J Plant Growth Regul (1989) 8:81–92

Journal of
Plant
Growth
Regulation

Influence of Calcium and Calcium and Calmodulin Antagonists on the Cytokinin-Induced Amaranthin Accumulation in *Amaranthus tricolor*

U. Vallon, J. A. Graf, and U. Kull

Biology Institute, University of Stuttgart, Ulmer Strasse 227, D-7000 Stuttgart 60, West Germany

Received March 9, 1988; accepted September 7, 1988

Abstract. The concentration of kinetin and kinetinriboside plays an essential role in the induction of amaranthin accumulation in cotyledons of *Amaranthus tricolor* during germination. The dose/effect ratio shows that kinetin induced 3- to 3.5-fold more amaranthin than kinetinriboside at the same molecular concentration. Various concentrations of exogenous Ca^{2+} did not influence the effects of kinetin on the betacyanin synthesis. However, when Ca^{2+} was applied together with kinetinriboside, the amaranthin production was stimulated. Time-course experiments show a lag phase of 16 h starting from the incubation with kinetin and a distinct increase of amaranthin thereafter. If the seedlings were treated simultaneously with kinetin and Ca^{2+}, the increase of amaranthin started after 12 h. At 16 h of incubation in kinetin/Ca^{2+}, the amount of amaranthin increased significantly compared to controls incubated with kinetin alone. If Ca^{2+} ions (16 h kinetin/Ca^{2+} incubation) were removed from the medium after 2 h, 4 h, and up to 14 h, the amaranthin content was enhanced compared to controls without Ca^{2+}. The stimulating effect was highest in the presence of Ca^{2+} for 8 h. These data show that exogenous Ca^{2+} stimulated the amaranthin synthesis mainly during the first 12 h of incubation. The Ca^{2+} antagonists EGTA, chlorotetracycline, and $CoCl_2$ reduced the amaranthin content up to 80%. The calmodulin antagonists chloropromazine and trifluoperazine inhibited the betacyanin accumulation up to 97% when applied at the beginning of the incubation. Neither Co^{2+} nor trifluoperazine after 12 h of preincubation in kinetin had inhibiting effects on the amaranthin production. Therefore, we presume that a specific period of competence is required for calmodulin-mediated Ca^{2+} effects on the accumulation of amaranthin induced by cytokinins in the seedlings of *Amaranthus tricolor*.

The accumulation of the betacyanin in cotyledons of *Amaranthus* seedlings is induced either by phytochrome (Bamberger and Mayer 1960, Piatelli et al. 1969) or by cytokinins (Elliott et al. 1972, Elliott and Murray 1975). The importance of Ca^{2+} ions in mediating many cellular and developmental processes in plants and in activating the Ca^{2+}-binding protein calmodulin is well known (Dieter and Marmé 1980, 1981, Hetherington and Trewavas 1984, Schäfer et al. 1985, Elliott and Skinner 1986). Elliott (1980, 1983) and Elliott et al. (1983) showed that the cytokinin-mediated amaranthin synthesis in *Amaranthus* is dependent on changes in intracellular calcium level and on the activation of calmodulin. In the present investigation, the influence of exogenous Ca^{2+} on the cytokinin-induced betacyanin synthesis in seedlings of *Amaranthus tricolor* was examined by varying the Ca^{2+} concentrations and the time of incubation with kinetin and Ca^{2+}. Calmodulin and Ca^{2+} antagonists were used to support the hypothesis of Elliott (1980) that endogenous Ca^{2+} and calmodulin are involved in the amaranthin synthesis. Furthermore, this study should reveal whether there is a specific period of competence for the Ca^{2+} and calmodulin effects on the regulation of the amaranthin biosynthesis. A preliminary report of some of the results was published earlier (Vallon et al. 1987).

Materials and Methods

The *Amaranthus* bioassay according to Elliot (1979) was used. Seeds of *Amaranthus tricolor* (from the Botanical Garden of the University of Stuttgart) were germinated in protoplast culture boxes at 25°C for 96 h in the dark on wet cellulose paper. After germination the cotyledons and the top 5 mm of the hypocotyls (half-seedlings) were transferred under green light conditions (Elliott and Murray 1975) to filter paper disks (7 mm) in protoplast culture boxes containing 10 ml of the incubation medium [10 mM Na_2HPO_4-KH_2PO_4 (pH 6.8) containing 5 mM L-tyrosin (Serva Feinbiochemica, Heidelberg, FRG)]. The seed coats were removed from the cotyledons. Experiments were accomplished in the presence of variable concentrations of either kinetin (Merck, Darmstadt, FRG) or kinetinriboside (Sigma). In some experiments the Ca^{2+} antagonists EGTA (ethyleneglycol-bis-(β-aminoethylether)N,N′-tetraacetic acid) (Merck), chlorotetracycline (Merck), and $CoCl_2$ or the calmodulin antagonists chloropromazine (Merck) and trifluoperazine (Sigma) were applied simultaneously to the phytohormone. After 24 h of incubation (if not indicated otherwise), the half-seedlings were homogenized at 4°C. For extraction of amaranthin 3 ml of 3.33 mM acetic acid was added. The cell debris was removed by centrifugation at 12,000 rpm for 55 min (WKF centrifuge). Amaranthin was determined by difference spectroscopy ($A_{537nm}-A_{620nm}$) using a Zeiss spectral photometer. The molecular extinction coefficient (ϵ) of 5.66×10^4 was used (Piatelli et al. 1969). Each point of the curves was measured in nine separate assays. The values shown in the diagrams are means of these nine experiments ± standard deviation.

