Adenine Phosphoribosyltransferase in Plant Tissues: Some Effects of Kinetin on Enzymic Activity

P. B. Nicholls and A. W. Murray
School of Biological Sciences, Flinders University of South Australia.
Bedford Park, South Australia 5042

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Abstract. Adenine phosphoribosyltransferase activity was measured in extracts of soybean (Glycine max var. Aeme) callus and of senescing barley leaves (Hordeum distichon c.v. Prior). The enzyme from soybean callus had Michaelis constants for adenine and 5-phosphoribosyl pyrophosphate of 1.5 and 7.5 μM respectively and was inhibited by AMP and stimulated by ATP. The presence of kinetin was found to considerably increase the activity of adenine phosphoribosyltransferase in extracts of soybean callus and senescing barley leaves.

Studies on the involvement of macromolecular biosynthesis in cell division and cell elongation led to the current concept that plant hormones exert their control of growth through the regulation of RNA biosynthesis (7). Hormone-stimulated growth can be inhibited with compounds such as 2,6-diaminopurine, 8-azaguanine, 6-methylpurine, and 5-fluorodeoxyuridine which inhibit nucleic acid synthesis (2, 8, 14). In bacterial and tumor-cell systems, these purine and pyrimidine analogues are considered to act after conversion to their nucleotides (3). Similarly natural purine and pyrimidine bases supplied to study the hormonally induced stimulation of RNA biosynthesis must be converted to their nucleotide derivatives before incorporation into nucleic acid. In the case of orotic acid the pathway has been described (17). Incorporation of purines into nucleotides can occur by 2 pathways: A) purine + ribose-1-P → nucleoside + Pi (nucleoside phosphorylase), nucleoside + ATP → nucleoside + ADP (nucleotide kinase); B) purine + 5-phosphoribosyl pyrophosphate (PRPP) → nucleotide + P-P (purine phosphoribosyltransferase).

This paper describes the occurrence of adenine phosphoribosyltransferase (AMP-pyrophosphate phosphoribosyltransferase; EC 2.4.2.7) in 2 plant tissues and the stimulation of enzyme activity in tissues treated with kinetin.

Materials and Methods

Cell-free extracts were made by suspending the plant tissue in 10 volumes of 50 mM-tris (Cl−; pH 7.8) at 1°, homogenizing for 10 seconds with an Ultra Turrax homogenizer and centrifuging at 20,000 × g for 30 minutes. Protein determinations were carried out by the Biuret method (4) on 0.4 ml subsamples of the supernatant. Extracts could be stored at −15° for at least 2 weeks without loss of enzymic activity. Adenine phosphoribosyltransferase activity was measured using the DEAE-cellulose disc technique described by Atkinson and Murray (1). Assays contained 20 μmoles of tris (Cl−; pH 7.8), 2 μmoles of MgCl2, 0.018 μmole of PRPP (Sigma Chemical Company), 0.05 or 0.1 ml of extract (containing about 0.2–0.3 mg protein) and 0.03 μmole of (8-14C) adenine (specific radioactivity 4 μc/μmole) in a final volume of 0.4 ml; assays were carried out for 2 minutes at 25°. The (8-14C) AMP formed in the reaction was separated from residual adenine on DEAE-cellulose discs and the radioactivity measured with a liquid scintillation spectrometer (1). Assays containing no PRPP were routinely carried out as controls. The control activities were subtracted from activities in the presence of PRPP to obtain rates of PRPP-dependent nucleotide formation. To determine the nature of the reaction products with soybean callus extracts, standard assays were carried out at 25° for 5 minutes and portions (0.05 ml) were chromatographed on Whatman 3 MM paper in 5% (w/v) Na2HPO4. Radioactivity associated with adenine (Rf 0.37) and AMP (Rf 0.75) was estimated by scanning in a gas-flow counter (Actigraph III; Nuclear Chicago, Inc., Chicago, Illinois); adenosine had an Rf of 0.64 in this solvent.

