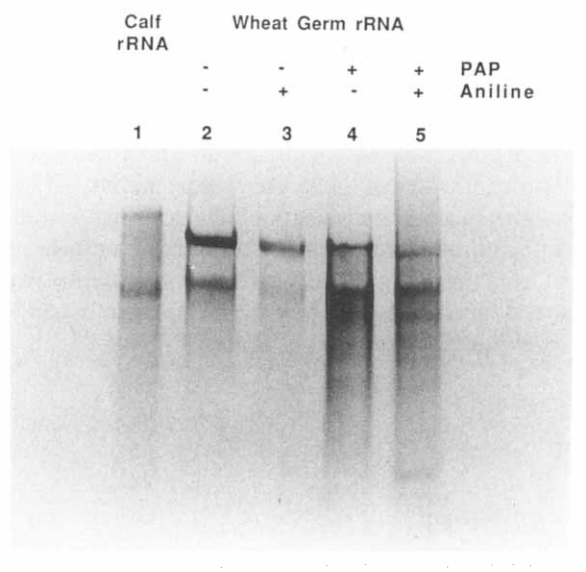

Fig. 1. Electrophoresis of wheat germ rRNA after various treatments. Samples, 6 μg, of rRNA from either control or PAP treated ribosomes were electrophoresed directly or treated with aniline prior to analysis as indicated in the figure and as described in section 2. A 6 μg sample of calf liver rRNA was included for reference.

electrophoretic results. The results indicate that pokeweed ribosomes contain the conserved target sequence found in all 23–28 S rRNAs which is thought to be essential for elongation factor interaction [20].

These results give substantial support to the hypothesis that the function of PAP is to provide a means of cellular suicide upon invasion by viruses. Un-

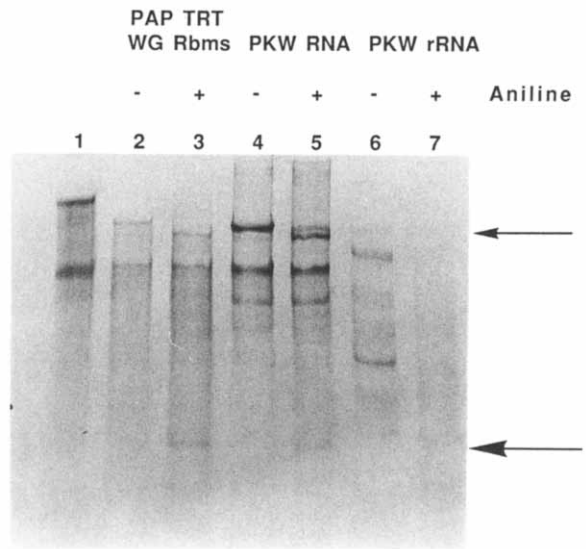

Fig. 2. Electrophoretic analysis of rRNAs from wheat germ and pokeweed. The following samples, 6 μg each, were analyzed by gel electrophoresis as described in section 2. Lane 1, calf liver rRNA; lanes 2 and 3, wheat germ rRNA; lanes 4 and 5, pokeweed RNA; and lanes 6 and 7, rRNA isolated from pokeweed ribosomes, all with the indicated treatments. The arrows indicate the positions of the fragments produced by aniline treatment.

fortunately these results are not readily extrapolated to other RIPs especially in the case of those like tritin [21], the RIP isolated from wheat. Though tritin is present in the extract during ribosome isolation the wheat germ ribosomes are very active and are not depurinated (Fig. 1). Our results demonstrate that the sensitivity of native ribosomes to an endogenous RIP can be tested by isolating undegraded rRNA followed by aniline treatment and electrophoresis rather than attempting to measure the activity of ribosomes which can become extensively damaged during isolation.

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