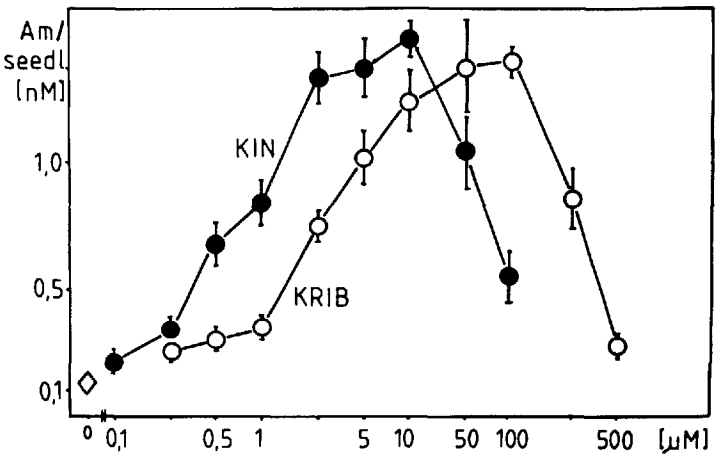

Fig. 1. Effects of kinetin (KIN, ●) and kinetinriboside (KRIB, ○) on the amaranthin (Am) accumulation. Control: incubation without application of cytokinins (◇).

Results

Application of Kinetin and Kinetinriboside

The cytokinin concentrations were chosen according to Conrad (1971) and Piatelli et al. (1971). Kinetin was applied to the seedlings at concentrations ranging from 0.1 to 100 μM and kinetinriboside from 0.25 to 500 μM. The dose-response curves of the two phytohormones (Fig. 1) show the course of optimum curves. When the concentration of the cytokinin exceeds its optimum, the synthesis of amaranthin is inhibited. With kinetin the amaranthin synthesis is 3- to 3.5-fold more intense than with kinetinriboside at the same molar concentration. Therefore, to obtain the same amount of the betacyanin, the concentration of kinetinriboside should be 5–10 times higher than that of kinetin. The most effective concentration in enhancing amaranthin production was found to be 10 μM kinetin. Amaranthin concentrations induced by 0.1 μM kinetin are comparable to those found in control incubations in the absence of cytokinin. The optimal kinetinriboside concentration inducing the highest increase in the amount of amaranthin was found to be 100 μM. On the other hand, 0.25 μM kinetinriboside induced an amaranthin production similar to that of control incubations without any cytokinin. The amaranthin synthesis was inhibited by kinetinriboside concentrations higher than 100 μM. In the following experiments, 0.5 μM kinetin was used (resulting in a half-maximal accumulation of amaranthin), because a physiological concentration of the phytohormone should exist in order to avoid the inhibiting effects caused by nonphysiological cytokinin concentrations. Kinetinriboside was applied at the same molar concentration (0.5 μM) as kinetin for purposes of comparison.

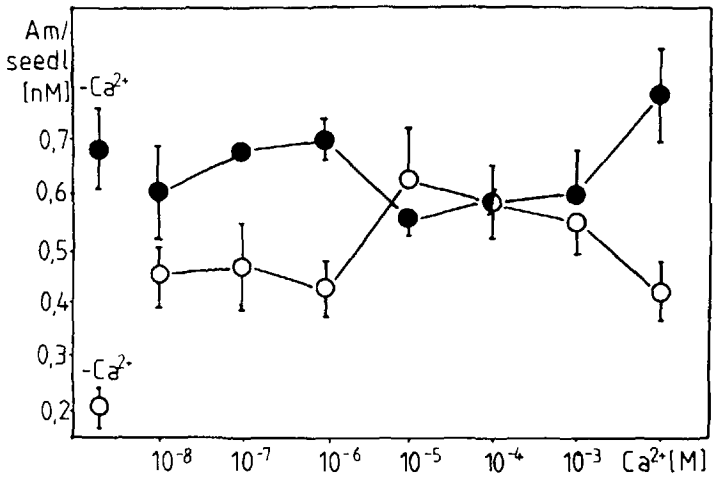

Fig. 2. Effects of Ca^{2+}, applied simultaneously with either kinetin (●) or kinetinriboside (○), on the amaranthin accumulation.

