Depurination of ribosomal RNA and inhibition of viral RNA translation by an antiviral protein of *Celosia cristata*

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An antiviral protein (25 kD) isolated from leaves of *Celosia cristata* (CCP 25) was tested for depurination study on ribosomal RNA from yeast. Ribosomal RNA yielded 360 nucleotide base fragment after treatment with CCP 25 indicating that CCP 25 was a ribosome inactivating protein. CCP 25 also inhibited translation of bromo mosaic virus (BMV) and pokeweed mosaic virus (PMV) RNAs in rabbit reticulocyte translation system. The radioactive assay showed that incorporation of [35S]methionine was less in translation proteins of BMV nucleic acid when CCP 25 was added to translation system. This indicated that antiviral protein from *Celosia cristata* not only depurinated ribosomal RNA but also inhibited translation of viral RNA in vitro.

Aqueous leaf extract as well as purified antiviral proteins from *Celosia cristata* have been shown to inhibit effectively the lesion formation by Tobacco mosaic virus, Sunnhemp rosette virus and Potato virus x in their hypersensitive hosts except in *Chenopodium aamaranticolar*1.2. These proteins have also been shown to completely inhibit Indian citrus ring spot virus in its systemic host, *Phaseolus vulgaris*. Two antiviral proteins have been isolated from the leaves of different growth stages of *Celosia cristata*. Mechanism of action of antiviral proteins from *Celosia cristata* is not clearly understood. Many of the antiviral proteins from plants are ribosome inactivating proteins (RIPs). The ribosome inactivating proteins inactivate ribosomes by depurination of a specific adenine from the highly conserved, surface exposed sarcin/ricin (S/R) loop of large rRNA of eu- karyotic and prokaryotic ribosomes. Based on depurination activity and extracellular location of some RIPs like pokeweed antiviral protein (PAP), a general antiviral mechanism has been suggested according to which PAP and other RIPs might enter the cell along with the virus at the site of infection and inhibit virus multiplication by inactivating host ribosomes. Antiviral activity of these proteins was attributed to ribosome inactivation in heterologous plants. Subsequent studies have shown that ribosome inactivating activity of PAP could be dissociated from its antiviral activity. The present study was aimed at to find out whether the antiviral protein isolated from *Celosia cristata* is a ribosome inactivating protein, and whether it inhibited viral translation in vitro.

**Purification of Antiviral Proteins**—Purification of antiviral proteins from *Celosia cristata* with molecular mass of 25 kD (CCP 25 ) and from pokeweed (*Phytophaca americana*) with molecular mass of 29 kD (PAP) has already been described.8,9 Ammonium sulphate was used to sediment CCP 25 and PAP proteins from homogenized leaves of *Celosia cristata* (harvested at pre-flowering stage) and *Phytophaca americana* (harvested at flowering stage) respectively. The sediment was dialyzed and subjected to DEAE cellulose column chromatography. The active unabsorbed fractions were pooled. The pooled fractions were fractionated by CM-sepharose cation exchange chromatography in case of CCP 25 and Bio- Rad S2, cation exchange column in case of PAP. For CCP25 proteins, fractions exhibiting maximum antiviral activity were pooled and subjected to size exclusion chromatography on Sephadex G-75. This resulted in a single protein peak with antiviral activity and it showed a prominent band of 25 kD on linear gradient (10-20 %) SDS-PAGE. For PAP, fractions were assayed by immunoblot analysis and those containing PAP were pooled and used for study.

**Ribosomal RNA depurination assay**—Total ribosomes (50 µg) isolated from yeast (*Saccharomyces
cells were incubated with 100 ng of CCP 25 or PAP in RIP buffer (167 mM, KCl, 100 mM, Tris HCl, pH 7.2, 100 mM, MgCl₂) at 30°C for 30 min. Following incubation, CCP 25 and PAP were removed from the mixture by phenol and phenol chloroform extraction. The ribosomal RNA (rRNA) was precipitated in ethanol at -80°C. Half of the rRNA was incubated with 1 M aniline/acetate mixture on ice for 30 min and then precipitated with ethanol. Non-aniline and aniline treated rRNAs were run on 7 M urea, 6%, polyacrylamide gel. Depurination of rRNA was confirmed by the presence of a 360-nucleotide fragment visible on the urea-acrylamide gel (Fig. 1).

Inhibition of in vitro viral RNA translation — Brome mosaic viral RNA (BMV RNA) which has been studied as a model system for RNA replication was purchased from Promega. Pokeweed mosaic viral RNA (PMV RNA) was isolated from intact virus. Both these RNAs were used for studying the effect of CCP 25 on in vitro translation studies of plant viral RNAs. PAP was used as positive control. CCP 25 and PAP (80 ng each) were added directly to rabbit lysate (25 μl) in vitro translation system (Promega) with 500 ng of BMV or PMV RNA. Following incubation at 30°C for 1.5 hr, 3 μl of translation reaction mixture was separated by 12% SDS-PAGE, washed in Entensify autoradiography solutions (New England Nuclear) and exposed to X-ray film. The level of translation was also measured by [³⁵S]-incorporation after precipitating the total protein products. Aniline treatment of rRNA is an established method of detecting the depurination of large rRNA by ribosome inactivating proteins. Ribosome inactivating proteins inactivate ribosomes with their RNA N-glycosidase activity cleaving a specific N-glycosylic bond of an adenosine residue in the highly conserved sarcin/ricin loop of large rRNA of eukaryotic and prokaryotic ribosomes. Depurination results in increased susceptibility of the sugar phosphate backbone to hydrolysis at depurination sites in the presence of aniline under acid conditions. When the depurinated rRNA was treated with aniline, a small fragment is released. This fragment was about 360 nucleotides in length in yeast 25S and rat 28S rRNA. Figure 1 shows that CCP 25 was able to remove 360-nucleotide base fragment from depurination site in the same way as PAP did after aniline treatment. Thus, it was clear that the antiviral protein from Celosia cristata was also able to depurinate ribosomal RNA and could be grouped under broad category of ribosome inactivating proteins.

CCP 25 also inhibited viral RNA translation in vitro. BMV proteins were not translated from BMV RNA in presence of CCP 25 as well as PAP (Fig. 2). PAP was used as positive control. The amount of translation product as measured by per cent incorpora-

![Fig. 1 — Electrophoretic analysis of rRNAs from yeast ribosomes incubated with PAP (100 ng) and CCP 25 (100 ng). Following incubation, rRNAs were precipitated and divided in half. Half was treated with aniline (+), and the other half was without aniline (-). Arrow indicates small fragments (360 nucleotides) released from the ribosomes by the treatment(s).]

![Fig. 2 — Autoradiogram of translation proteins of BMV RNA when PAP or CCP 25 was directly added to rabbit reticulocyte translation system. [RIP- ribosome inactivating protein; PAP- Pokeweed antiviral protein; CCP- Celosia antiviral protein]
Fig. 3—Per cent incorporation of $^{35}$S-methionine in vitro translation product of BMV RNA in presence and absence of RIPS

Further studies are still required to understand that how these antiviral ribosome inactivating proteins including the one from *Celosia cristata* function in inhibition of plant viruses in plant system. Nevertheless, these ribosome inactivating proteins do possess potential for their utilization in development of resistance against virus infection particularly because they offer broad spectrum resistance in plants.

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References