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# Cytokinin Structure-Activity Relationships and the Metabolism of $N^6$-($\Delta^2$-Isopentenyl)Adenosine-8-$^{14}$C in *Phaseolus* Callus Tissues[1]

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## ABSTRACT

**The activities of the free base and ribonucleoside forms of cytokinins bearing saturated and unsaturated $N^6$-isoprenoid side chains have been examined in callus cultures derived from *Phaseolus vulgaris* cv. Great Northern, *P. lunatus* cv. Kingston, and the interspecific hybrid Great Northern × Kingston. In callus of cv. Great Northern, cytokinins bearing saturated side chains ($N^6$-isopentyladenine, $N^6$-isopentyladenosine, dihydrozeatin, and ribosyldihydrozeatin) were always more active than the corresponding unsaturated analogs ($N^6$-[$\Delta^2$-isopentenyl]adenine, $N^6$-[$\Delta^2$-isopentenyl]adenosine, zeatin, and ribosylzeatin). In callus of cv. Kinston, the cytokinins bearing unsaturated side chains were either more active or equally as active as the saturated compounds. These differences in cytokinin structure-activity relationships were correlated with differences in the metabolism of $^{14}$C-$N^6$-($\Delta^2$-isopentenyl)adenosine. In Great Northern tissues, this cytokinin was rapidly degraded to adenosine; in Kingston tissues, the major metabolite was the corresponding nucleotide. The growth responses of callus of the interspecific hybrid were intermediate between the parental tissues, and the metabolism of $^{14}$C-$N^6$-($\Delta^2$-isopentenyl)-adenosine by the hybrid callus exhibited characteristics of both parental tissues. The results are consistent with the hypothesis that the weak activity of cytokinins with unsaturated side chains in promoting the growth of Great Northern callus is due to the rapid conversion of these cytokinins to inactive metabolites.**

As part of a continuing investigation of the genetic regulation of cytokinin metabolism in *Phaseolus*, we have employed tissue culture techniques to screen *Phaseolus* genotypes for traits indicative of possible intrinsic genetic variations in cytokinin metabolism (2, 10, 12, 13). The traits identified to date include pronounced differences in the cytokinin responses of callus tissues derived from *P. vulgaris* cv. Great Northern and *P. lunatus* cv. Kingston (12). Callus cultures of these two genotypes were found to differ dramatically in their response to cytokinins bearing unsaturated isoprenoid side chains (io$^6$Ade and i$^6$Ade).[2] In Great Northern callus tissues, io$^6$Ade and i$^6$Ade were 30- to 100-fold less active than their saturated analogs (hio$^6$Ade and hi$^6$Ade) in promoting the growth of the cytokinin-dependent tissue cultures. In Kingston callus tissues, on the other hand, the cytokinins with unsaturated side chains were either more active (io$^6$Ade) or equally as active (i$^6$Ade) as their saturated counterparts. The activities of the cytokinins with saturated side chains were comparable in the two systems, and the observed differences in cytokinin structure-activity relationships could be attributed to the relatively low activities of cytokinins with unsaturated side chains in promoting the growth of *P. vulgaris* cv. Great Northern callus tissues.

In the work reported here, we have attempted to further characterize differences in cytokinin structure-activity relationships in *Phaseolus* callus tissues and to examine the physiological basis of these differences. Both the free base and ribonucleoside forms of cytokinins bearing $N^6$-isoprenoid side chains have been tested and compared for their ability to promote the growth of callus tissues derived from *P. vulgaris* cv. Great Northern, *P. lunatus* cv. Kingston, and the interspecific hybrid Great Northern x Kingston. In addition, the metabolism of $^{14}$C-i$^6$Ado has been examined in callus tissues derived from all three genotypes. The weak activity of i$^6$Ado (and other cytokinins with unsaturated side chains) in promoting the growth of Great Northern callus tissues correlates with a rapid degradation of i$^6$Ado to Ado in callus tissues of this genotype.

## MATERIALS AND METHODS

**Chemicals.** Zeatin, dihydrozeatin, ribosylzeatin, $N^6$-($\Delta^2$-isopentenyl)adenine, and $N^6$-($\Delta^2$-isopentenyl)adenosine were obtained from Sigma. $N^6$-isopentyladenine, $N^6$-isopentyladenosine, and ribosyldihydrozeatin were synthesized according to the procedures described by Leonard *et al.* (7), Fleysher *et al.* (4), and Matsubara *et al.* (8), respectively. Picloram (4-amino-2,3,5-trichloropicolinic acid) was a gift from Dow Chemical. Adenine, adenosine, kinetin, alkaline phosphatase (calf intestinal mucosa type VII) and Sephadex LH-20 were purchased from Sigma. Porapak Q was acquired from Waters Associates, Inc. Adenosine-8-$^{14}$C (54 mCi/mmol) was obtained from Amersham Co. Isopentenyl bromide was purchased from K and K. Omnifluor is a product of New England Nuclear.