Influence of Ca^{2+} Concentration of the Cytokinin-Induced Amaranthin Synthesis

At first, seedlings were treated with incubation medium containing variable concentrations of Ca^{2+} between 10^{-5} M and 10^{-2} M without cytokinin application. Ca^{2+} stimulated the amaranthin production at concentrations of 10^{-4} M by 47%, 10^{-3} M by 55%, and 10^{-2} M by 34%, so these seedlings accumulated amounts of amaranthin in the range of kinetinriboside incubations (1 μM) and kinetin incubations (0.1–0.25 μM).

Seedlings were incubated in 0.5 μM kinetin or 0.5 μM kinetinriboside, and variable Ca^{2+} concentrations between 10^{-8} M and 10^{-2} M. Figure 2 shows that no significant differences in the amount of amaranthin were found in Ca^{2+}/kinetin incubations compared to seedlings incubated in kinetin only. Furthermore, Ca^{2+} of 10^{-2} M (a very high and nonphysiological concentration) induced the highest level of amaranthin production in all Ca^{2+}/kinetin incubations, whereas Ca^{2+} of 10^{-7} M and 10^{-6} M (physiological concentrations) caused the production of the same amount of amaranthin, similar to incubations with 0.5 μM kinetin alone.

When seedlings were treated with kinetinriboside solutions (0.5 μM) containing Ca^{2+} concentrations between 10^{-8} and 10^{-2} M, more amaranthin (50–68%) was found than in controls. The maximum amount of amaranthin was induced by Ca^{2+} at 10^{-5} M concentration together with 0.5 μM kinetinriboside. The amaranthin accumulation was lowest when seedlings were incubated in 10^{-6} M of Ca^{2+} or in 10^{-2} M of Ca^{2+} plus 0.5 μM kinetinriboside.

Nonphysiological Ca^{2+} concentrations (10^{-5} M or higher) stimulated the betacyanin accumulation more than the physiological concentrations. By comparing the two dose-response curves, one can see that the amount of amaranthin in Ca^{2+}/kinetin and Ca^{2+}/kinetinriboside incubations did not differ at

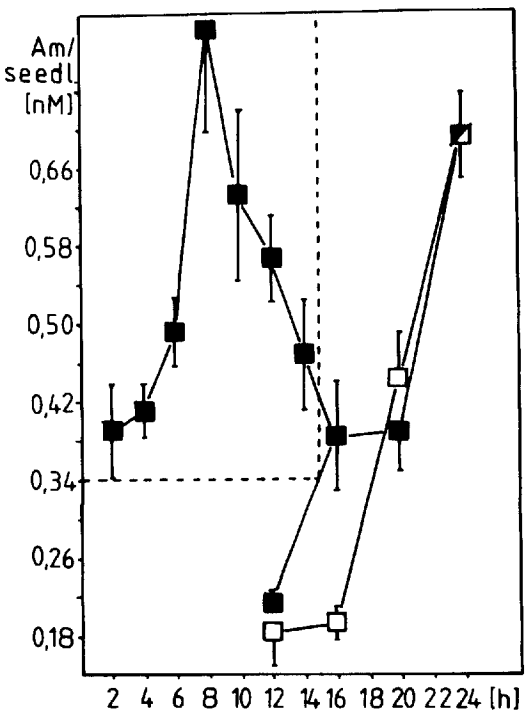

Fig. 3. Time course of the amaranthin accumulation. Seedlings were incubated either with kinetin plus 10^{-7} M Ca^{2+} (■) or with kinetin alone (□) for 12, 16, 20 or 24 h. Inset: Ca^{2+} was removed from the medium containing kinetin after 2, 4, and up to 14 h. Seedlings were homogenized after a total incubation time of 16 h.

Ca^{2+} concentrations from 10^{-5} M to 10^{-3} M. Our data show that exogenous Ca^{2+} alone and applied together with kinetinriboside stimulated the betacyanin accumulation in seedlings depending on its concentration, whereas Ca^{2+} had no stimulating effect at all on kinetin incubated seedlings.

Time Course Experiments

Piatelli et al. (1971) had found that the amaranthin accumulation shows a lag phase of about 10 h from the time of kinetin application. In experiments in which the dependence of amaranthin accumulation on the time course of cytokinin and Ca^{2+} application was tested, incubation periods of more than 10 h were used. Seedlings were incubated for 12, 16, 20, and 24 h in 0.5 μM kinetin. A parallel run was performed using seedlings incubated in 0.5 μM kinetin plus Ca^{2+} (10^{-7} M). At the end of the incubation periods (12, 16, 20, and 24 h), the seedlings were homogenized. The Ca^{2+} concentration of 10^{-7} M was chosen for the following experiment, since it certainly has no inhibiting effect on the amaranthin synthesis (see Fig. 2). After 12 h of incubation with 0.5 μM kinetin or 0.5 μM kinetin plus Ca^{2+}, the accumulated amount of amaranthin was found to be in the same range as that found in the controls without cytokinin (Fig. 3).

