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# Modification of Cytokinins by Cauliflower Microsomal Enzymes[1]

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## ABSTRACT

**$N^6$-($\Delta^2$-isopentenyl)Adenine and $N^6$-($\Delta^2$-isopentenyl)adenosine were hydroxylated, respectively, to 6-(4-hydroxy-3-methyl-*trans*-2-butenylamino)purine and 6-(4-hydroxy-3-methyl-*trans*-2-butenylamino)-9-α-ribofuranosylpurine in the presence of NADPH and the microsomal fraction from cauliflowers (*Brassica oleracea* L.). The hydroxylating reaction was completely inhibited by 10 millimolars metyrapone and partially inactivated by 10-minute treatment of the microsomal preparation with ethylene. The cytokinins were also dealkylated by the microsomal enzymes and formed adenine from cytokinin base and adenosine from cytokinin nucleoside. These results suggest that plant cytochrome P-450 is involved in the conversion of one type of cytokinin to another, and in the modification of cytokinin molecules.**

Cytokinins, such as i$^6$Ade,[2] io$^6$Ade, and closely related derivatives, are a group of plant hormones which promote cell division and differentiation (13, 14, 28). The biosynthesis, interconversion, and degradation of cytokinins as well as the enzymic regulation of these processes have been studied (6, 16, 17). However, the modification of cytokinin molecules by microsomal enzymes is still unknown. In animal tissues, xenobiotics as well as endogenous substrates are oxidized by the microsomal mixed function oxidases (3, 12). The involvement of these enzymes, particularly Cyt P-450, in the oxidation of several endogenous and exogenous compounds in the tissues of higher plants has been demonstrated (*e.g.* 19). Also, Cyt P-450 has been shown to metabolize the plant hormones gibberellin (23).

Despite the potential significance of Cyt P-450 enzymes in regulating plant cell metabolism, little is known about the relationship between Cyt P-450 and cytokinin metabolism. This paper reports the modification of cytokinin molecules by cauliflower Cyt P-450 enzymes, particularly the hydroxylation of i$^6$Ado to io$^6$Ado and i$^6$Ade to io$^6$Ade. Ethylene, a plant hormone which regulates many growth responses (1), also destroys Cyt P-450 and lowers the rate of the hydroxylation of i$^6$Ade and i$^6$Ado.

## MATERIALS AND METHODS

**Materials.** Fresh cauliflowers (*Brassica oleracea* L.) were purchased from a local farm or market on the day of the experiment. Metyrapone, io$^6$Ade, i$^6$Ade, various nucleosides and bases were from Sigma Chemical Co.; *trans*-io$^6$Ade, *cis*-io$^6$Ade, and their corresponding ribonucleosides were from Calbiochem. The preparations of [8-$^{14}$C]i$^6$Ado (53 mCi/mmol) and [8-$^{14}$C]i$^6$Ade (53 mCi/mmol) were as previously described (7). Ethylene and CO were purchased from Union Carbide Corp. and used without further purification.

**Preparation of Microsomal Fraction.** All operations were performed at 4°C. Cauliflower tissues (30 g) were mixed with 1.2 volumes (w/v) of Buffer A (50 mM Tris·HCl, pH 7.2, 10 mM $MgCl_2$, 50 mM KCl, 400 mM sucrose, 15 mM 2-mercaptoethanol, 0.1% BSA, 0.2% sodium cholate, 0.1% phosphatidyl choline) and homogenized in a Waring Blendor for 45 s at low speed. The homogenate was filtered through four layers of cheesecloth and centrifuged at 10,000*g* for 20 min and pellet discarded. The resulting supernatant was centrifuged at 100,000*g* for 1 h, and the pellet was resuspended in Buffer A and dispersed gently with a 1-ml pipette. The suspension was then centrifuged at 10,000*g* for 20 min and this pellet was also discarded. Finally, the resultant supernatant was centrifuged at 100,000*g* for 1 h. This pellet suspended in Buffer A is referred to as the microsomal fraction. The microsomal preparation was used immediately or stored at 0 to 4°C under $N_2$ atmosphere and used the next day.

