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Short Communication

Polygalacturonase mRNA of Tomato: Size and Content in Ripe Fruits

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In ripe tomato fruits, polygalacturonase (PG) mRNA comprised about 1% of the translatable RNAs in the poly(A)(+)RNA fraction. Sucrose density gradient centrifugation showed that this PG mRNA is similar in size to 18*S* rRNA, which suggests the presence of a non-coding region.

Key words: *Lycopersicon esculentum* — Polyadenylated RNA — Polygalacturonase (mRNA) — Tomato (fruit.)

PG (EC 3.2.1.15) is one of the enzymes formed in tomato fruits during ripening (Hobson 1965, Grierson et al. 1981, Brady et al. 1982, Yoshida et al. 1984), and it is assumed to have an important function in the softening of tomato fruits. Two forms of PG, PG-1 (100 kDa) and PG-2 (44 kDa), appear after the onset of ripening, but only PG-2 increases dramatically (Tucker et al. 1980, Grierson et al. 1981, Yoshida et al. 1984). The increase in PG activity is due to the de novo synthesis of PG molecules (Tucker and Grierson 1982, Brady et al. 1982), and mRNA-specifying PG is thought to accumulate in ripe tomato fruits.

We previously reported that the poly(A)(+)RNA from ripe tomato fruits produces the 54 kDa precursor polypeptide of PG in the wheat germ translation system (Sato et al. 1984). In the experiments reported here, we measured the content of PG mRNA in the poly(A)(+)RNA fraction from ripe tomato pericarp tissue and analyzed its size on a sucrose density gradient.

Mature green tomato (*Lycopersicon esculentum* Mill, cv. Omiya) fruits were ripened for 20 days at 22°C as described previously (Yoshida et al. 1984). Nucleic acids were isolated from the ripe tomato pericarp tissue (100 g) by the SDS-phenol method described elsewhere (Sato et al. 1984). Nucleic acids were dissolved in distilled water then precipitated with 3 M LiCl at 0°C for at least 2 h. The precipitate collected by centrifugation at 30,000 × *g* for 30 min at 0°C was dissolved in distilled water then precipitated with ethanol. Poly(A)(+)RNA was separated by column chromatography using poly(U)-Sepharose (Pharmacia Fine Chemical) (Sato et al. 1978). The concentration of the poly(A)(+)RNA was estimated by measuring the abosrbance at 260 nm.

In vitro translation of tomato poly(A)(+)RNAs was done at 30°C for 60 min in a wheat germ translation system (25 μl) in the presence of ^{3}H-leucine (5 μCi, 146 Ci/mmol, New England Nuclear) as described elsewhere (Sato et al. 1980). The PG polypeptide produced in vitro was

Abbreviations: DMSO, dimethyl sulfoxide; PAGE, polyacrylamide gel electrophoresis; PG, polygalacturonase; poly(A)(+)RNA, polyadenylated RNA; poly(U), polyuridylic acid; prePG, precursor peptide of PG.

Table 1 Contents of PG mRNA in the poly(A)(+)RNA isolated from ripe tomato pericarp tissue

Poly(A)(+)RNA (μg/ml)	Total incorporation (cpm $\times 10^{-3}$)	Incorporation into immunoprecipitates (cpm)		
		Non-immunized serum (A)	Anti-PG-2 antiserum (B)	B−A
0	19.6	158	186	28
5	372.0	1,232	5,124	3,892
10	545.4	1,935	9,326	7,391
15	859.3	2,554	14,129	11,575
20	1,054.7	2,257	12,736	10,479

Poly(A)(+)RNA was translated in a wheat germ system. The polypeptides produced in vitro were treated with anti-PG-2 antiserum or non-immunized serum.

immunoprecipitated with anti-PG-2 antiserum (5 μl) then analyzed by SDS-PAGE followed by fluorography (Sato et al. 1984).

The radioactivity incorporated into the immunoprecipitable polypeptide increased with the amount of poly(A)(+)RNA used (Table 1). A previous study showed that this polypeptide represents a precursor molecule of PG-2 (Sato et al. 1984). The radioactivity incorporated into this polypeptide accounted for about 1% of the total radioactivity in the translation products (Table 1). Assuming a comparable efficiency of translation for different mRNAs, we estimated that the PG mRNA in the ripe pericarp tissue of tomato is about 1% of the translatable RNAs in the poly(A)(+)RNA fraction.