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2 Present address: Department of Plant Physiology, Waite Agricultural Research Institute, University of Adelaide, Glen Osmond, South Australia 5064.
Barley plants (* Hordeum distichon* c.v. Prior) were grown in potting mixture in a growth room at 22°C under a 12 hour photoperiod from a fluorescent light source. Chlorophyll retention tests were carried out essentially as described by Kende (6). One cm sections from the first leaf were cut 3 cm from the leaf apex (2-3 days after second leaf had appeared) and floated on 2 mM-phosphate (K⁺; pH 5.7) in the dark for 18 to 24 hours and then transferred to the test solutions. In these experiments the kinetin concentration was 1 mg/l (4.6 µM) in phosphate buffer and 500 µg of penicillin G was added to each petri dish containing 15 ml of solution. The sections (20/petri dish), when harvested, were surface dried, weighed, and homogenized as described before. The pellet obtained by centrifuging at 20,000 X g for 30 minutes was extracted in a small volume of ethanol and centrifuged. The pellet was washed in a small volume of ethanol and again centrifuged. The combined supernatants were made up to 10 ml with ethanol and the optical density at 665 mµ was determined in a 1 cm cuvette. A chlorophyll unit is defined as 1 optical density unit at 665 mµ/g fresh weight of tissue/10 ml of ethanolic extract. The initial supernatant was assayed for adenine phosphoribosyltransferase activity as described before.

**Results and Discussion**

Properties of Adenine. Under the conditions described no measurable amounts of (8-14C) adenine or (8-14C) AMP were formed in the absence of added PRPP. In the presence of PRPP the only detectable radioactive product was AMP. Activity was decreased by about 90% when Mg²⁺ was omitted from the reaction mixture; the residual activity was presumably due to Mg²⁺ present in the extracts and because PRPP was used as the Mg²⁺ salt. These results clearly established the presence of adenine phosphoribosyltransferase activity in soybean extracts.

Under standard assay conditions the rate of the reaction was proportional to protein concentration (up to 0.4 mg of protein/assay) and with time (up to 3 min). The values of the Michaelis constant for adenine and PRPP were 1.5 and 7.5 µM, respectively. These values are similar to those obtained with adenine phosphoribosyltransferase from Ehrlich ascites-tumour cells (1,5). In common with the tumour cell enzyme, adenine phosphoribosyltransferase from soybean callus was inhibited by AMP and stimulated by low concentrations of ATP indicating a pattern of regulation similar to that with the mammalian enzyme (12,13). Thus in the presence of 31 µM-PRPP, activity was inhibited 92% by 0.62 mM-AMP and stimulated 21% by 60 µM-ATP. In the presence of 31 µM-PRPP, 67 mfl.14N-benzyl AMP inhibited the reaction by 13% and in the presence of 12.4 µM-PRPP by 35%. This finding is of interest as 14N-benzyl AMP has been reported to be formed following treatment of * Xanthium* leaves with the cytokinin benzyladenine (9).

**Effect of Kinetin on Enzyme Activity in Soybean Callus.** The soybean (*Glycine max* var Acme) callus strain, which was used in experiments investigating the change in time of adenine phosphoribosyltransferase activity in freshly subcultured tissue, was developed and had been maintained for 6 months by routinely subculturing every 5 weeks onto the fresh medium containing 2.3 µM-kinetin described by Miller (11). At the end of 5 weeks of culture the callus pieces have almost stopped increasing their fresh weight and such tissue was always taken for subculturing onto fresh stock or experimental media. In the time course experiment reported in figure 1 callus pieces were excised and 3 pieces (about 40 mg each) were placed into each flask holding 50 ml of medium which contained either no kinetin or 2.3 µM-kinetin. At the indicated times 2 flasks each containing 3 pieces of callus were taken from each treatment; the tissue pooled and prepared for enzyme assay as described and assayed in duplicate. The data in figure 1 is from 1 of 2 such experiments and was analyzed by conventional statistical methods; the least significant difference (LSD P=0.05) between points is 0.091 units of enzyme specific activity. For 5 days of culture the specific activity of the enzyme

![Fig. 1. Effect of the presence or absence of kinetin in the culture medium on adenine phosphoribosyltransferase activity in extracts from soybean callus of increasing age from subculture. ○—○. — kinetin; ■—■. + kinetin (23 µM). The least significant difference (L.S.D.; P=0.05) between any 2 points is given.](https://www.plantphysiol.org/)

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in the tissue maintained on kinetin was 2 to 3 times greater than that in tissue which had been transferred to medium deficient in kinetin. By the seventh day the specific activity of the enzyme in each treatment was not significantly different.

A similar amount of growth was observed in the non-harvested flasks from both (+)kinetin and (-)kinetin treatments when the flasks were examined 7 days after the last harvest. This confirms an earlier observation by Kende (personal communication) that the response to applied kinetin is greatly reduced in strains which are greater than 6 months old.