**Determination of Cyt P-450.** Spectrophotometric measurements of hemoproteins were performed at room temperature (about 22°C) in a Cary 14 spectrophotometer. Cyt P-450 concentrations were estimated by CO difference spectra using an absorption coefficient of 91 $cm^{-1}$ $mM^{-1}$ (24). Microsomal proteins were solubilized in 0.1% Triton X-100, and concentrations were determined according to the method of Bradford (4) using BSA as a standard. The concentrations of soluble microsomal protein quantified by Bradford's method generally agree with the concentrations measured by the method of Lowry *et al.* (18) if thiol reagent is not present in the buffer. However, the thiols interfered with the method of Lowry *et al.* (18). From six different preparations of microsomal proteins, it was estimated that Cyt P-450 content was 0.2 to 0.6% of the total microsomal proteins. The CO difference spectra of the prepared cauliflower Cyt P-450 were very similar to the published data of Rich and Bendall (26). Metyrapone [2-methyl-1,2-bis(3-pyridyl)-1-propanone]-induced difference spectra of reduced Cyt P-450 were measured as described by Mitani *et al.* (20). The spectroscopically observable change of Cyt P-450 was recorded with a Cary 14 spectrophotometer.

**Effect of CO and Ethylene on Enzyme Activity.** To determine whether the conversion of i$^6$Ado by the microsomal enzymes was affected by CO or ethylene, the enzymes were treated with the gas. Incubations were done in 10-ml vials. After addition of substrate, buffer, and Cyt enzyme preparation (total volume, 3 ml), the vials were first equilibrated with $N_2$, and then flushed with 100% CO, or ethylene gas, at flow rate of 20 ml/min for 10 min. The control experiment contained identical components except that 96% $N_2$, 4% $O_2$ gas was bubbled through the vials. The vials were then sealed with parafilm and incubated at 37°C for various periods of time, and the conversion of i$^6$Ado to

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[2] Abbreviations: i$^6$Ade, $N^6$-($\Delta^2$-isopentenyl)adenine; i$^6$Ado, $N^6$-($\Delta^2$-isopentenyl)adenosine; io$^6$Ade, 6-(4-hydroxy-3-methyl-2-butenylamino)-purine; io$^6$Ado, 6-(4-hydroxy-3-methyl-2-butenylamino)-9-α-ribofuranosylpurine; Ade, adenine; Ado, adenosine.

io$^6$Ado was determined.

**Assays of Cytokinin Metabolism.** The metabolism of radioactive cytokinins was assayed in 3 ml of Buffer A with 3 to 3.5 mg of microsomal protein, 1 mM NADPH, 4.5 mM glucose-6-P, 25 units glucose-6-P dehydrogenase and 42 nM [8-$^{14}$C]i$^6$Ado. The reaction was initiated by addition of the cytokinin to the reaction mixture at 37°C which appears to be an optimum temperature for the enzymic reaction. After various periods (15–210 min) of incubation at 37°C, the reaction was stopped by the addition of an equal volume of 95% ethanol. The mixture was centrifuged at 20,000*g* for 20 min and the resultant pellet was extracted with 5 volumes 50% ethanol, and then with 5 volumes of water saturated ethyl acetate. These extracts were combined with the 20,000*g* supernatant and reduced *in vacuo* to about 0.5 ml. Five $A_{269}$ units of unlabeled io$^6$Ado were added as a marker to the concentrated extracts and analyzed by Sephadex LH-20 column (2.5 × 32 cm) in 35% ethanol. Each radioactive peak eluted from the column was chromatographed on paper chromatography in solvent systems A to C. In the control experiment the microsomal preparation was boiled prior to incubation.

Paper chromatography (Whatman No. 1 MM) was carried out in a descending fashion in the following solvent system (v/v): (A) ethyl acetate:1-propanol:$H_2O$ (4:1:2); (B) 2-propanol:$H_2O$: concentrated $NH_4OH$ (7:2:1); (C) 1-propanol:concentrated $NH_4OH$:$H_2O$ (60:20:20).

**Analytical Methods.** Radioactivity was measured in a Tracor Analytic Mark III liquid scintillation system. For liquid samples, an aliquot of no more than 0.1 ml was added to 10 ml of Bray's solution (5). For paper chromatograms, 1 cm sections were placed in vials containing scintillation fluid (7).

HPLC was performed on a Waters Associates ALC-200 liquid chromatograph using a LiChrosorb (RP-18, 10 μm) column (250 mm × 4.6 mm), a Model 660 solvent programmer, and a Model 440 absorbance detector working at 254 nm. The mobile phase consisted of a gradient of (A) 4% aqueous acetic acid and (B) distilled 100% methanol. Cytokinins were eluted with a linear gradient of increasing methanol concentration (0 to 100%) at 2 ml/min over 20 min.