**Plant Materials.** Seeds of *Phaseolus vulgaris* cv. Great Northern were obtained from Dr. D. P. Coyne, University of Nebraska, Lincoln, NE. Seeds of *P. lunatus* cv. Kingston were obtained

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[2] Abbreviations: Ade, adenine; Ado, adenosine; i$^6$Ade, $N^6$-($\Delta^2$-isopentenyl)adenine; i$^6$Ado, $N^6$-($\Delta^2$-isopentenyl)adenosine; $^{14}$C-i$^6$Ado, 8-$^{14}$C-$N^6$-($\Delta^2$-isopentenyl)adenosine; hi$^6$Ade, $N^6$-isopentyladenine; hi$^6$Ado, $N^6$-isopentyladenosine; io$^6$Ade, zeatin, $N^6$-(4-hydroxy-3-methyl-*trans*-2-butenyl)adenine; io$^6$Ado, ribosylzeatin, $N^6$(4-hydroxy-3-methyl-*trans*-2-butenyl)adenosine; hio$^6$Ade, DL-dihydrozeatin,$N^6$-(4-hydroxy-3-methylbutyl)adenine; hio$^6$Ado, DL-ribosyldihydrozeatin, $N^6$-(4-hydroxy-3-methylbutyl)adenosine; picloram, 4-amino-3,5,6-trichloropicolinic acid.

locally. Hybrid embryos from the cross *P. vulgaris* cv. Great Northern (female) × *P. lunatus* cv. Kingston (male) were produced as described previously (9).

**Tissue Culture Medium.** The tissue culture medium consisted of the mineral nutrients described by Murashige and Skoog (14) with the following organic substances added: sucrose (30 g/L), *myo*-inositol (100 mg/L), thiamine.HCl (1 mg/L), nicotinic acid (5 mg/L), pyridoxine. HCl (0.5 mg/L), and picloram (2.5 μM). (The latter compound is used to supply the auxin requirement of *Phaseolus* tissue cultures [11].) Kinetin (5 μM) was included in the medium used for callus initiation and stock cultures. The pH of the medium was adjusted to 5.7 and Difco Bacto-agar (10 g/L) was added. The medium was dispensed into 125-ml Erlenmeyer flasks (50 ml/flask) and autoclaved at 120°C for 15 min. The compounds tested for cytokinin activity were dissolved in dimethylsulfoxide (17) and added to the autoclaved tissue culture flasks prior to solidification of the medium. (The final amount of dimethylsulfoxide in the tissue culture medium was 0.025 ml/flask.)

**Growth and Harvest of *Phaseolus* Callus Cultures.** Callus cultures of the parental genotypes were established from the hypocotyls of 5-d-old seedlings as described previously (11). Callus cultures of the hybrid were derived from immature embryos. Hybrid seeds were excised from surface-sterilized pods 21 to 24 d after pollination (9). The immature embryos (approximately 0.4 mm long) were removed from the seeds under aseptic conditions and transferred to the medium used for callus initiation. Four replicate callus culture lines, each derived from a different seedling (or embryo), were established for each genotype for each experiment. The callus tissues that formed on the initial explants were transferred once (first passage) on medium containing 5 μM kinetin. Tests for cytokinin activity were performed in the second passage of the callus tissue using 4-week-old first-passage cultures as stock tissue. Three pieces of callus weighing approximately 10 mg each were planted/flask. Four replicate flasks, each corresponding to one of the four replicate lines of stock callus, were used for each treatment. Tissues were harvested and weighed after 35 d of growth at 27°C in the dark. All tests were repeated at least once.

**Preparation of $^{14}$C-i$^6$Ado.** The $^{14}$C-i$^6$Ado was synthesized using a modification of the method described by Pačes *et al.* (15). An aqueous solution of $^{14}$C-Ado (100 μCi; 1.82 μmol) was mixed with 2.18 μmol unlabeled Ado and evaporated to dryness *in vacuo* at room temperature. The mixture was dried from 100% ethanol and placed in a desiccator over $P_2O_5$ for 24 h. The dry Ado preparation (4 μmol, 25 μCi/μmol) was dissolved in 40 μl dimethylformamide, and 10 μl of 10% (v/v) isopentenyl bromide in dimethylformamide was added. (Isopentenyl bromide and dimethylformamide were distilled before use, the former *in vacuo*. The dimethylformamide was dried over anhydrous calcium sulfate prior to distillation.) The reaction mixture was kept in the dark at room temperature for 24 h. At the end of that time, 50 μl dimethylamine and 50 μl methanol were added. The mixture was left for 40 h and then evaporated to dryness *in vacuo*. The $^{14}$C-i$^6$Ado was dissolved in 4 ml 33% (v/v) ethanol and purified by chromatography on a Sephadex LH-20 column (1.5 × 30 cm) equilibrated with the same solvent, followed by rechromatography on Sephadex LH-20 in distilled $H_2O$. The compound was dried, redissolved in $H_2O$, and stored at −20°C. The yield was approx. 50% and the final specific radioactivity was 25 μCi/μmol.

