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Short Communication

Pyruvate Orthophosphate Dikinase mRNA Organ Specificity in Wheat and Maize[1]

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ABSTRACT

Polyadenylated RNA was isolated from leaves and seeds of a C_3 plant (*Triticum aestivum* L. cv Cheyenne, CI 8885) and from a C_4 plant (*Zea mays* L. cv Golden bantam). Each polyadenylated RNA preparation was translated *in vitro* with micrococcal nuclease-treated reticulocyte lysate. When the *in vitro* translation products were probed with antibodies to pyruvate orthophosphate dikinase (PPDK) (EC 2.7.9.1), two sizes of polypeptide were identified. A 110 kilodalton polypeptide was found in the *in vitro* translation products of mRNA isolated exclusively from leaves of both wheat and maize. A 94 kilodalton polypeptide, similar to the PPDK polypeptide which can be extracted after *in vivo* synthesis in maize and wheat leaves and seeds, was found in the *in vitro* translation products obtained from wheat seeds and maize kernels.

These results indicate that the mRNAs for PPDK polypeptides are organ-specific in both a C_4 and a C_3 plant. Hague *et al.* (1983 Nucleic Acids Res 11: 4853–4865) proposed that the larger size polypeptide of the *in vitro* translation polypeptide from maize leaf RNA contains a 'transit sequence' which permits entry into the chloroplasts of a polypeptide synthesized *in vivo* in maize leaf cell cytoplasm. It appears that in wheat leaves also the transit of synthesized PPDK polypeptide through an intracellular membrane may be required, while such a transit sequence seems not to be required within cells of wheat and maize seeds.

Pyruvate orthophosphate dikinase (EC 2.7.9.1) is an essential enzyme for photosynthetic carbon dioxide fixation in C_4 (8) and in some CAM (11) plants, where it catalyzes formation of phosphoenolpyruvate, the substrate for the initial carboxylation reaction. In C_4 leaves, PPDK[2] is found in the chloroplasts of the mesophyll cells (8). PPDK is found also in seeds (1, 12) and leaves (1) of wheat and in some other C_3 plant tissues (for references, see 2). The role of PPDK in C_3 leaf tissues is not clearly known, although it has been suggested that it might be involved in the control of stomatal opening (16). The occurrence of PPDK in developing wheat seeds does not correlate quantitatively with photosynthetic enzymes (2), and it was suggested that one role of the enzyme might be in the conversion of C_3 amino acids, especially alanine, to C_4 and C_5 amino acids.

Hague *et al.* (9) employed immunoprecipitation by PPDK antibody to identify a 110 kD polypeptide among the *in vitro* translation products of RNA extracted from maize leaf. Since the *in vivo* PPDK polypeptide from maize has a mol wt of about 94 to 97 kD, it was proposed that the polypeptide is nuclear encoded and is synthesized on cytoplasmic ribosomes as a 110 kD polypeptide with the extra 16 kD section serving as a 'transit' sequence to facilitate entry into the chloroplasts. Evidence that PPDK polypeptide is synthesized on cytoplasmic ribosomes in maize was found when PPDK was detected by an immunoprecipitation probe in the maize mutant iojap which is devoid of chloroplast ribosomes (6).

The sizes of PPDK polypeptide(s) formed by *in vitro* translation of polyadenylated RNA from C_3 leaf cells and from seed cells of C_4 and C_3 plants were not previously reported and are the subject of this study.

MATERIALS AND METHODS

Plants. Wheat (*Triticum aestivum* L. cv Cheyenne CI 8885) and maize (*Zea mays* L. cv Golden bantam) were grown in a growth chamber with a radiant flux density of 250 $\mu E\ m^{-2}\ s^{-1}$ with a photoperiod of 8 h and at 15°C. The plants were watered every other day with modified half-strength Hoagland solution. The leaves were harvested for RNA extraction when the plants were 45 d old. Maize kernels were harvested from a mature ear of corn. Wheat seeds were harvested from plants about 25 d after anthesis. Corn and wheat seeds tissues were frozen in liquid N_2 and stored at −80°C until used.

Preparation of mRNA. Total RNA was extracted according to the method of Nelson *et al.* (13). In a coffee grinder, 8 g of frozen tissues was ground into powder in dry ice and then thawed to room temperature in 4 mM guanidinium thiocyanate (1 ml/g tissue) (5). Polyadenylated RNA was prepared by fractionation of total RNA on a column of oligo-dT cellulose (Collaborative Research) (3), followed by ethanol precipitation.