GLC analyses were conducted in a Hewlett-Packard 5750 dual-column gas chromatograph equipped with flame-ionization detectors. The techniques used for the preparation of trimethylsilyl derivatives were described previously (8).

## RESULTS

**Metabolism of $N^6$-($\Delta^2$-isopentenyl)adenosine by Cauliflower Microsomal Enzymes.** Purification of animal Cyt P-450 enzymes which catalyze hydroxylation of several compounds has been reported (15, 29), and the purified enzyme, which is often inactive, has to be reconstituted to form an active microsomal enzyme system. Furthermore, these authors have shown that it is necessary to induce an increase in the enzyme level in liver by treating animals with phenobarbital or ethanol to obtain adequate amounts of the enzyme. Since the primary goal of this investigation is to show that the plant microsomal enzyme system is capable of converting i$^6$Ado to io$^6$Ado or i$^6$Ade to io$^6$Ade, crude microsomal preparation was used as a microsomal enzyme source; dissociation of the membrane-bound mixed function oxidases resulted in inactivation of the enzyme system. Furthermore, the amount of Cyt P-450 protein/mg microsomal proteins in cauliflower is estimated to be about 35- to 50-fold less than that of rat liver.

Unlabeled i$^6$Ado or [8-$^{14}$C]i$^6$Ado was metabolized by NADPH-dependent microsomal enzymes and yielded at least three metabolites. Figure 1B shows the Sephadex LH-20 column elution profiles of metabolites formed from 2-h incubation of unlabeled i$^6$Ado or 8-$^{14}$C i$^6$Ado in the presence of native microsomal enzymes. The control experiment with boiled microsomal preparation is shown in Figure 1A. Each peak area (peak I to VII) was pooled and reduced to about 1 ml. About 10,000 dpm of the radioactive sample for each peak was mixed with an unlabeled marker and chromatographed on Whatman No. 1 paper in solvent systems A to C. The pooled unlabeled i$^6$Ado metabolites were also analyzed in the same manner. A comparison of the Sephadex LH-20 elution profiles of authentic samples and cytokinin metabolites, and paper chromatographic analysis of the metabolites in three solvent systems led to the preliminary identification of the metabolites. The $R_F$ values in solvent systems A, B, and C, respectively, were io$^6$Ado: 0.69, 0.77, and 0.87; i$^6$Ado: 0.83, 0.87, and 0.88; Ado: 0.19, 0.57, and 0.58. Tentatively, the peak IV compound was identified as io$^6$Ado; peak V

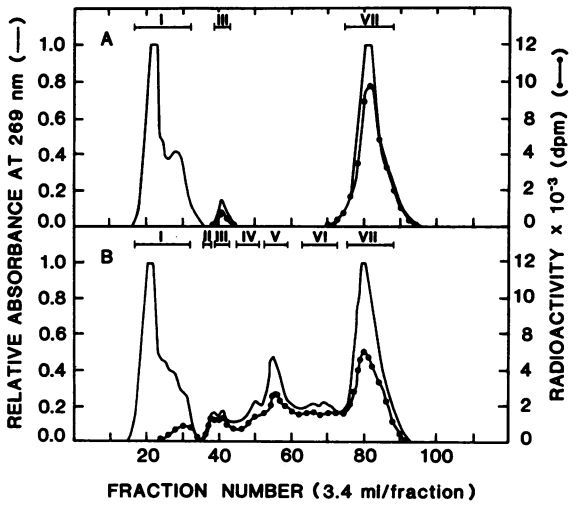


FIG. 1. Elution profile of [8-$^{14}$C]i$^6$Ado and unlabeled i$^6$Ado metabolites on Sephadex LH-20 columns. The $^{14}$C-labeled or unlabeled cytokinin was incubated with microsomal enzymes from cauliflowers and NADPH for 2 h at 37°C. The metabolites were extracted with ethanol and ethyl acetate-water, reduced to 0.5 ml, and applied onto a column (2.5 × 32 cm) previously equilibrated with 35% ethanol. The radioactive materials were eluted with the same ethanol solution. Panel A shows a control experiment with boiled microsomal preparations.