**Metabolism of $^{14}$C-i$^6$Ado.** The metabolism of $^{14}$C-i$^6$Ado was examined in second passage callus tissues of *P. vulgaris* cv. Great Northern, *P. lunatus* cv. Kingston, and their hybrid. Callus tissues were selected for use in the labeling studies when the average fresh weight per flask was equal to 10 g (±1 g). At this point, the tissues were rapidly growing and had reached approximately half of their final weight. Culture age at the 10 g stage averaged 23 d for *P. vulgaris* cv. Great Northern, 21 d for *P. lunatus* cv. Kingston, and 21 d for the Great Northern × Kingston hybrid tissue. The $^{14}$C-i$^6$Ado was applied as an aqueous solution, aseptically, and directly to the surface of the callus pieces. A total of 0.05 μCi (0.002 μmol) in 1 ml distilled $H_2O$ was applied to the tissue in each flask. The tissues were incubated at 27°C in the dark for periods of 0.5, 1, 2, 4, and 8 h. Metabolites were extracted by homogenizing the tissue with 2.5 parts (v/w) of cold 95% (v/v) ethanol. The cell debris was removed by successive filtrations through Whatman No. 1 filter paper and Millipore filters. The ethanol extract was taken to dryness *in vacuo* at 35°C, redissolved in 4 ml 33% (v/v) ethanol and centrifuged at 23,500*g* for 20 min. The supernatant was fractionated on a Sephadex LH-20 column (2 × 60 cm) in 33% (v/v) ethanol. Fractions of 9 ml (one-twentieth bed volume) were collected. Aliquots (4 ml) of each fraction were evaporated to dryness in scintillation vials and counted in a toluene-based scintillation fluid (Omnifluor) with a Beckman LS 7000 scintillation counter. Authentic cytokinin standards were chromatographed on the same column immediately following the experimental samples. Each experiment was repeated at least once. In the case of 1-h time points, the labeling studies were also repeated using a second extraction procedure involving homogenization of tissues in cold 1 N $HClO_4$ (1:1, w/v) and isolation of the cytokinin metabolites as described by Laloue *et al.* (6).

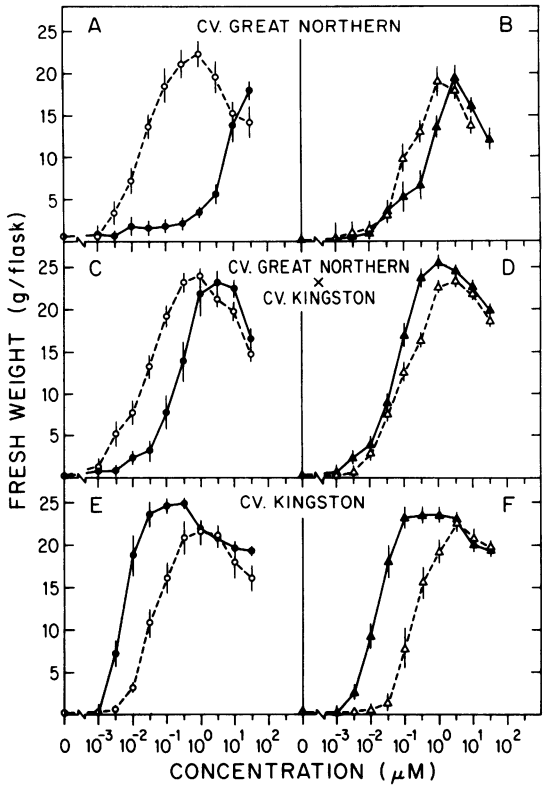


FIG. 1. Cytokinin activities of io$^6$Ade (●), hio$^6$Ade (○), io$^6$Ado (▲), and hio$^6$Ado (△) in promoting callus growth of *P. vulgaris* cv. Great Northern (A, B), *P. vulgaris* cv. Great Northern × *P. lunatus* cv. Kingston (C, D) and *P. lunatus* cv. Kingston (E. F). The vertical bars indicate SE.

**Identification of i$^6$Ado Metabolites.** Porapak Q chromatography (3) was employed in the separation of Ado and io$^6$Ado. (These two compounds are not resolved by chromatography on Sephadex LH-20 in 33% [v/v] ethanol.) The peak of radioactivity that eluted from the Sephadex LH-20 column at the position of the Ado and io$^6$Ado standards was taken to dryness *in vacuo* at 35°C, redissolved in 1 ml 24% (v/v) ethanol, and chromatographed on a Porapak Q column (0.9 × 30 cm) in 24% (v/v) ethanol.