Kinetin incubations showed a lag phase of 16 h in the amaranthin production from the time of cytokinin application. This led to an increase in the amount of

the betacyanin. After 20 h of incubation, more amaranthin (56%) was isolated than in the 16-h incubation. Furthermore, a 40% additional increase in amaranthin at 24 h of incubation was observed in seedlings incubated in 0.5 μM kinetin compared to the 20-h incubation.

Results of treated seedlings (0.5 μM kinetin and 10^{-7} M Ca^{2+}) indicate a distinct increase in amaranthin after 12 h of incubation. These seedlings show a lag phase of only 12 h after the combined application of kinetin and Ca^{2+}. As shown in Fig. 3, the amount of amaranthin produced at 16 h of incubation is 45% higher than at 12 h. Hence, these seedlings accumulated 50% more amaranthin than those incubated in kinetin alone. Seedlings incubated in Ca^{2+} and kinetin for 20 and 24 h were found to produce amounts of betacyanin in the same range as those found in seedlings incubated in kinetin alone.

Removal of Ca^{2+}

In the following experiments the seedlings were incubated in 0.5 μM kinetin. Furthermore, Ca^{2+} of 10^{-7} M was added but removed from the medium (by washing) after 2, 4, up to 14 h. Then, the seedlings were transferred into a kinetin-containing medium without Ca^{2+}. Therefore, the exogenous Ca^{2+} is allowed to stimulate the amaranthin synthesis only for a limited time. The seedlings were homogenized after a total incubation period of 16 h. The results are charted in Fig. 3. The stimulation of amaranthin production occurred after 2 h of Ca^{2+} activity. The amaranthin concentration measured was in the range of that found in Ca^{2+}/kinetin incubations for 16 h and was 50% more than that found in kinetin incubations without Ca^{2+}. The longer Ca^{2+} stayed in the medium, the more the amount of amaranthin increased. After 6 h of Ca^{2+} effect, the amount of synthesized amaranthin was 20% greater than that after 4 h. The maximum amaranthin concentration was induced when Ca^{2+} was present in the medium for 8 h. These seedlings produced about 55% more amaranthin than Ca^{2+}/kinetin incubations after 16 h of incubation.

The stimulating effect of Ca^{2+} diminished when Ca^{2+} was present for more than 8 h. After 14 h of Ca^{2+} effect, the degree of amaranthin accumulation was not significantly different from that observed in the Ca^{2+}/kinetin incubations of 16 h. These series of experiments indicate that exogenous Ca^{2+} stimulated the amaranthin synthesis during the first 8–12 h of incubation. To show that Ca^{2+} is most stimulating during the first 8–12 h of incubation, removal experiments were carried out using the Ca^{2+} antagonist Co^{2+}.

Seedlings were incubated in 0.5 μM kinetin and 10^{-3} M Co^{2+}. Co^{2+} was removed (by washing) after 2, 4, 6, up to 14 h, and the seedlings were homogenized after a total incubation period of 24 h. After 2 h, Co^{2+} caused a reduction in the amount of amaranthin (−50%) as compared to controls. When Co^{2+} was present in the medium for 4 h, 25% less amaranthin was found. The longer Co^{2+} stayed in the medium, the more the amount of amaranthin diminished (6 h, −55%; 8 h, −62%). The same amount of amaranthin as after incubation in kinetin and Co^{2+} for 24 h was detected when Co^{2+} was present in the medium for 10–12 h.

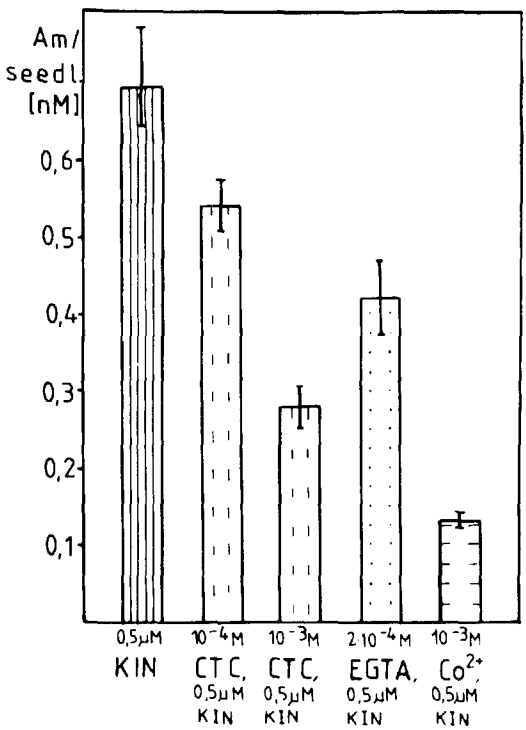

Fig. 4. Effects of the Ca^{2+} antagonists EGTA, CTC, and Co^{2+} on the kinetin-stimulated amaranthin synthesis.