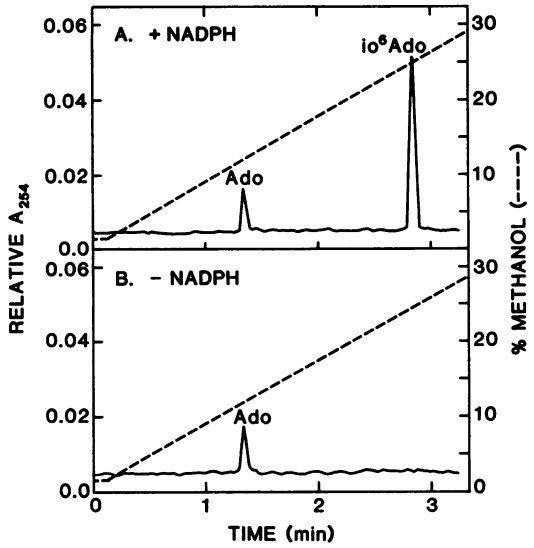


FIG. 2. HPLC of peak IV components from Sephadex LH-20 chromatography (Fig. 1). Column: LiChrosorb (RP-18, 10 μm; 25 cm × 4.6 mm). Gradient: generated from pump A 4% aqueous acetic acid and pump B 100% methanol with linear gradient of 0 to 100% methanol over 20 min. Flow rate: 2 ml/min.

as Ado; and peak VII, the substrate i$^6$Ado. Peak I contained endogenous compounds and NADPH which were also found in the control experiment and peak III is a chemical degradation product of the substrate i$^6$Ado. Peaks II and VI compounds were metabolites not identifiable with any known authentic marker used.

**Identification of Metabolites.** To further identify the reaction products, large quantities of unlabeled cytokinin metabolites were obtained by scaling up the above experiments. After Sephadex LH-20 column separation (elution profile was similar to Fig. 1, peaks I to VII), each major UV absorption peak was collected, reduced to a small volume, and further characterized by HPLC. Figure 2A shows the HPLC elution profile of peak IV compound (overlapping with peak V) obtained from the Sephadex LH-20 column. High pressure liquid chromatogram shows that the peak IV was further resolved into two peaks: the relative

Table 1. *Ultraviolet Absorption Spectra*

The ultraviolet absorption spectra for authentic compounds were determined in 50% ethanol. The peak II to VII metabolites as shown in Figure 1 were purified further by paper chromatography in solvent A system. Each major UV band on the paper was cut, eluted with 50% ethanol, and its absorption spectra determined.

| Compound | pH 1.5 | | pH 7.0 | | pH 12.0 | |
|---|---|---|---|---|---|---|
| | $\lambda_{max}$ | $\lambda_{min}$ | $\lambda_{max}$ | $\lambda_{min}$ | $\lambda_{max}$ | $\lambda_{min}$ |
| | | | *nm* | | | |
| Ado | 257 | 230 | 260 | 227 | 259 | 227 |
| i$^6$Ado | 265 | 232 | 269 | 234 | 269 | 234 |
| i$^6$Ade | 273 | 235 | 269 | 225 | 275 | 240 |
| io$^6$Ado | 265 | 233 | 269 | 232 | 269 | 235 |
| io$^6$Ade | 274 | 235 | 269 | 233 | 275 | 242 |
| Peak II | 275 | 242 | 278 | 243 | 278 | 249 |
| Peak III | 276 | 245 | 268, 278 | 248 | 279 | 249 |
| Peak IV | 265 | 233 | 269 | 233 | 270 | 235 |
| Peak V | 257 | 231 | 260 | 227 | 259 | 228 |
| Peak VII | 265 | 233 | 269 | 233 | 269 | 234 |

Table II. *GLC Retention Times of Trimethylsilyl Cytokinins, Bases and Nucleosides*

| Compound | Retention Time | Relative Retention Time[a] |
|---|---|---|
| | *min* | |
| Gua | 11.30 | 1.00 |
| Ade | 0.86 | 0.08 |
| Ado | | |
| Peak I | 5.45 | 0.48 |
| Peak II | 6.92 | 0.61 |
| i$^6$Ade | 2.96 | 0.26 |
| i$^6$Ado | 14.91 | 1.32 |
| *cis* io$^6$Ade | 3.88 | 0.34 |
| *trans* io$^6$Ade | 4.65 | 0.41 |
| *cis* io$^6$Ado | 26.43 | 2.34 |
| *trans* io$^6$Ado | 32.81 | 2.90 |
| Dihydro io$^6$Ade | 20.35 | 1.80 |
| Peak IV compound | 33.08 | 2.92 |
| Peak V compound | | |
| Peak I | 5.48 | 0.48 |
| Peak II | 6.98 | 0.62 |
| Peak VII compound | 14.88 | 1.32 |

[a] Relative retention time refers to the retention time with respect to guanosine (Gua). Chromatographic conditions: a 0.3 × 300 cm aluminum or glass column of 2% QF-1 on 80–150 mesh gas Chrom Q. Helium flow rate 100 ml/min. Temperature: column at 200°C, inlet at 300°C, and flame ionization detector at 300°C.