To confirm the identity of the major peak of radioactivity that eluted from the Sephadex LH-20 column prior to Ado, the cor-

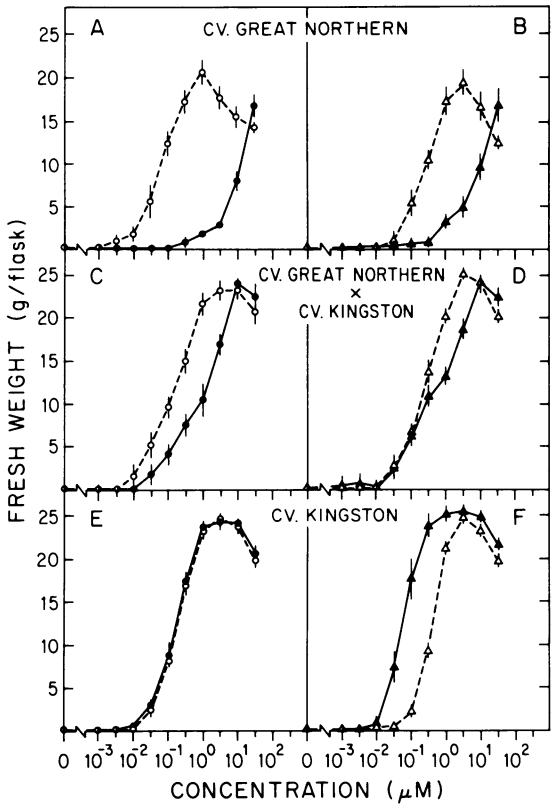


FIG. 2. Cytokinin activities of i$^6$Ade (●), hi$^6$Ade (○), i$^6$Ado (▲), and hi$^6$Ado (△) in promoting callus growth of *P. vulgaris* cv. Great Northern (A, B), *P. vulgaris* cv. Great Northern × *P. lunatus* cv. Kingston (C, D) and *P. lunatus* cv. Kingston (E, F). The vertical bars indicate SE.

responding fractions were dried and incubated with five units of calf intestinal alkaline phosphatase in 0.5 ml 0.05 M Tris-HCl (pH 8.6) containing 0.005 M $MgCl_2$ for 1 h at 37°C. Ethanol (2 ml) was added, and the solution was centrifuged (12,000*g*, 5 min) and dried *in vacuo* at 35°C. The residue was dissolved in 2 ml 33% (v/v) ethanol and chromatographed on a Sephadex LH-20 column (1.5 × 30 cm). Fractions of 2.25 ml (one-twentieth bed volume) were collected and taken to dryness in scintillation vials for determination of radioactivity.

**Uptake and Metabolism of $^{14}$C-i$^6$Ado in Liquid Cultures.** Liquid cultures were established by transferring 10 g of second passage callus tissue (selected in the same manner as the tissue used for the labeling studies described above) to a 125-ml Erlenmeyer flask containing 10 ml of liquid medium having the same composition as the semi-solid medium except for the omissions of kinetin and agar. The cultures established in this manner were incubated with $^{14}$C-i$^6$Ado (0.1 μCi; 0.004 μmol) on a rotary shaker (120 rpm) for periods of 0.5, 1, 2, and 4 h in the dark at 27°C. The cells and medium were then separated by centrifugation at 6,000*g* for 3 min. The pelleted cells were resuspended in 10 ml $H_2O$ and recentrifuged. The medium and washing were combined, evaporated to dryness *in vacuo* at 35°C, suspended in 4 ml 33% (v/v) ethanol, centrifuged, and the supernatant chromatographed on Sephadex LH-20 in 33% (v/v) ethanol as described above. Cytokinin metabolites were extracted from the pelleted cells by homogenization in ethanol. The extracts were prepared and chromatographed on Sephadex LH-20 in the same manner as the extracts from tissue incubated on semi-solid medium.

## RESULTS

**Response of *Phaseolus* Callus Tissues to Cytokinins with $N^6$-Isoprenoid Side Chains.** The free base and ribonucleoside forms