Experiments with Antagonists

Ca^{2+} Antagonists. The calcium antagonists chlorotetracycline (CTC), EGTA, and $CoCl_2$ were used. Seedlings were incubated in 0.5 µM kinetin and 10^{-4} M CTC for 24 h (Fig. 4). They accumulated 25% less amaranthin than control incubations with 0.5 µM kinetin alone. When the concentration of CTC was increased to 10^{-3} M, the amaranthin production was reduced by up to 60%. EGTA (2×10^{-4} M) also inhibited the amaranthin synthesis by about 45%. When seedlings were incubated in 0.5 µM kinetin and 10^{-3} Co^{2+}, the amount of amaranthin was reduced by up to 80% compared to that of controls.

It was shown in the Ca^{2+} removal experiments that Ca^{2+} has its greatest effects on the amaranthin accumulation during the first 8–12 h of incubation. For this reason, we varied the time of addition of the antagonist Co^{2+}. The seedlings were first preincubated in 0.5 µM kinetin for 12 h. Then 10^{-3} Co^{2+} (according to Markmann-Mulisch and Bopp 1985) was added to the medium. These seedlings produced an amount of amaranthin in the range of that found in incubations with kinetin alone. Hence, Co^{2+} has no effect on the amaranthin synthesis after 12 h of preincubation in kinetin. The same results were found when Co^{2+} was added after 16 h.

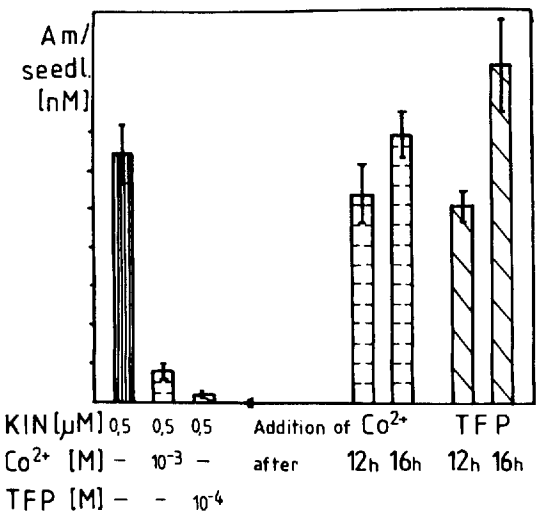

Fig. 5. Effects of Co^{2+} and TFP on the amaranthin accumulation of seedlings preincubated in kinetin for 12 or 16 h. Seedlings were homogenized after 24 h.

Calmodulin Antagonists. The calmodulin antagonists chloropromazine (CP) and trifluoperazine (TFP) were used at concentrations chosen according to Elliott (1980, 1983). A concentration of 10^{-6} M CP in the incubation medium had no influence on the amaranthin accumulation, but 10^{-5} M CP caused a reduction of the amaranthin synthesis by about 55%. The amaranthin production was totally inhibited (−96%) when seedlings were incubated in 0.5 μM kinetin and 10^{-4} M CP. Similarly, TFP of 10^{-6} M had no significant effect. Seedlings incubated in 10^{-5} M TFP plus kinetin exhibited a decrease in amaranthin production by 56%. On the other hand, 10^{-4} M TFP inhibited the amaranthin synthesis by 97%.

In the following experiments, seedlings were preincubated in 0.5 μM kinetin for 12 h (the same as in the Ca^{2+} antagonist experiments). Afterward, TFP (10^{-4} M) was added to the medium (Fig. 5). This treatment allowed the seedlings to accumulate 30% less amaranthin than in kinetin incubations. When TFP (10^{-4} M) was added to the medium after 16 h of preincubation in 0.5 μM kinetin, the amaranthin synthesis in the seedlings was found to be in the same range as in the incubations with kinetin alone. This proves that TFP no longer had an inhibitory effect when added after 16 h preincubation in kinetin. From these experiments, we may conclude that Ca^{2+} and calmodulin stimulate the amaranthin synthesis in the first 8–12 h of incubation.

Discussion

The concentration-dependent effects of the two cytokinins on amaranthin accumulation show the course of optimum curves. Kinetin started to affect the amaranthin synthesis at a concentration of 10^{-7} M, as had been found by Munsche et al. (1968) and Conrad (1971). If the applied concentration exceeds

the optimal concentration of the hormone, the amaranthin synthesis is reduced by 30–60%. Such inhibitory effects concerning the amaranthin synthesis have also been described by the authors mentioned above.

