INCREASES IN ACTIVITIES OF AMINOACYL-tRNA SYNTHETASES DURING COLD-TREATMENT OF DORMANT PEAR EMBRYO*

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Summary

Aminoacyl-tRNA synthetases from a pH 5 enzyme fraction from dormant or non-dormant pear embryos were studied by the pyrophosphate exchange assay. Synthetases from dry embryos exchanged 18.6 n mol/min/mg protein. This value was slightly increased by the moist treatment, however a greater increase was obtained by cold-treatment. The activity of individual synthetases was generally higher after cold-treatment. The rate of pyrophosphate exchange was enhanced by kinetin or gibberellic acid treatment of dormant embryos and additional enhancement was observed when both hormones were used in combination. Abscisic acid inhibited synthetase activity in non-dormant embryos. The presence of 6-methylpurine during a 5 day cold-treatment resulted in a 50% decrease in enzyme activity compared to the control.

Many seeds in the Rosaceae (1) and other families (2) require an extended period of cold, moist pretreatment (stratification) to break the embryo dormancy. An application of exogenous GA_3 ** can replace the cold treatment of certain seeds such as beech (3) and partially release pear embryo dormancy (4). In order to determine the controlling mechanism of seed dormancy, numerous works have focused attentions on the action of promotor, inhibitor or their interaction on seed germination (1, 3, 5, 6). The dormancy of seeds with chilling requirement appears to be controlled by a balance between endogenous inhibitors, notably ABA, and promotors, particularly gibberellic acid and/or cytokinin (1, 2). It has been suggested that cold-treatment shifts the inhibitor - promotor balance in favor of the promotor, thus causing germination (3, 6, 7, 8). However, only limited work has been done on the molecular

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** Abbreviation: GA_3, gibberellic acid; ABA, abscisic acid.

level concerning the mechanism of stratification. Higher rates of RNA synthesis and more DNA available for transcription were found in hazel seeds following GA_3 treatment (9). During stratification and following GA_3 or kinetin treatment of dormant pear embryos, the nucleic acid synthesizing capacity as well as chromatin - bound RNA polymerase activity were increased (10, 11). This paper describes the relationship between dormancy release by cold or hormonal treatments of pear embryos and aminoacyl - tRNA synthetases.

MATERIAL AND METHODS

Pear seeds, Pyrus communis cv. Bartlett, were kept at 5° or 25° on moist blotters for various lengths of time. Before and after the above treatments, seeds were soaked for 10 minutes in 2% sodium hypochlorite solution and then washed thoroughly with distilled water. Embryos were then dissected out, homogenized, centrifuged and the aminoacyl-tRNA synthetases were isolated from supernatant by acidification to pH 5 as described by Hall and Tao (12). The only exception was that 50 embryos were homogenized with 10 ml of solution in a mortar. Protein was determined by the method of Lowry et al (13). For the pryophosphate - exchange assay, the procedure of Attwood and Cocking (14) was followed.

RESULTS AND DISCUSSION

The aminoacyl - tRNA synthetases in the pH 5 enzyme fraction from the unchilled, dry embryos exchanged 18.6 n mol/min/mg protein (Table 1). This value increased only slightly in embryos from seeds kept at 25°C. The enzyme activity, however, increased dramatically when the embryos were chilled and this increase was highly dependent on the length of cold treatment. After 30 days of cold treatment when dormancy release was near its maximum (4), the enzyme activity was twice that of unchilled embryos and three times that of dry embryos. The lower activity of enzymes from embryos kept at 25°C is not due to embryo deterioration as embryo excised after 30 days grew better than 1 day embryo in presence or absence of GA_3. This better growth might be due

Table 1. Effects of treatment time and temperature on the activity of pear embryo aminoacyl-tRNA synthetases

Time of moist treatment (days)	Temperature during moist treatment	Radioactivity in ATP (cpm)	Amount of pryophosphate exchanged (n mole/min/mg protein)
0 (Dry)	---	1164	18.6
5	25°	1531	24.6
30	25°	1704	27.4
5	4°	2755	44.2
30	4°	4211	67.7

Each reaction mixture contained, in a final volume of 1 ml, 10 mM ATP, 10 mM $MgCl_2$, 8 mM KCl, 1 M Tris, 0.5 mg enzymes, 2 μCi ^{32}P pyrophosphate (0.2 m Ci/m mole) and 20 mM amino acid mixture. Incubation was for 30 min at pH 8.0 and 30°C. The radioactivity is the mean of 2 replicates. The values are obtained after subtracting the radioactivity of 0 time control (about 1100 cpm).

to a partial loss of germination inhibitors by diffusion during the prolonged wet treatment. However, these embryos were still dormant.