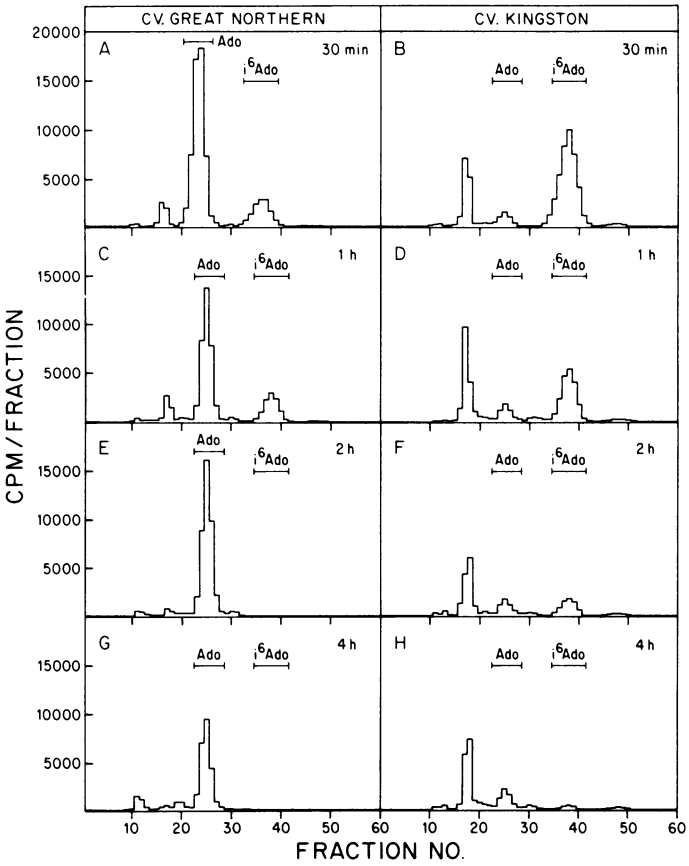


FIG. 3. Sephadex LH-20 chromatography of extracts prepared from callus tissues of *P. vulgaris* cv. Great Northern (A, C, E, G) and *P. lunatus* cv. Kingston (B, D, F, H) after incubation with $^{14}$C-i$^6$Ado. The extracts were chromatographed in 33% (v/v) ethanol as described under "Materials and Methods."

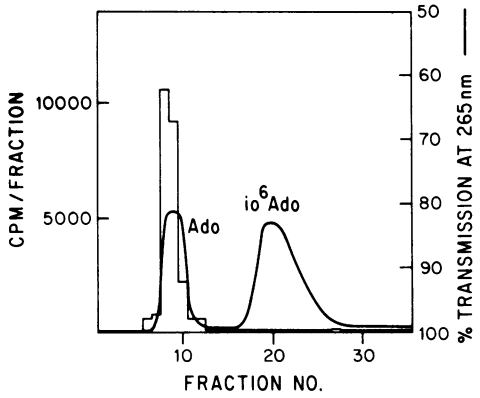


FIG. 4. Rechromatography on Porapak Q of the radioactive metabolite present in the Ado region of a Sephadex LH-20 fractionation of an extract prepared from *P. vulgaris* cv. Great Northern callus tissues after incubation for 1 h with $^{14}$C-i$^6$Ado. The sample was chromatographed in 24% (v/v) ethanol as described under "Materials and Methods."

of cytokinins bearing saturated and unsaturated $N^6$-isoprenoid side chains were tested for their ability to promote the growth of callus tissues derived from *P. vulgaris* cv. Great Northern, *P. lunatus* cv. Kingston, and their interspecific hybrid. The results are shown in Figures 1 and 2. (The activities of the saturated and unsaturated cytokinin bases in cv. Great Northern and cv. Kingston have been reported previously [12], but are included in Figs. 1 and 2 for comparison with the hybrid.) In Great Northern

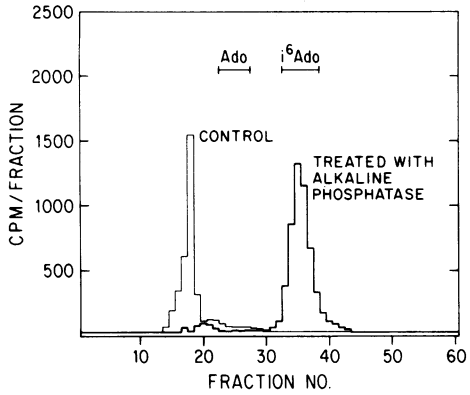


FIG. 5. Effect of alkaline phosphatase treatment on the major radioactive metabolite produced by *P. lunatus* cv. Kingston tissues during a 1-h incubation with $^{14}$C-i$^6$Ado. Aliquots of the sample (fractions 17 and 18, Fig. 3D) were incubated in Tris-HCl buffer with and without (control) alkaline phosphatase and then rechromatographed on Sephadex LH-20 in 33% (v/v) ethanol as described under "Materials and Methods."

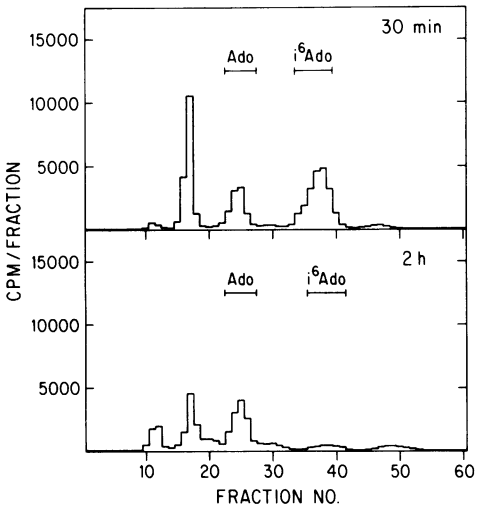


FIG. 6. Sephadex LH-20 chromatography of extracts prepared from callus tissues of *P. vulgaris* cv. Great Northern × *P. lunatus* cv. Kingston after incubation with $^{14}$C-i$^6$Ado. Extracts were chromatographed in 33% (v/v) ethanol as described under "Materials and Methods."