Inhibiting effects due to high cytokinin concentrations are perhaps mainly caused by an excessive stimulation of the metabolism (Simpkins and Street 1970, Kull 1972, Moore and Miller 1973). Our findings reveal that kinetinriboside started to stimulate the amaranthin synthesis at 10^{-6} M in *Amaranthus tricolor* and that at 10^{-4} there was a decline. Other results were obtained by Conrad (1971). He reported the kinetinriboside started to affect the amaranthin synthesis at 10^{-7} M.

Data from our experiments show that kinetin stimulated the amaranthin synthesis 3- to 3.5-fold more than kinetinriboside at the same molar concentration. This is comparable to findings of Conrad (1971). It is not yet known if cytokininribosides are active hormones themselves or if their activity is caused by deribosylation to the free base or by phosphorylation (Moore 1981, McGaw and Horgan 1985). The differences in activity between kinetin and kinetinriboside may be due to structural differences or differences in uptake and transport. Chong-maw-Chen (1981) and Letham and Palni (1983) suggest that different cytokinins could serve different functions: as active hormones, as transport forms, or/and as storage forms.

Exogenous Ca^{2+} did not stimulate the amaranthin synthesis when applied simultaneously with kinetin. In *Raphanus* seedlings, the Ca^{2+} uptake was strongly reduced when incubated in kinetin and Ca^{2+} (Bittner and Buschmann 1983). These authors concluded that kinetin itself is involved in regulating the Ca^{2+} uptake. Perhaps the same is true for the *Amaranthus* seedlings. On the other hand, the amaranthin synthesis of seedlings incubated in kinetinriboside was stimulated by the exogenous Ca^{2+}. Kubowicz et al. (1982) showed that Ca^{2+} uptake into membrane vesicles was inhibited when incubated in Ca^{2+} plus kinetin. This inhibition was reduced when zeatinriboside instead of kinetin was applied. If kinetinriboside as well has a less inhibitory effect on Ca^{2+} uptake than kinetin, then this would explain our results.

According to Piatelli et al. (1971), the amaranthin synthesis shows a lag phase of about 10 h starting from the time of kinetin application. We found that the lag phase lasted almost 16 h when seedlings were incubated in kinetin alone. Seedlings treated with kinetin and Ca^{2+} showed a lag phase of only about 12 h. We conclude that Ca^{2+} ions stimulate the amaranthin synthesis mainly during the first 12 h of incubation and have no influence after 16 h of incubation. If the seedlings were incubated in kinetin for 16 h and Ca^{2+} was removed from the medium in 2-h intervals, the amaranthin amount continuously increased and reached its maximum in the presence of Ca^{2+} for 8 h. Thereafter, the amaranthin production declined. This is comparable to the findings of Carbonell and Jones (1984), who investigated gibberellic acid effects.

The Co^{2+} removal experiments support the findings of the Ca^{2+} removal experiments and confirm the hypothesis that Ca^{2+} has its period of competence during the first 8–12 h of the incubation. Plant tissues contain different pools for Ca^{2+}, which are variously affected by the diverse Ca^{2+} antagonists. Chlorotetracycline for the first part chelates membrane-bound Ca^{2+} (Caswell

and Hutchinson 1971). The inhibition of the amaranthin synthesis by chlorotetracycline indicates that membrane-bound Ca^{2+} possibly has a function in the signal chain between the plant hormone and the betacyanin. The signal for the release of Ca^{2+} is probably connected to a breakdown of phosphatidylinositol and phosphatidylinositol-4,5-phosphate. The hydrolysis of these lipids results in a release of inositol-1,4,5-trisphosphate and diacylglycerol (Hesketh 1983). Elliott (1986) found that exogenous diacylglycerol stimulated the amaranthin synthesis in *Amaranthus tricolor*. This result indicates that inositol-1,4,5-trisphosphate, and probably diacylglycerol as well, plays an important role in the signal chain between the hormone and the betacyanin by acting as a stimulus for releasing membrane-bound Ca^{2+}.

EGTA predominantly chelates extracellular free Ca^{2+}. Many authors (Hetherington and Trewavas 1984, Mitsui et al. 1984, Sane et al. 1984, Köhle et al. 1985, Veluthambi and Poovaiah 1986) showed that different Ca^{2+}-dependent enzymes and processes in plants are inhibited by EGTA. Since the accumulation of amaranthin was reduced by EGTA in a distinct way, it may be dependent on extracellular Ca^{2+} as well.