***In Vitro* Protein Synthesis.** From 0.2 to 0.5 μg of polyadenylated RNA was translated in micrococcal nuclease-treated reticulocyte lysate (Amersham) in the presence of [^{35}S]methionine (New England Nuclear) at 30°C for 70 min (15).

Immunoprecipitation of *In Vitro* Translation Products. Antibody to PPDK (1) was added to the *in vitro* translation products and the mixture was incubated over night at 4°C. Prewashed Protein A (Bethesda Research Laboratories) was added to the immunoprecipitated protein according to the method of Kessler (10). After the precipitate was washed, PPDK was released from the mixture by heating at 80°C for 3 min. Following SDS-PAGE of a sample of the supernatant solution, the gel was fixed in protein fixation solution containing 10% TCA (w/v), 10% glacial acetic acid (v/v), and 30% methanol (v/v) for 1 h. The gel was then immersed in En^{3}hance (New England Nuclear) for 1 h and then in H_2O for 45 min. After the gel was vacuum-dried, it was

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[2] Abbreviation: PPDK, pyruvate orthophosphate dikinase.

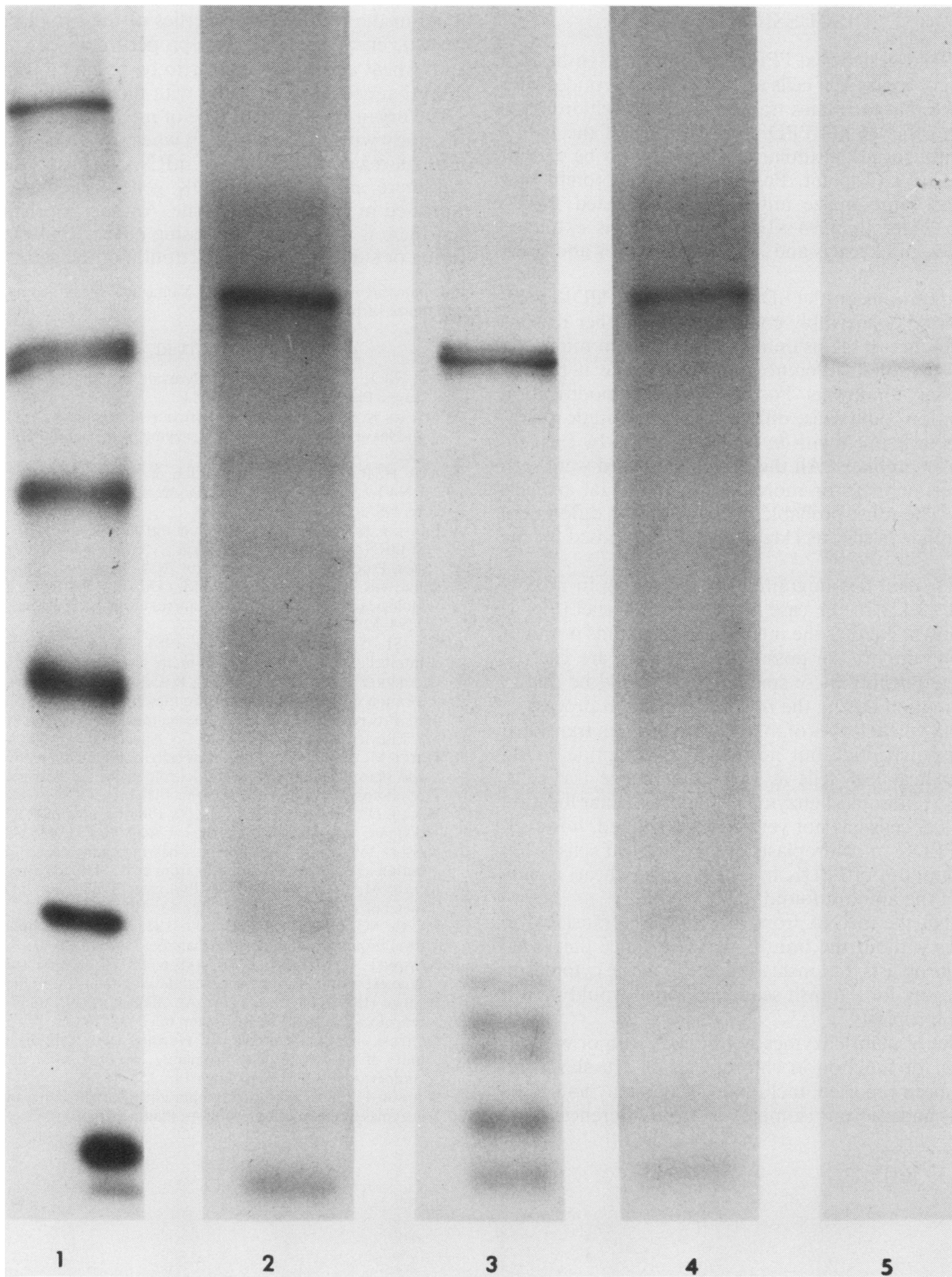

FIG. 1. *In vitro* translation of PPDK mRNA from leaves and seeds from maize and wheat. Analysis was by SDS-PAGE followed by fluorography. Lane 1, ^{14}C-labeled mol wt standards. Lanes 2–5, immunoprecipitated polypeptides synthesized in rabbit reticulocyte lysate system in the presence of poly(A)RNA from: maize leaf (lane 2), maize kernel (lane 3), wheat leaf (lane 4), and wheat seed (lane 5).

placed in contact with Kodak X-AR film with an intensifying screen at −80°C for autoradiography. For estimating the size of the polypeptides, ^{14}C mol wt standards (Bethesda Research Laboratories) were run on the same gel adjacent to the samples.

RESULTS

With antibody to maize leaf PPDK as a probe against the polypeptides in the *in vitro* translation products of the poly(A)RNA extracted from the several sources (Fig. 1) only approximately 110 kD polypeptides were detected when the poly(A)RNA was obtained from either maize leaves (lane 2) or wheat leaves (lane 4). In contrast, only approximately 94 kD polypeptides were immunoprecipitated among the translation products of mRNA from either maize kernels (lane 3) or wheat seed (lane 5). In the case of maize kernel translation products, there was an indication of a doublet of polypeptides differing from each other only very slightly in mol wt (lane 3), and several smaller mol wt bands. Each result reported here was reproducible from at least six experiments.

DISCUSSION

Hague *et al.* (9) suggested that PPDK subunits are synthesized in the cytoplasm of maize leaf cells as 110 kD polypeptides with a transit sequence that facilitates transport into the chloroplasts and processing to the 94 kD PPDK polypeptide of the native protein. Our results suggest a similar mechanism may be present in leaves of wheat, a C_3 plant. Previously, using protein blot analysis with the same maize antibody, we detected PPDK polypeptides no larger than 94 kD among proteins extracted from maize leaves and kernels and from wheat leaves and seeds (2).

