Plant Cell Physiol. 27(7): 1419–1422 (1986)

Short Communication

Cell-Free Synthesis of Rice Prolamin

Hiroshi Yamagata[1], Koichi Tamura[2], Kunisuke Tanaka[1,4] and Zenzaburo Kasai[3]

[1] *Department of Biochemistry, College of Agricultural Chemistry, Kyoto Prefectural University, Shimogamo, Kyoto 606, Japan*
[2] *Central Research Division, Fujisawa Pharmaceutical Co. Ltd., Osaka 532, Japan*
[3] *Department of Agricultural Chemistry, College of Agriculture, Kinki University, Higashi Osaka, Osaka 577, Japan*

Polyadenylated RNA was isolated from the protein-body rich fraction of developing rice (*Oryza sativa*. L) seeds. In a wheat germ cell-free system, the isolated polyadenylated RNA produced polypeptides with the same solubility as prolamin subunits. The electrophoretic mobility of the polypeptides suggested the presence of a signal peptide.

Key words: mRNA — *Oryza sativa* — Prolamin — Rice (seed) — Signal peptide — Storage protein.

Prolamin, an alcohol soluble protein, is one of the principal proteins of PB in the endosperm of cereals (Miflin and Shewry 1979, Larkins 1981). However, rice prolamin comprises only ca 3% of milled rice protein and has been shown to have one major subunit (Juliano and Boulter 1976, Mandac and Juliano 1978). Rice prolamin is poor in lysine, histidine, cystine and methionine but rich in glutamic acid, tyrosine and proline (Mandac and Juliano 1978, Padhye and Salunkhe 1979).

Previously, we reported that rice prolamin accumulated in the type I protein body (PB-I) in the starchy endosperm and that its major component was a 13 kDa polypeptide (Tanaka et al. 1980). The prolamin appeared to be synthesized as a precursor molecule by membrane-bound polysomes attached to PB-I and RER and to pass through the membrane into the lumen where they were deposited as aggregates (Yamagata and Tanaka 1986).

Several prolamin polypeptides of cereals including maize, wheat and barley undergo co-translational modification, such as removal of the NH_2-terminal signal sequence (Kreis et al. 1985, Larkins 1981). Rice glutelin is also synthesized as a precursor which appears to have a signal sequence (Furuta et al. 1986). However, it has not been established whether rice prolamin is synthesized as a precursor with a signal sequence. Here we describe the isolation of poly(A)$^+$RNA that is enriched with mRNA of prolamin and the synthesis in a cell-free translation system of a prolamin precursor with an extra peptide.

Rice (*Oryza sativa* L. Japonica cv Koshihikari) was field-grown and developing seeds were harvested about 2 weeks after flowering, as described previously (Yamagata and Tanaka 1986). The frozen immature grains (600 g) were pulverized as described previously (Furuta et al. 1986) and suspended in 150 ml of a homogenizing buffer (Yamagata et al. 1982) containing 30% (w/v)

Abbreviations: DAF, days after flowering; β-ME, β-mercaptoethanol; m^{7}G^5'p, 7-methylguanosine 5'-phosphate; PAGE, polyacrylamide gel electrophoresis; PB, protein body(s); poly(A)$^+$RNA, polyadenylated RNA; RER, rough endoplasmic reticulum.

[4] To whom correspondences should be addressed

sucrose. The homogenate was then filtered through four layers of gauze, and centrifuged at 1,900×*g* for 20 min. The resulting pellet containing mainly starch granules and PB (PB-rich fraction) was washed with the homogenizing buffer containing 30% (w/v) sucrose and suspended in water. Total RNA was extracted from this pellet with SDS and phenol at a neutral pH as described by Brawerman (1974), precipitated by adding 2 volumes of cold ethanol and 0.25 volume of 1 M K-acetate (pH 5.0), and then chromatographed on oligo(dT)-cellulose (type II, Collaborative Research Inc. Waltham, MA.) as described by Wienand and Feix (1978). The poly(A)$^+$RNA thus obtained, which we will refer to as PB-mRNA, was precipitated with ethanol and K-acetate, washed with absolute ethanol, dried, dissolved in a small volume of water and stored at −80°C. About 31.2 mg (624 A_{260} units) of total RNA was obtained from 600 g of rice grains and poly(A)$^+$RNA accounted for about 0.42% of the total RNA, as estimated from the UV absorption spectra using $\varepsilon_{260}^{1\%}=20$ (Rhoads et al. 1973).