Co^{2+} ions are bound to Ca^{2+} channels in animal cells (Lee and Tsien 1983, Reuter 1983). Our results reveal that Co^{2+} reduced the amaranthin accumulation drastically, which suggests that calcium carrier systems transporting extracellular Ca^{2+} into the cells also may exist in plant cells. This accentuates the presumption that physiological effects mediated through cytokinins may be dependent also on extracellular Ca^{2+}. The cytokinin-induced bud formation in the mosses *Funaria* (Saunders and Hepler 1982, Markmann-Mulisch and Bopp 1985, Saunders 1986) and *Pylaisiella selwynii* (Spiess et al. 1985) was found to be inhibited by the Ca^{2+} antagonists La^{3+} and Co^{2+} (without EGTA treatment) and the Ca^{2+} channel blocker verapamil. These results support the hypothesis that changes in intracellular Ca^{2+} mediate cytokinin induced processes. These changes seem to be a result of cytokinin activation of ion carrier systems (Saunders 1986).

The loss of the inhibitory effect of Co^{2+} on the amaranthin synthesis upon its addition after 12 or 16 h of preincubation supports the suggestion that Ca^{2+} stimulates the amaranthin synthesis only in the first 8–12 h of incubation. Therefore, this time interval is regarded as a period of competence for the Ca^{2+} effect.

Elliott (1979, 1980, 1983), using benzyladenine instead of kinetin, showed that the amaranthin synthesis is dependent on activated (Ca-binding) calmodulin when the calmodulin antagonists CP and TFP (5×10^{-3} M to 5×10^{-5} M) are used. Our results confirm and broaden their data. When TFP was added to the medium after 12 h of preincubation in kinetin, only a small effect could be seen. After 16 h, no effect was exhibited at all. Therefore, we conclude that since the amaranthin synthesis could be inhibited totally by the simultaneous incubation with TFP and kinetin, the calmodulin activation must be occurring during the first 10 h of incubation. It is possible that TFP still occupies calmodulin molecules after 12 h of preincubation in kinetin. This would explain why the amaranthin synthesis continued to be reduced by about 30%. From these results we presume that a specific period of competence plays an essential role

in calmodulin-mediated effects on the accumulation of amaranthin induced by cytokinins in the seedlings of *Amaranthus tricolor*.

Acknowledgments. Thanks are due Mrs. B. Schreiter for correction of the English text.

References

Bamberger E, Mayer AM (1960) Effect of kinetin on formation of red pigment in seedlings of *Amaranthus retroflexus*. Science 131:1094–1095

Bittner A, Buschmann C (1983) Uptake and translocation of K^+, Ca^{2+} and Mg^{2+} by seedlings of *Raphanus sativus* L. treated with kinetin. Z Pflanzenphysiol 109:181–189

Carbonell J, Jones RL (1984) A comparison of the effects of Ca^{2+} and gibberellic acid on enzyme synthesis and secretion in barley aleurone. Physiol Plant 63:345–350

Caswell AH, Hutchinson JD (1971) Visualization of membrane bound cations by a fluorescent technique. Biochem Biophys Res Commun 42:43–49

Chong-maw-Chen (1981) Biosynthesis and enzymic regulation of the interconversion of cytokinins. In: Guérn J, Péaud-Lenoel C (eds) Metabolism and molecular action in plants 1981. Springer-Verlag, New York, pp 34–43

Conrad K (1971) Zur Spezifität des *Amaranthus* -cytokinin-tests. V. Adenin- und Adenosinderivate. Biochem Physiol Pflanzen 162:327–333

Dieter P, Marmé D (1980) Partial purification of plant NAD kinase by calmodulin-sepharose affinity chromatography. Cell Calcium 1:279–286

Dieter P, Marmé D (1981) A calmodulin-dependent microsomal ATPase from corn (*Zea mays* L.). FEBS Lett 125:245–248

Elliott DC (1979) Analysis of variability in the *Amaranthus* bioassay for cytokinins. Effects of water stress on benzyladenine- and fusicoccin-dependent responses. Plant Physiol 63:269–273

Elliott DC (1980) Calmodulin inhibitor prevents plant hormone response. Biochem Int 1:290–294

Elliott DC (1983) Inhibition of cytokinin-regulated responses by calmodulin-binding compounds. Plant Physiol 72:215–218

Elliott DC (1986) Calcium involvement in plant hormone action. NATO ASI Ser A 104:285–292

Elliott DC, Murray AW (1975) Evidence against involvement of cyclic nucleotides in the induction of betacyanin synthesis by cytokinins. Biochem J 146:333–337

Elliott DC, Skinner JD (1986) Calcium-dependent, phospholipid-activated protein kinase in plants. Phytochemistry 25:39–44

Elliott DC, Murray AW, Saccone GT, Atkinson MR (1972) Use of structural analogues in the study of cytokinin action. In: Carr DJ (ed) Plant growth substances 1970. Springer-Verlag, New York, pp 459–466