To determine its approximate size of the PG mRNA, we fractionated poly(A)(+)RNA under fully denaturing conditions. A linear gradient of sucrose (5 to 20% (w/v)) in 95% (v/v) DMSO and 4% (v/v) formamide containing 1 mM EDTA, 10 mM LiCl and 10 mM Tris-HCl buffer (pH 7.5) was prepared in a polyallomer tube (4.8 ml, Hitachi Co Ltd.) (Forde and Miflin 1983). Poly(A)(+)RNA (70 μg) was taken up in 100 μl of 10 mM Tris-HCl buffer (pH 7.5) containing 10 mM LiCl, 1 mM EDTA and 10% DMSO then heated at 60°C for 5 min, after which it was chilled on ice then loaded on the gradient. The gradient was centrifuged at 20°C and 190,400 $\times g$ for 26 h with a Hitachi RPS 50-2 rotor. Fractions of 0.25 ml were collected, and the amount of

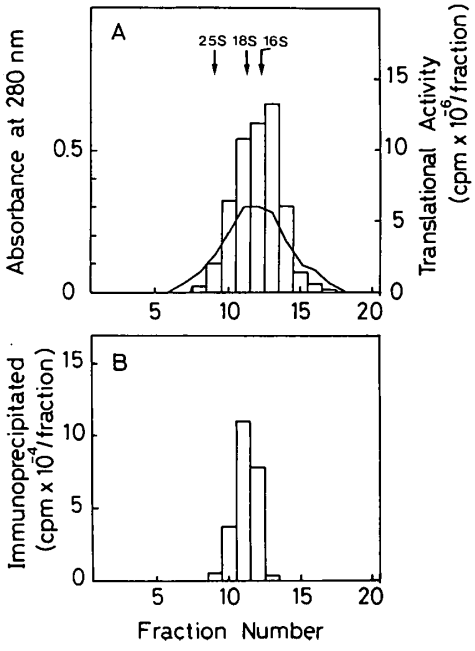

Fig. 1 Fractionation of tomato poly(A)(+)RNA by sucrose density gradient centrifugation. Poly(A)(+)RNA was fractionated on a 5 to 20% sucrose gradient as described in the text. RNA (10 μg/ml) from each fraction was translated in the wheat germ system. The polypeptides produced were treated with anti-PG-2 antiserum. A: Absorbance at 280 nm (——), and translational activity (bar graph). B: Radioactivity in the translation product immunoprecipitated by anti-PG-2 antiserum. Positions of size markers (spinach leaf rRNAs) are indicated by arrows.

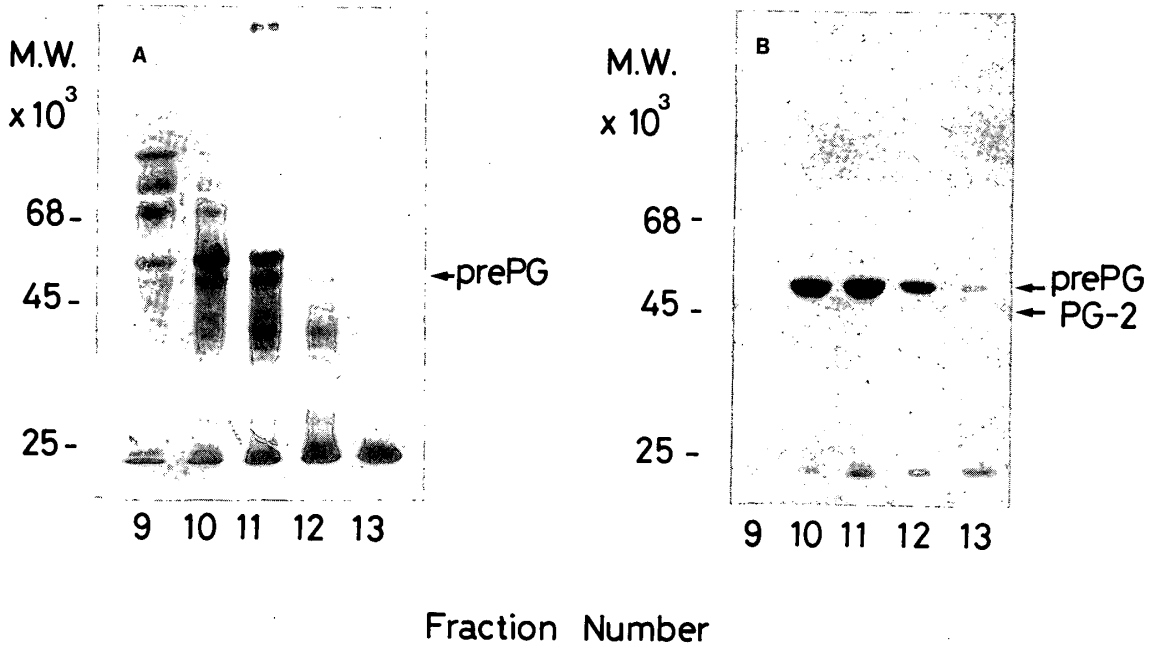

Fig. 2 Gel electrophoresis of in vitro translation products directed by poly(A)(+)RNA fractions from the sucrose density gradient. Translation products produced in vitro were analyzed by 10% SDS-PAGE, followed by fluorography. The mobilities of the prePG and PG-2 polypeptides are indicated by arrows. A: Total translation products (about 2×10^5 cpm). B: Immunoprecipitated translation products. Translation products (about 1×10^6 cpm) were treated with anti-PG-2 antiserum.