In vitro protein synthesis using the wheat germ cell-free system was carried out as described previously (Yamagata et al. 1982, Yamagata and Tanaka 1986). The maximal incorporation of labeled amino acid into protein was obtained with a reaction mixture containing 4 mM Mg^{2+}, 100 mM K^+ and 18 μg poly(A)$^+$RNA/100 μl. Translation of the PB-mRNA was inhibited by 74% by 1 mM $m^7G^5'p$ (P-L Biochemicals Inc., Milwaukee, Wis.) at 82 mM K^+, suggesting that most of the poly(A)$^+$RNAs are capped (Hickey et al. 1976). Poly(A)$^-$RNA showed a much lower level of template activity than poly(A)$^+$RNA.

The polypeptides synthesized in vitro were processed for SDS-PAGE as described previously (Yamagata and Tanaka 1986), and electrophoresis was carried out according to Yamagata et al. (1982). Radioactive proteins were located by fluorography (Yamagata and Tanaka 1986). Immunoprecipitate of cell-free translation products were prepared using antibodies against a crude mixture of the subunits of glutelin-prolamin as described previously (Yamagata and Tanaka 1986). When the cell-free translation system was directed by PB-rich fraction, the immunoprecipitate contained three PB-I polypeptides of 10, 13 and 16-kDa (data not shown).

Electrophoretic mobilities of the [^{14}C]leucine-labelled translation products of PB-mRNA did not coindice with those of the in vivo [^{14}C]-labeled prolamin (Fig. 1). The mol wt of two major polypeptides synthesized in vitro by PB-mRNA was calculated to be 11,600 and 14,900.

When [^{35}S]methionine was used for both in vivo and in vitro labeling, the products showed a different pattern (Fig. 2). The 13-kDa polypeptide, a major prolamin component, was not labeled in vivo with [^{35}S]methionine (Fig. 2, lane 5), indicating that it contains little, if any, methionine. The possible relationship between the [^{35}S]methionine-labeled polypeptides synthesized in vivo and in vitro were examined by comparing their *n*-propanol solubility (Fig. 2). For extraction of prolamin after in vitro translation, 1.6 volumes of absolute *n*-propanol containing 1.7% (w/v) β-ME were added to the reaction mixture with stirring. An equal volume of purified prolamin solution (4 g/ml) in 60% (v/v) *n*-propanol and 1% (w/v) β-ME was then added as a carrier. Following incubation at 60°C for 1 h and centrifugation at 10,000×*g*, the protein in the supernatant was precipitated by adding 3 volumes of acetone at −20°C overnight, washed with acetone and water, and dissolved in the sample buffer for SDS-PAGE. Of the total [^{35}S]methionine-labeled translation products directed by PB-mRNA, about 55% were soluble in 60% (v/v) *n*-propanol but insoluble in water. The [^{35}S]-labeled total in vitro products contained five smaller polypeptides ranging between 10 and 20 kDa in size and a 29-kDa polypeptide (Fig. 2, lane 3). Judging from their propanol solubility, all of these polypeptides, with the exception of the 29-kDa polypeptide, appear to be prolamins; prolamin thus seems to be a complex of several polypeptides that are between 10 and 20 kDa in size (Fig. 2, lane 4).