tissues, cytokinins with saturated side chains (hi$^6$Ade, hi$^6$Ado, hio$^6$Ade, hio$^6$Ado) were always more active than the corresponding compounds with unsaturated side chains (i$^6$Ade, i$^6$Ado, io$^6$Ade, io$^6$Ado). The effect of the side chain was pronounced except in the case of io$^6$Ado, which was only slightly less active than hio$^6$Ado. In Kingston tissues, on the other hand, the unsaturated cytokinins were approximately 10 times more active than the corresponding saturated compounds except in the case of i$^6$Ade and hi$^6$Ade, which were approximately equally active. The difference in cytokinin structure-activity relationships in the two genotypes was largely due to the differences in their responses to cytokinins bearing unsaturated side chains. Cytokinins with saturated side chains exhibited roughly equivalent activity in both parental lines of callus tissue as well as in callus tissues of the Great Northern × Kingston hybrid. In the hybrid tissues, the response to cytokinins bearing unsaturated side chains was intermediate between those of the parental tissues.

In both Great Northern and Kingston tissues, the free base form of cytokinins bearing saturated side chains was always more active than the corresponding ribonucleoside form. However, the relative activity of the free base and ribonucleoside forms of cytokinins bearing unsaturated side chains was more variable. Thus, io$^6$Ade was more active than io$^6$Ado in Kingston callus tissues but less active than io$^6$Ado in Great Northern. In the case of i$^6$Ade and i$^6$Ado, the ribonucleoside form was actually more active than the free base in Kingston, and the two compounds were approximately equally active in Great Northern callus tissues.

**Metabolism of $^{14}$C-i$^6$Ado in *P. lunatus* cv. Kingston and *P. vulgaris* cv. Great Northern Callus Tissues.** Ethanol extracts prepared from Great Northern and Kingston callus tissues after incubation with $^{14}$C-i$^6$Ado were fractionated by chromatography on Sephadex LH-20 columns. Elution profiles are shown in Figure 3. In Great Northern callus tissues, $^{14}$C-i$^6$Ado was relatively rapidly metabolized, and after 2 h, only a trace of the original $^{14}$C-i$^6$Ado could be detected. The rapid metabolism of $^{14}$C-i$^6$Ado in Great Northern was associated with the appearance of a major peak of radioactivity that was evident at all incubation times and eluted at the position of the Ado standard (fractions 22–25). (As noted previously, Ado and io$^6$Ado are not resolved under these conditions of chromatography.) In Kingston tissues, $^{14}$C-i$^6$Ado was metabolized at a somewhat slower rate than in Great Northern, and the major radioactive metabolite eluted from the Sephadex LH-20 column at an earlier position (fractions 17 and 18) than Ado.

The results presented in Figure 3 were obtained using the ethanol extraction procedure, but essentially identical profiles were obtained with the $HClO_4$ extraction procedure. The latter procedure would be expected to minimize possible phosphatase activity (6). Thus, the difference in the elution profiles of the metabolites from the two lines of callus tissue does not appear to be due to differences in phosphatase activity in the extracts.

**Identity of Major $^{14}$C-i$^6$Ado Metabolites.** The identity of the major radioactive metabolite of $^{14}$C-i$^6$Ado present in extracts of Great Northern tissues was further examined using Porapak Q chromatography (3). (This chromatographic system resolves Ado and i$^6$Ado, which chromatograph together on Sephadex LH-20 in 33% ethanol.) The results of rechromatography on Porapak Q of the radioactive material recovered from Sephadex LH-20 fractions 22 to 25 (Fig. 3, 1 h incubation sample) are shown in Figure 4. The radioactivity chromatographed with Ado; no label could be detected at the elution position of io$^6$Ado. Similar results were obtained with the equivalent Great Northern samples from other incubation times. Thus, under the conditions employed here, the major metabolite of $^{14}$C-i$^6$Ado in Great Northern callus tissues appears to be $^{14}$C-Ado.