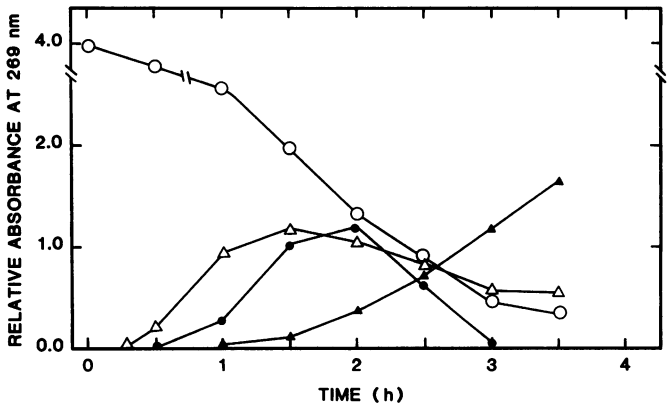


FIG. 3. Time courses of i$^6$Ado metabolites production catalyzed by NADPH-dependent cauliflower microsomal enzymes. The experimental conditions were identical to Fig. 1 except that at the indicated times the reactions were terminated. The purified metabolites were identified by their UV absorption spectra in 50% ethanol at pH 1.5, 7.0 and 12.0; i$^6$Ado (○——○); Ado (△——△); io$^6$Ado (●——●); unidentified compound ($\lambda_{max}$ at pH 1.5, 275; at pH 7.0, 278; at pH 12.0, 278) (▲——▲).

Table III. *Effect of Metyrapone, CO, Ethylene, and Heat Denaturation on io$^6$Ado Formation*

Complete system contained 3 ml Buffer A, 3.5 mg microsomal protein, 1 mM NADPH, 4.5 mM glucose-6-P, 25 units glucose-6-P dehydrogenase, and 42 nM [8-$^{14}$C]i$^6$Ado. The reaction mixture was flushed with 4% $O_2$, 96% $N_2$ for 10 min with the exceptions of ethylene and CO treatment, and incubated at 37°C for 2 h. The radioactive metabolites were extracted, mixed with 5 $A_{269}$ units of unlabeled io$^6$Ado, and separated by Sephadex LH-20 column (2.5 × 32 cm) in 35% ethanol. The radioactive io$^6$Ado peak area was further separated by paper chromatography in solvent system A. The values of the ranges are from 3 experiments. 100% represents 880 dpm.

| Treatment | io$^6$Ado Formed |
|---|---|
| | % |
| Complete system | 100 |
| Complete system + 1 mM metyrapone | 26–31 |
| Complete system + 10 mM metyrapone | 0 |
| Complete system flushed with 100% CO for 10 min | 0 |
| Complete system flushed with ethylene for 10 min | 57–72 |
| Microsomal enzymes heated at 100°C for 10 min | 0 |

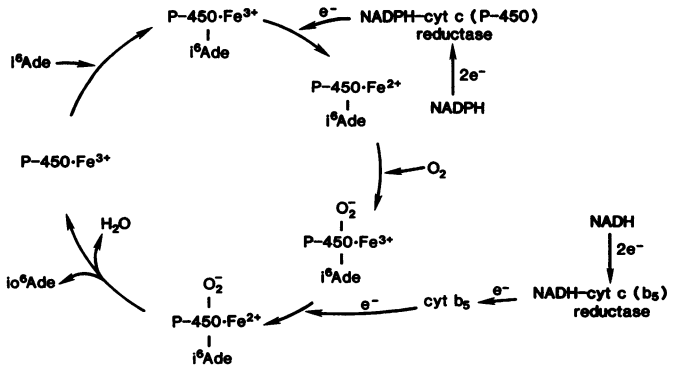


FIG. 4. Proposed mechanism of the hydroxylation of i$^6$Ade to io$^6$Ade catalyzed by NADPH-dependent cauliflower Cyt P-450. The same mechanism can be applied to i$^6$Ado.

mobility of the first peak (1.3 min) corresponds to Ado (peak V compound) and the second peak (2.8 min), io$^6$Ado. Each major UV absorption peak from Sephadex LH-20 column was also purified by paper chromatography in solvent system A. Major UV absorption bands on the paper were cut and eluted with 50% ethanol. The eluted compounds were characterized by UV absorption spectra in 50% ethanol at pH 1.5, 7, and 12 (Table I), and by GLC (Table II).