The results presented here indicate that PB-mRNA, the poly(A)$^+$RNA isolated from the PB-rich fraction, is rich in mRNAs for prolamin. The differences in the mobilities between

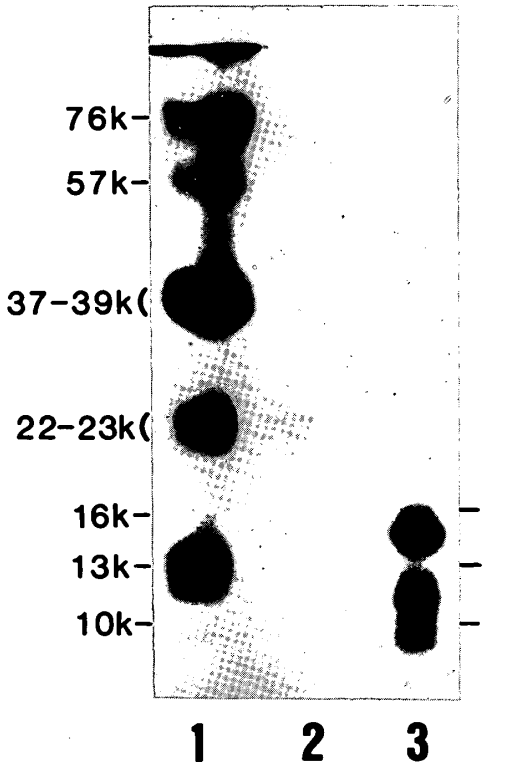

Fig. 1

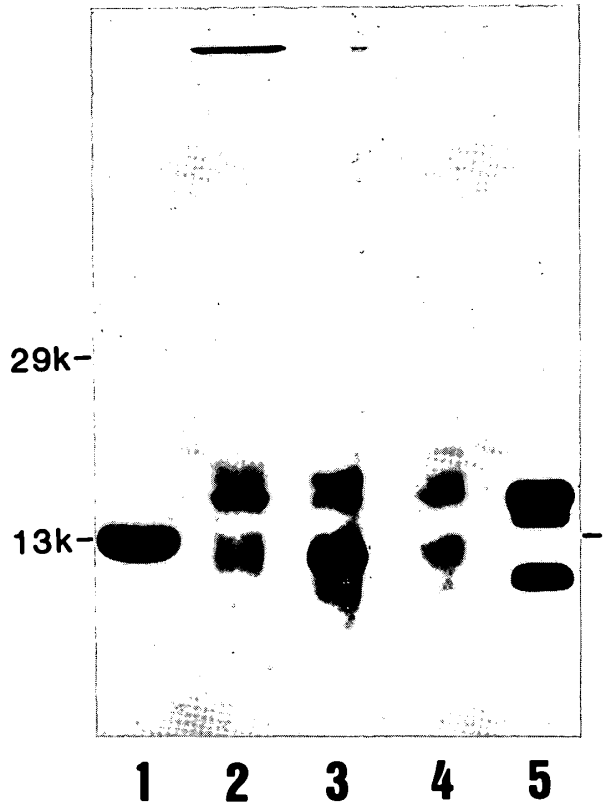

Fig. 2

Fig. 1 SDS-PAGE analysis of [^{14}C]leucine labeled translation products by PB-mRNA. Lane 1, in vivo labeled protein extracted from the endosperms of 10 DAF that had been administered the [^{14}C]leucine for 1 day; lane 2, polypeptides in the PB isolated from endosperms of 10 DAF labeled with [^{14}C]leucine for 1-day; lane 3, products synthesized in vitro with PB-mRNA isolated from PB-rich fraction of developing rice endosperm.

Fig. 2 SDS-PAGE analysis of [^{14}C]leucine- and [^{35}S]methionine-labeled translation products of PB-mRNA. Lanes 1 and 5, in vivo labeled prolamin extracted from the starchy endosperm about 10 DAF that had been administered [^{14}C]leucine (lane 1) or [^{35}S]methionine (lane 5) for 1 day; lanes 2 and 3, total products synthesized in vitro by PB-mRNA, labeled with [^{14}C]leucine (lane 2) and [^{35}S]methionine (lane 3); lane 4, *n*-propanol soluble and water insoluble [^{35}S]methionine labeled products directed by PB-mRNA.