The major radioactive metabolite of $^{14}$C-i$^6$Ado present in extracts of Kingston callus tissues eluted prior to the Ado standard

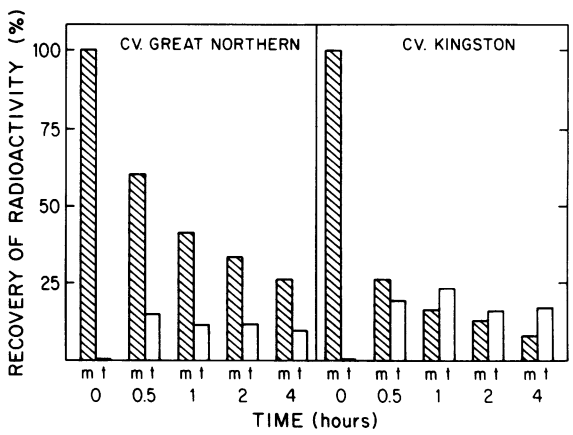


FIG. 7. Change in distribution of total radioactivity in media (*m*) and tissues (*t*) during a 4-h incubation of *P. vulgaris* cv. Great Northern and *P. lunatus* cv. Kingston callus tissues with $^{14}$C-i$^6$Ado in liquid cultures.

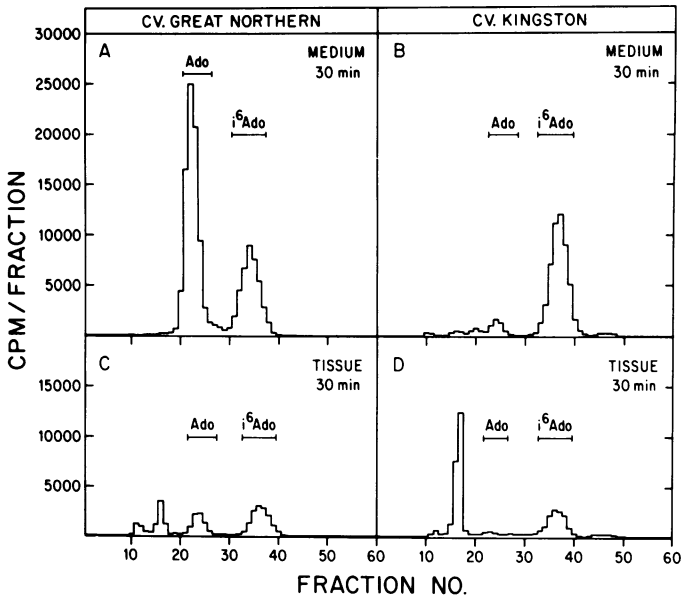


FIG. 8. Sephadex LH-20 chromatography of radioactive metabolites recovered from the media and from extracts of *P. vulgaris* cv. Great Northern (A, C) and *P. lunatus* cv. Kingston (B, D) after incubation with $^{14}$C-i$^6$Ado for 30 min. The samples were chromatographed in 33% (v/v) ethanol as described under "Materials and Methods."

when chromatographed on Sephadex LH-20 in 33% ethanol. The early elution position of this metabolite suggested that it might be a nucleotide. The appropriate Sephadex LH-20 fractions (fractions 17 to 18, Fig. 3) from the various incubation times were incubated with alkaline phosphatase and rechromatographed on Sephadex LH-20. The results of phosphatase treatment and rechromatography of a 1 h incubation sample are shown in Figure 5. A large peak of radioactivity (95% of total) eluted at the same position as the i$^6$Ado standard. Thus, $^{14}$C-i$^6$Ado appears to be rapidly phosphorylated to its nucleotide in Kingston callus tissues.

**Metabolism of $^{14}$C-i$^6$Ado in Hybrid Callus Tissues.** Callus tissues of the hybrid Great Northern × Kingston were incubated with $^{14}$C-i$^6$Ado in the same manner as the parental tissues. The Sephadex LH-20 elution profiles of ethanol extracts of the hybrid tissues are shown in Figure 6. The rate of disappearance of $^{14}$C-i$^6$Ado from the tissues was intermediate between the rates observed with the parental tissues. The major radioactive metabolite was the nucleotide of $^{14}$C-i$^6$Ado (identified by phosphatase treatment and rechromatography) and a smaller portion of radioactivity was associated with fractions corresponding to the elution position of Ado (confirmed as Ado by rechromatography on Porapak Q). The distribution of label between the two peaks was intermediate between that observed with the parental lines but more closely resembled the Kingston pattern.

**Uptake and Metabolism of $^{14}$C-i$^6$Ado from Liquid Medium.** To test further for possible differences in the cytokinin metabolism of Great Northern and Kingston cultures, callus tissues of the two genotypes were suspended in liquid medium containing $^{14}$C-i$^6$Ado. Changes in the distribution and composition of the label were followed over a 4-h time period. The radioactivity in the medium in contact with Kingston callus tissue dropped precipitously within the first 30 min incubation (Fig. 7). In the case of Great Northern callus tissue, the radioactivity in the medium decreased more slowly. However, Figure 8 demonstrates that after 30 min incubation, most of the label in the Great Northern medium had been converted to $^{14}$C-Ado. Thus, the slower rate of disappearance of counts from the Great Northern medium is somewhat misleading and appears to reflect differences in the metabolism of the label by the two tissues rather than differences in rate of uptake.