Elliott DC, Batchelor SM, Cassar RA, Marinos NG (1983) Calmodulin-binding drugs affect responses to cytokinin, auxin, and gibberellic acid. Plant Physiol 72:219–224

Hesketh R (1983) Inositol triphosphate: Link or lability? Nature 306:16–17

Hetherington AM, Trewavas A (1984) Activation of pea membrane protein kinase by calcium ions. Planta 161:409–417

Köhle H, Jeblick W, Poten F, Blaschek W, Klauss H (1985) Chitosan-elicited callose synthesis in soybean cells as a calcium-dependent process. Plant Physiol 77:544–551

Kubowicz BD, Vanderhoff LN, Hanson JB (1982) ATP-dependent calcium transport in plasmalemma preparations from soybean hypocotyls. Plant Physiol 69:187–191

Kull U (1972) Wirkungen von Wuchsstoffen auf Speicherung und Stoffwechsel in vegetativen Pflanzenteilen. Bot Studien 19:1–163

Laloue M, Pethe-Terrine C, Guérn J (1981) Uptake and metabolism of cytokinins in tobacco cells: Studies on relations to the expression of their biological activities. In: Péaud-Lenoël C, Guérn J (eds) Metabolism and molecular activities in plants 1981. Springer-Verlag, New York, pp 218–227

Lee KS, Tsien RW (1983) Mechanism of calcium channel blockade by verapamil, D 600, diltiazem, and nitrendipine in single dialysed heart cells. Nature 302:790–794

Letham DS, Palni LMS (1983) The biosynthesis and metabolism of cytokinins. Annu Rev Plant Physiol 34:163–197

Markmann-Mulisch U, Bopp M (1985) Role of Ca^{2+} in cytokinin-dependent bud formation of *Funaria hygrometrica* Hedw. In: Bopp M, Knoop B, Rademacher W (eds) Abstracts of the 12th International Conference on Plant Growth Substances, 1985, Heidelberg, FRG, p 58

McGaw BA, Horgan R (1985) Cytokinin metabolism and the control of cytokinin activity. Biol Plant (Praha) 27:180–187

Mitsui T, Christeller JT, Hara-Nishimura I, Akazawa T (1984) Possible roles of calcium and calmodulin in the biosynthesis and secretion of α-amylase in rice scutellar epithelium. Plant Physiol 75:21–25

Moore TC (1981) Cytokinins. In: Biochemistry and physiology of plant hormones. Springer-Verlag, New York, pp 147–178

Moore TS Jr, Miller CO (1973) Effects of cytokinins on the respiration of soybean callus tissues. Plant Physiol 52:50–53

Munsche D, Engelbrecht L, Göhler K-D, Conrad K (1968) Beitrag zur Frage der Beziehungen zwischen Struktur und Cytokininwirksamkeit. Flora 159:268–278

Piatelli M, Guidici de Nicola M, Castrogiovanni V (1969) Photocontrol of amaranthin synthesis in *Amaranthus tricolor*. Phytochemistry 8:731–736

Piatelli M, Giudici de Nicola M, Castrogiovanni V (1971) The effect of kinetin on the amaranthin synthesis in *Amaranthus tricolor* in darkness. Phytochemistry 10:289–293

Reuter H (1983) Calcium channel modulation by neurotransmitters, enzymes and drugs. Nature 301:569–574

Sane PV, Kochhar S, Kumar N, Kochhar VK (1984) Activation of plant aspartate kinase by calcium and calmodulin-like factor from plants. FEBS Lett 175:238–242

Saunders MJ (1986) Cytokinin activation and redistribution of plasmamembrane ion channels in *Funaria*. Planta 167:402–409

Saunders MJ, Hepler PK (1982) Calcium ionophor A 23187 stimulates cytokinin-like mitosis in *Funaria*. Science 217:943–945

Schäfer A, Bygrave F, Matzenauer S, Marmé D (1985) Identification of a calcium and phospholipid-dependent protein kinase in plant tissues. FEBS Lett 187:25–28

Simpkins F, Street HE (1970) Studies on the growth in culture. VII. Effect of kinetin on the carbohydrate and nitrogen metabolism of *Acer pseudoplatanus* L. cells grown in suspension culture. J Exp Bot 21:170–185

Spiess LD, Lippincott BB, Lippincott JA (1985) Calcium and bud development in *Pylaisiella selwynii*. In: Bopp M, Knoop B, Rademacher W (eds) Abstracts of the 12th International Conference on Plant Growth Substances, Heidelberg, FRG, p 57

Vallon U, Graf JA, Kull U (1987) Cytokinin-calcium interaction as detected by *Amaranthus* bioassay. Plant Physiol 84 (Suppl), No. 976

Veluthambi K, Poovaiah BW (1986) In vivo and in vitro protein phosphorylation in *Avena sativa* L. coleoptiles. Plant Physiol 81:836–841