the polypeptides synthesized in vitro by the isolated PB-mRNA (11.6 and 14.9 kDa) and the PB-I polypeptides synthesized in vivo (10 and 13 kDa) are probably due to cotranslational modification in vivo to produce the final products. These findings support the idea that the newly synthesized prolamin passes through the membrane of PB, as proposed from ultrastructural observations (Yamagata and Tanaka 1986). The storage protein of maize, zein, is also synthesized on the polysomes attached to the outer surface of PB, and deposited within the PB. mRNAs derived from PB are capable of directing the in vitro synthesis of zein (Burr and Burr 1976, Burr et al. 1978). The amino acid sequences of the signal peptides of several cereal prolamins including maize zein, barley hordein and wheat gliadin have been reported (Kreis et al. 1985).

References

Brawerman, G. (1974) The isolation of messenger RNA from mammalian cells. *Methods Enzymol.* 30: 605–612.

Burr, B. and F. A. Burr (1976) Zein synthesis in maize endosperm by polyribosomes attached to protein bodies. *Proc. Natl. Acad. Sci. USA* 73: 515–519.

Burr, B., F. A. Burr, I. Rubenstein and M. N. Simon (1978) Purification and translation of zein messenger RNA from maize endosperm protein bodies. *Proc. Natl. Acad. Sci. USA* 75: 696–700.

Furuta, M., H. Yamagata, K. Tanaka, Z. Kasai and S. Fujii (1986) Cell-free synthesis of the rice glutelin precursor. *Plant Cell Physiol.* 27: 1201–1204.

Hickey, E. D., L. A. Weber and C. Baglioni (1976) Inhibition of initiation of protein synthesis by methylguanosine-5′-monophosphate. *Proc. Natl. Acad. Sci. USA* 73: 19–23.

Juliano, B. O. and D. Boulter (1976) Extraction and composition of rice endosperm glutelin. *Phytochemistry* 15: 1601–1606.

Kreis, M., R. R. Shewry, B. G. Forde, J. Forde and B. J. Miflin (1985) Structure and evolution of seed proteins and their genes with particular reference to those of wheat, barley and rye. *In* Oxford Surveys of Plant Molecular & Cell Biology. Edited by B. J. Miflin. Vol. 2. pp. 253–317.

Larkins, B. A. (1981) Seed storage proteins: characterization and biosynthesis. *In* The Biochemistry of Plants. Edited by A. Marcus. Vol. 6. Chap. 11. pp. 449–489. Academic Press, New York.

Mandac, B. E. and B. O. Juliano (1978) Properties of prolamin in mature and developing rice grain. *Phytochemistry* 17: 611–614.

Miflin, B. J. and P. R. Shewry (1979) The biology and biochemistry of cereal seed prolamins. *In* Seed Protein Improvement in Cereals and Grain Legumes. Vol. 1. IAEA, Vienna, pp. 137–158.

Padhye, V. W. and D. K. Salunkhe (1979) Extraction and characterization of rice proteins. *Cereal Chem.* 56: 389–393.

Rhoads, R. E., G. S. McKnight and R. T. Schimke (1973) Quantitative measurement of ovalbumin messenger ribonucleic acid activity. *J. Biol. Chem.* 248: 2031–2039.

Tanaka, K., T. Sugimoto, M. Ogawa and Z. Kasai (1980) Isolation and characterization of two types of protein bodies in the rice endosperm. *Agric. Biol. Chem.* 44: 1633–1639.

Wienand, U. and G. Feix (1978) Electrophoretic fractionation and translation in vitro of poly (rA)-containing RNA from maize endosperm. *Eur. J. Biochem.* 92: 605–611.

Yamagata, H., T. Sugimoto, K. Tanaka and Z. Kasai (1982) Biosynthesis of storage proteins in developing rice seeds. *Plant Physiol.* 70: 1094–1100.

Yamagata, H. and K. Tanaka (1986) The site of synthesis and accumulation of rice storage proteins. *Plant Cell Physiol.* 27: 135–145.

(Received May 13, 1986; Accepted July 25, 1986)