RNA was estimated from the absorbance at 280 nm (strong absorbance by formamide interfered with the measurement at 260 nm). Carrier tRNA (10 μg wheat germ tRNA, Sigma) was added to each fraction, after which RNA was precipitated with 2 volumes of ethanol. The precipitated RNA was washed with 70% ethanol then taken up in sterile distilled water. The poly(A)(+)-RNA measured from the absorbance at 280 nm ranged in size from about 7*S* to more than 28*S* with a peak at 16–18*S* (Fig. 1). Translational activity also was distributed in RNAs of 7*S* to more than 28*S* with a peak at 15*S* (Fig. 1-A). The activity for the direction of the synthesis of PG in the fractionated RNA peaked in fraction 11 which corresponded to 18*S* rRNA (Fig. 1-B).

The total polypeptides and immunoprecipitated polypeptides produced in vitro by the fractionated RNAs also were analyzed by SDS-PAGE, followed by fluorography (Fig. 2). The RNAs in fractions 10–12 from the sucrose density gradient centrifugation contained mRNA for the prePG polypeptide (Fig. 2-B). The fluorograph of the total polypeptides (Fig. 2-A) showed that prePG polypeptide was one of the major polypeptides produced in vitro from the RNAs in fractions 10–12.

Our results indicate that the size of the PG mRNA is similar to that of 18*S* rRNA (6.7×10^5 Da) which has about 1,950 nucleotides (Tumer et al. 1981) (Fig. 2). This size is large enough to contain the structural information for 54 kDa prePG, the minimum size required for an mRNA to encode a 54 kDa polypeptide being about 1,470 nucleotides. If our assumption is valid, PG mRNA would contain an additional noncoding segment of about 480 nucleotides.

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References

Brady, C. J., G. MacAlpine, W. B. McGlasson and Y. Ueda (1982) Polygalacturonase in tomato fruits and the induction of ripening. *Aust. J. Plant Physiol.* 9: 171–178.

Forde, J. and B. J. Miflin (1983) Isolation and identification of mRNA for the high-molecular-weight storage proteins of wheat endosperm. *Planta* 157: 567–576.

Grierson, D., G. A. Tucker and N. G. Robertson (1981) The molecular biology of ripening. *In* Recent Advances in Biochemistry of Fruit and Vegetables. Edited by J. Friend and M. J. C. Rhodes. pp. 147–158. Academic Press, London.

Hobson, G. E. (1965) The firmness of tomato fruit in relation to polygalacturonase activity. *J. Hortic. Sci.* 40: 66–72.

Sato, T., A. Watanabe and H. Imaseki (1978) RNA synthesis in the early stage of aerobic incubation of potato tuber discs. *Plant Cell Physiol.* 19: 609–616.

Sato, T., M. Ishizuka, A. Watanabe and H. Imaseki (1980) The synthesis and properties of polysomal RNA in potato tuber slices during the early stage of aging. *Plant Cell Physiol.* 21: 137–147.

Sato, T., S. Kusaba, H. Nakagawa and N. Ogura (1984) Cell-free synthesis of a putative precursor of polygalacturonase in tomato fruits. *Plant Cell Physiol.* 25: 1069–1071.

Tucker, G. A. and D. Grierson (1982) Synthesis of polygalacturonase during tomato ripening. *Planta* 155: 64–67.

Tucker, G. A., N. G. Robertson and D. Grierson (1980) Change in polygalacturonase isoenzymes during the 'ripening' of normal and mutant tomato fruit. *Eur. J. Biochem.* 112: 119–124.

Tumor, N. E., V. H. Thanh and N. C. Nielsen (1981) Purification and characterization of mRNA from soybean seeds. *J. Biol. Chem.* 256: 8756–8760.

Yoshida, O., H. Nakagawa, N. Ogura and T. Sato (1984) Effect of heat treatment on the development of polygalacturonase activity in tomato fruit during ripening. *Plant Cell Physiol.* 25: 505–509.

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