The different positions on the SDS-PAGE gel of PPDK polypeptides synthesized conceivably could be due to other reasons than mol wt difference. It seems unlikely that different migration is due to conformational difference, however, since it persists through denaturing conditions. Post-translational modification such as glycosylation could cause different electrophoretic migration, but for this to happen with *in vitro* translation by the cell-free system appears unlikely. All the results presented were seen in repeated experiments (6 or more), so experimental artifacts are improbable. The most probable explanation for differential mobility is difference in size, as Hague *et al.* (9) proposed for the maize leaf case.

The origin of several fast-migrating bands ranging in mol wt between 14 and 23 kD in the case of the maize kernel (Fig. 1, lane 3) is not known. Neither the intensity of the bands nor their apparent mol wt support the possibility that they are cleaved transit sequences. Possibly these smaller bands could be due to premature termination during the *in vitro* translation process.

The presence in wheat leaves of mRNA which when translated gives a 110 kD polypeptide but no 94 kD polypeptide would suggest that in wheat leaf cells as well as in maize leaf cells, PPDK might be a chloroplast enzyme. The intracellular location of PPDK in C_3 leaf cells has not yet been determined, however. A function for PPDK in chloroplasts of C_3 plant leaf cells is not clear. Also, the amount of PPDK in C_3 leaf cells (wheat) is only about 1 to 2% of the amount found in C_4 leaves (2).

We propose that the mRNA from seed tissue is translated to give polypeptides without the transit sequence, since they need not enter the chloroplasts, but instead function in the cytoplasm. There is no necessity for a transit sequence which would permit entry into the chloroplasts.

Other examples of plant enzymes synthesized with or without transit sequences for function in chloroplasts or cytoplasm, respectively, have been reported, including glyceraldehyde-3-P dehydrogenases (4) and triose-P isomerases (15). Differences were noted in the regulatory properties of the cytoplasmic and chloroplastic enzymes. So far, the properties of seed and leaf PPDK from wheat and maize appear to be remarkably similar (2, 12), but differences may be found with further study.

The organ specificity of type of mRNA for PPDK observed in this study with maize and with wheat suggests some mechanism of organ regulation of type of mRNA formed. One possibility is that there are different PPDK genes which are differentially expressed in leaf and seed tissue. Another mechanism might be that there is a different processing of the RNA sequence, either during or subsequent to transcription of the gene.

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