UV absorption spectra, GLC, HPLC, and paper chromatographic data indicate that peak IV contains mainly io$^6$Ado; peak V, Ado; and peak VII, the substrate i$^6$Ado. Compounds in the other peaks (I, II, III, and VI) were not identified.

As a final confirmation of the identity of the putative radioactive io$^6$Ado which was separated by Sephadex LH-20 column (peak IV) and purified by paper chromatography in solvent A, the radioactive sample was mixed with 3.0 $A_{269}$ units of unlabeled io$^6$Ado and the mixture was oxidized with 1 M $KMnO_4$ in a sealed tube at 100°C for 30 min. The reaction products were chromatographed on Whatman No. 1 MM paper in solvent B. There are three radioactive spots comigrating with three oxidation products of unlabeled io$^6$Ado ($R_F$ values: 0.14, 0.18, and 0.55) which can be detected by UV absorption. These results show that the radioactive metabolite of purified peak IV compounds is indeed io$^6$Ado.

In less extensive studies, unlabeled i$^6$Ade, the corresponding base of i$^6$Ado, was also metabolized by the microsomal enzymes. One of the i$^6$Ade metabolites was characterized to be io$^6$Ade by UV absorption spectra (Table I), GLC (Table II), HPLC (relative mobility: io$^6$Ade, 8.7 min; i$^6$Ade, 13.7 min), in addition to Sephadex LH-20 column mobilities.

Conversion of i$^6$Ado to io$^6$Ado or i$^6$Ade to io$^6$Ade requires NADPH. In the absence of NADPH, the elution profile of i$^6$Ado metabolites on Sephadex LH-20 column was similar to Figure 1, panel B, except that (a) peak IV was absent, (b) peak I area was reduced to about 20% of that of Figure 1, panel B, and (c) the production of io$^6$Ado was not detected after rechromatography of the peak IV area on HPLC (Fig. 2B), or paper on solvent A.

**Time Course Studies.** Time course studies of i$^6$Ado metabolism catalyzed by microsomal enzymes are shown in Figure 3. The conversion of i$^6$Ado to io$^6$Ado increases to a maximum at 2 h of incubation at 37°C, and then is followed by a slow decrease in the amount of io$^6$Ado production. No io$^6$Ado was detected after 3-h incubation. Apparently the product, io$^6$Ado, was metabolized further by the enzymes. The microsomal enzyme preparation also catalyzes the dealkylation of i$^6$Ado or io$^6$Ado to form Ado. After 2-h incubation, the yields of io$^6$Ado and Ado were about 1 and 25%, respectively.

Time course studies of [8-$^{14}$C]i$^6$Ade metabolism indicate that conversion of i$^6$Ade to io$^6$Ade reaches its peak after 1 h incubation, and decreases thereafter (results not shown). After 2.5-h incubation, no io$^6$Ade was detected in the metabolites.

**Effect of Metyrapone, Ethylene, and CO on $N^6$-($\Delta^2$-isopentenyl)adenosine Hydroxylation.** Metyrapone has been shown to be an inhibitor of Cyt P-450 catalyzed steroid hydroxylation reactions (27). Experiments were designed to examine whether this compound is also an inhibitor of i$^6$Ado hydroxylation. Inclusion of 1 or 10 mM of metyrapone in the standard cytokinin metabolism assay resulted in 69 to 74 or 100% inhibition of the conversion of i$^6$Ado to io$^6$Ado over a 2-h period of incubation (Table III). Since adenosine was still produced, the dealkylation reaction was not inhibited by the drug. It appears that intact microsomal membranes may be required for the conversion of i$^6$Ado to io$^6$Ado or i$^6$Ade to io$^6$Ade, because disruption of the microsomal membranes by longer homogenation (2 to 3 min) resulted in the disappearance of the conversion. These results along with the fact that NADPH is required for this conversion (Fig. 2), suggest that Cyt P-450 is involved in the hydroxylation of i$^6$Ado to io$^6$Ado or i$^6$Ade to io$^6$Ade.