A comparison of the labeled metabolites recovered from the two tissues after 30 min incubations is shown in Figure 8. As in the case of labeling studies on semi-solid medium, most of the counts recovered from Kingston callus tissue were in the form of i$^6$Ado and a compound with chromatographic properties resembling the corresponding nucleotide. The predominant metabolite of i$^6$Ado recovered from the Great Northern callus tissues was Ado. The total radioactivity recovered from the incubation systems (medium + tissue) decreased with time, presumably as the result of conversion of the label to ethanol-insoluble products in the tissues.

## DISCUSSION

The differences in response of callus tissues of *P. vulgaris* cv. Great Northern and *P. lunatus* cv. Kingston to cytokinins bearing saturated and unsaturated $N^6$-isoprenoid side chains are evident using either the free base or ribonucleoside forms of these cytokinins. Thus, io$^6$Ado and i$^6$Ado were more active than the corresponding saturated nucleosides (hio$^6$Ado and hi$^6$Ado) in promoting the growth of Kingston callus tissues, but they were less active than hio$^6$Ado and hi$^6$Ado in promoting the growth of Great Northern tissues. Similar structure-activity relationships were observed for the free base forms of these cytokinins as reported in our earlier study (12) of Great Northern and Kingston callus tissues. With a given $N^6$-side chain, the free base was generally more active than the corresponding ribonucleoside in promoting the growth of the *Phaseolus* callus tissues. However, this did not apply to all combinations of genotype and side chain structure. Thus, io$^6$Ado was more active than io$^6$Ade in Great Northern and i$^6$Ado was more active than i$^6$Ade in Kingston. Preliminary results with other *P. lunatus* genotypes indicate that in general the activity of i$^6$Ade is low in this species. We do not as yet have an explanation for this phenomenon.

We suggested previously (12) that the observed differences in cytokinin structure-activity relationships in Kingston and Great Northern callus tissues might be related to differences in cytokinin destruction in the two tissues. This suggestion was based on the observation of Whitty and Hall (21) that cytokinins with saturated side chains were relatively resistant to attack by a cytokinin oxidase from maize that readily cleaved the unsaturated side chains of i$^6$Ade and io$^6$Ade (and their ribonucleosides). Thus, the low activity of i$^6$Ade and io$^6$Ade in promoting the growth of Great Northern callus tissues could be explained if these cytokinins were rapidly converted to inactive metabolites in *P. vulgaris* callus tissues. The patterns of $^{14}$C-i$^6$Ado metabolism observed in the present study provide some support for this interpretation. In *P. vulgaris* cv. Great Northern tissues, $^{14}$C-i$^6$Ado was rapidly degraded to Ado, which constituted the predominant metabolite of i$^6$Ado in this tissue. In *P. lunatus* cv. Kingston tissues, the major product of $^{14}$C-i$^6$Ado metabolism was the corresponding cytokinin nucleotide, and only a small peak of $^{14}$C-Ado was detected. Both direct application of $^{14}$C-i$^6$Ado to callus tissues growing on semi-solid medium and incubation of the tissues in liquid medium containing $^{14}$C-i$^6$Ado gave essentially the same pattern of metabolites in the two tissues. We have not examined the metabolism of hi$^6$Ado (the cytokinin with the corresponding saturated side chain) in the two types of tissues. However, $N^6$-benzyladenine, which is approximately equally active in the two callus tissues (12), is not degraded to Ade to any significant extent in either tissue (unpublished).

The ability to induce callus formation from immature *Phaseolus* embryos has permitted us to examine the correlation between cytokinin structure-activity relationships and the metabolism of $^{14}$C-i$^6$Ado in tissues of the hybrid Great Northern × Kingston. The response of the hybrid callus tissues were intermediate be-

tween the two parental tissues, and the pattern of $^{14}$C-i$^6$Ado metabolism in this tissue exhibited characteristics of both parents.

Dihydrozeatin and its derivatives have been identified as naturally occurring cytokinins in *P. vulgaris* (5, 19, 20), and exogenously supplied io$^6$Ade has been shown to be converted to hio$^6$Ade by the axes of *P. vulgaris* embryos (18) and by leaf tissue (16). Because of the rapid degradation of $^{14}$C-i$^6$Ado in *P. vulgaris* callus tissues, it is possible that reduction of the $N^6$-side chain is necessary to protect against excessive cytokinin destruction during the normal growth and development of this species. The cytokinins produced by *P. lunatus* have not been examined, and it will be of interest to determine whether *P. vulgaris* and *P. lunatus* differ in their ability to reduce the side chains of unsaturated cytokinins. On the basis of the results obtained here, the two species appear to differ in major aspects of cytokinin metabolism, and further comparative studies of cytokinin metabolism in the two genotypes may be useful in elucidating the function of some of the various classes of cytokinin metabolites produced by the plant tissues.

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