Accordingly a second series of experiments to investigate the relationship between the growth rate and changes in enzyme activity were conducted on a new strain which had undergone 1 routine subculture onto stock medium containing kinetin. At the second subculture the tissue was transferred to kinetin free medium for 4 days and then some of the flasks were chosen at random and these cultures were transferred to medium containing 2.3 \( \mu \text{M} \) kinetin. The callus pieces were weighed in groups of 3 at subculture, and when harvested. For the first 4 days after subculture onto (-)kinetin medium (see fig 2) there was a decline in fresh weight but no significant change in enzyme specific activity. At the harvest 3 days after retransferring the tissue to (+)kinetin medium there is nearly a 2-fold rise in the specific activity of the enzyme. The mean value and standard deviation of the mean for the specific activity of the enzyme from tissue cultured on (-)kinetin medium was 0.111 \( \pm 0.011 \) units. A unit of enzyme activity is defined as 1 m\( \text{mole} \) of AMP formed per minute per mg of protein.

The difference between the specific activity of the enzyme from kinetin treated tissue (0.277 units) and the mean of the controls (0.111 units) is 6 times the population standard deviation (\( \pm 0.028 \)) of the control tissue.

In a similar experiment where the transfer from (-)kinetin medium to (+)kinetin medium was performed at 7 days, the comparable values were 0.064 \( \pm 0.010 \) units for the tissue grown on kinetin free medium and 0.154 units for tissue 3 days after retransfer back onto (+)kinetin medium. Again the difference between (+)kinetin and (-)kinetin values is 4 times the population standard deviation (\( \pm 0.024 \)).

The overall loss in fresh weight of the tissue on (-)kinetin medium was about 11% (initial mean fr wt/piece 46.34 \( \pm 1.13 \) mg) while the transfer to kinetin resulted in a gain in fresh weight of 32%. A similar increase in gain in fresh weight was observed in the second experiment where the rate of increase in fresh weight rose from 0.03 mg/mg/day to 0.09 mg/mg/day after transferring onto (+)kinetin medium. These results show a correlation between a raised enzyme activity and an increased growth rate of the callus tissue in the presence of kinetin under the experimental conditions described.

**Effect of Kinetin on Enzyme Activity of Senescing Barley Leaf Sections.** In another system in which the response to added kinetin is not one of growth but of chlorophyll retention the change in adenine phosphoribosyltransferase activity upon treatment of the tissue with kinetin was investigated.

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**FIG. 2. (A) Effect of transfer of soybean callus tissue from a 4 day old culture on (-)kinetin medium to (+) kinetin medium (2.3 \( \mu \text{M} \)) on activity of adenine phosphoribosyltransferase in extracts of the tissue. Sample mean and sample 95% confidence limits are indicated for enzyme activity in the tissue cultured on (-)kinetin medium. \( \boxed{\text{O}} \) enzyme activity in tissue cultured on (-) kinetin medium; \( \boxed{\text{\footnotesize{\textbullet}}} \) enzyme activity of tissue transferred to (+)kinetin medium.

**FIG. 2. (B) Effect of transfer of soybean callus tissue from a 4 day old culture on (-)kinetin medium to (+)kinetin medium (2.3 \( \mu \text{M} \)) on growth of the tissue. \( \boxed{\text{\footnotesize{\textsquare}}} \) change in fresh weight of tissue cultured on (-)kinetin medium; \( \boxed{\text{\footnotesize{\textblacksquare}}} \) change in fresh weight of tissue transferred to (+)kinetin medium.**
Barley leaf sections were prepared, treated, and extracted as described in the Materials and Methods section.

The results of 3 experiments are tabulated in Table I. The effective period for kinetin-induced retardation of chlorophyll breakdown appears to be less than 24 hours with this tissue. Although the specific activity of adenine phosphoribosyltransferase in leaf tissue extracts was about one-tenth of that in soybean extracts (see Fig 2) there was a consistent stimulation of enzyme activity in kinetin treated leaves. There was some evidence (see Table I, exp 3) that the kinetin-stimulated activity was again of relatively short duration as the enzyme activity at day 1 was more than twice that at day 2.

The results reported in this paper have shown a direct response of an adenine-metabolizing enzyme to treatment with kinetin in 2 plant tissues. Previous investigations have suggested a relationship between kinetin action and adenine metabolism (10,15,16). However, the observed increase in adenine phosphoribosyltransferase activity could simply reflect an overall stimulation of anabolic metabolism induced by kinetin rather than a direct primary effect of cytokinin treatment.

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Literature Cited