Ethylene, the most elemental unsaturated hydrocarbon, is a plant hormone which regulates plant growth and senescence (1). This gas has been shown to destroy hepatic Cyt P-450, accompanied by the formation of abnormal ('green') hepatic porphyrins (25). Bubbling ethylene gas for 10 min through the incubation mixture containing cauliflower Cyt P-450 preparation and unlabeled i$^6$Ado resulted in a 28 to 43% reduction of the conversion of i$^6$Ado to io$^6$Ado after 2-h incubation (Table III). When the incubation mixture was flushed with 100% CO for 10 min at a flow rate of 20 ml/min, conversion of i$^6$Ado to io$^6$Ado was completely inhibited (Table III). These results again suggest that Cyt P-450 enzymes are involved in the hydroxylation of i$^6$Ado to io$^6$Ado.

## DISCUSSION

The results reported here show that cytokinins are modified by crude microsomal fraction. Our results suggest that cyt P-450 is involved in the hydroxylation of i$^6$Ade and i$^6$Ado to form io$^6$Ade and io$^6$Ado, respectively. This conclusion is based on the fact that the hydroxylation reaction requires NADPH, that the reaction is inhibited by CO, and that metyrapone, a potent inhibitor of some Cyt P-450 catalyzed hydroxylation (27), inhibits io$^6$Ade and io$^6$Ado formation. After 2 h incubation of [8-$^{14}$C]i$^6$Ado or unlabeled i$^6$Ado with the Cyt P-450 preparation, about 15 to 25% of Ado was formed from the substrate (Figs. 1 and 3). Thus, microsomal enzymes also appear to catalyze dealkylation reactions which may be catalyzed by another type of oxidase, such as cytokinin oxidase (30), this enzyme catalyzes the removal of the isopentenyl side chain of cytokinins. Hydroxylation and dealkylation of various compounds by microsomal enzymes in animal cells have been demonstrated (12).

It has been reported by Miura and Hall (21) and Miura and Miller (22) that radioactive i$^6$Ade and i$^6$Ado were converted to io$^6$Ade and io$^6$Ado, respectively, in *Rhizopogan roseolus* and corn kernels. However, the putative enzyme system that catalyzed the hydroxylation reaction was not isolated. The cytokinins i$^6$Ade, i$^6$Ado and their corresponding nucleosides have been shown to be synthesized in various organisms using [$^{14}$C]Ade as a precursor (9, 11). The hormones i$^6$Ade and i$^6$Ado have been shown to be derived from i$^6$Ado-5′-monophosphate (10), although an alternative biosynthetic pathway(s) is not excluded.

Cyt P-450, in catalyzing hydroxylation of substrates, can act either as a mixed function oxidase or as a peroxygenase. The former mechanism involves the transfer of electrons from NADPH to P-450 and thus reduction of the hemoprotein during the catalytic cycle. CO, which binds to ferrous heme, is a good inhibitor of mixed function oxidase activity (24). As a peroxygenase, P-450 uses organic hydroperoxides or peracids in place of NADPH and molecular oxygen to support a variety of oxidations (2). No reduction of P-450 occurs during the catalytic cycle and CO does not inhibit the peroxygenase activity. The hydroxylation of i$^6$Ado or i$^6$Ade to form io$^6$Ado or io$^6$Ade by the cauliflower Cyt P-450 requires NADPH, and is inhibited by CO; hence, the cytokinin conversion is catalyzed by a mixed function oxidase. Based on the known mechanism of action of animal Cyt P-450 (3), a possible mechanism of i$^6$Ade hydroxylation reaction catalyzed by the plant Cyt P-450 is shown in Figure 4. This same mechanism can also be applied to another cytokinin, i$^6$Ado. It is still unknown if cauliflower cytosol fraction contains different types of i$^6$Ade or i$^6$Ado hydroxylating enzyme(s). If such an enzyme(s) exists in the cytosol, it has escaped our preliminary detection.

Ethylene has been shown to destroy hepatic microsomal Cyt P-450 (25). Similarly, cauliflower microsomal Cyt P-450 was partially destroyed by ethylene, and the hydroxylation of i$^6$Ade

or $i^6$Ado decreased (Table III). Ethylene affects plant growth in many ways, the destruction of Cyt P-450 by ethylene may be one of the many effects of ethylene on plant growth responses.

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