In order to study whether the high activity of enzymes in chilled embryos was caused by certain specific aminoacyl-tRNA synthetases, individual amino acid was added to the assay system instead of the amino acid mixture. The reaction rates, stimulated by each of the amino acid tested, were generally higher in the presence of enzymes from chilled than from unchilled embryos (Table 2). These suggested that an increase in activity is common for many, if not all, synthetases. The stimulation by amino acid mixture (Table 1) was less than the amount calculated by summing the stimulations by individual amino acids. This may be partially due to a lower concentration of individual

Table 2. Effects of cold-treatment on pyrophosphate exchange using individual amino acids.

Amino acid added	Temperature during moist treatment	Radioactivity (cpm)	Amount of pyrophosphate exchanged (n mole/min/mg protein)
Alanine	25°	793	9.6
	4°	1922	29.8
Arginine	25°	0	0
	4°	2599	41.8
Aspartic acid	25°	374	6.0
	4°	3427	55.0
Leucine	25°	0	0
	4°	494	7.9
Methionine	25°	0	0
	4°	302	4.8
Phenylalanine	25°	503	8.1
	4°	2779	44.6

Enzyme was extracted from embryos after 30 days of treatment. System is the same as in Table 1 except that 10 mM individual amino acid was used.

amino acids in the mixture. A similar phenomenon was observed in another plant system (12).

A 1 day-treatment with kinetin or GA_3 of dormant embryos enhanced the rate of pyrophosphate exchange and additional enhancement was observed when a combination of these hormones was used (Table 3). These data are consistent with the observations that kinetin and GA_3, alone or in combination, promote

Table 3. Effect of hormones on the activity of aminoacyl-tRNA synthetase from dormant and non-dormant embryos.

Hormonal treatments	Type of embryos	Radioactivity (cpm)	Amount of pyrophosphate exchanged (n mole/min/mg protein)
H_2O	non-dormant	3529	57.2
ABA	non-dormant	358	5.7
H_2O	dormant	884	14.1
GA_3	dormant	1916	30.7
Kinetin	dormant	1619	26.0
Kinetin + GA_3	dormant	2635	42.2

Fifty embryos were excised after a 30-day treatment at 5° or a 1-day treatment at 25° and were grown on filter papers moistened with 6 ml of water or hormonal solution (20 μM for each hormone) at 25°. Enzyme isolation was after 1 day of growth. Other procedures are the same as described in Table 1.

growth of the dormant pear embryos (4). When the non-dormant embryos were used, ABA treatment showed an inhibitory effect.

In order to determine if the increase in RNA synthesis played a primary role in the enhancement of the activity of aminoacyl-tRNA synthetases, 6-methylpurine, a powerful inhibitor of RNA synthesis was used during the cold-treatment; a reduction in enzyme activity of about 50% occurred after 5 days compared to water control (Table 4). This suggested that RNA synthesis was required for the high enzyme activity.

It has been reported that GA_3 increased the DNA template and RNA polymerase activity for RNA transcription in dormant hazel seeds (9). In dormant pear embryos nucleic acid synthesis increased during cold treatment (10). ABA inhibited labelling of P^{32} in certain nucleic acid fractions of chilled

Table 4. Effect of 6-methylpurine during cold treatment (4°) on the enzyme activity.

Treatment	Time of moist cold treatment (days)	Radioactivity (cpm)	Increase of radioactivity
---	0 (Dry)	1100	---
Water	5	2890	1790
6-Methylpurine (100 μg/ml)	5	1922	822

embryos and this effect was reversed selectively by kinetin and GA_3 (4). A similar interaction of hormones in pear embryos was observed at the level of chromatin-bound RNA polymerase activity (11). The results presented in this paper are in agreement with these data. It suggests that following cold-treatment an increase in RNA transcription by derepression of genetic material occurs and this is followed by an enhanced rate of translation of aminoacyl-tRNA synthetases which in turn lead to the release of dormancy. Experiments are in progress to further elaborate on the data presented here.

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