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Effects of *trans*- and *cis*-Zeatin and Optical Isomers of Synthetic Cytokinins on Protein Kinase Activity *in Vitro*

O. N. Kulaeva,[1,*] J. Corse,[2] and S. Y. Selivankina[1]

[1]Timiryazev Institute of Plant Physiology, Russian Academy of Sciences, Botanicheskaya St. 35, Moscow, 127276 Russia; and
[2]Western Regional Center, USDA Agricultural Research Service, 800 Buchanan St., Albany, California 94710, USA

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Abstract. The effects of *cis*- and *trans*-zeatin on the activity of the protein kinase associated with barley leaf chromatin were studied. Substances tested were added directly into the incubation medium for enzyme activity estimation. Only *trans*-zeatin activated the chromatin-associated protein kinase. Maximum activation was detected at a *trans*-zeatin concentration of 10^{-9} M. *cis*-Zeatin had no activity in a range of concentrations from 10^{-10} to 10^{-5} M. Comparison of the *R*-(−)-N^6-1-(1-naphthyl)ethyl-1H-purine-6-amine (*R*-NEPA) and *S*-(+)-N^6-1-(1-naphthyl)ethyl-1H-purine-6-amine (*S*-NEPA) effects on the enzyme activity showed that only *S*-NEPA activated the protein kinase from barley leaf chromatin, whereas *R*-NEPA had no such effect. The data on the effect of other synthetic analogues of cytokinin on the protein kinase activity are also presented. The results are discussed in terms of the specificity and sensitivity of the in vitro response of the chromatin-associated protein kinase from barley leaves to cytokinins. The advantages and limitations of this in vitro assay to test cytokinin activity are also considered.

Cytokinins play an important role in the regulation of plant ontogenesis. They induce cell division, participate in the regulation of plant growth and morphogenesis, activate chloroplast differentiation, prevent leaf senescence, and are involved in the regulation of many other physiologic processes in plants (Kulaeva 1973, Miller 1961, Skoog and Miller 1957). The prevalent cytokinins in plant tissues are *trans*-zeatin (*E*-zeatin) and its derivatives (Letham and Palni 1983). Plants also contain *cis*-zeatin (*Z*-zeatin) and its derivatives, which can appear in the cells during tRNA degradation, since *cis*-zeatin riboside is the predominant cytokinin in plant tRNA (Edwards et al. 1981, Skoog and Armstrong 1970, Vreman et al. 1972, 1978). *cis*-Zeatin was shown to be at least 100-fold less active than *trans*-zeatin in stimulating tobacco callus growth (Schmitz et al. 1972, Skoog and Ghani 1981). Such dramatic differences in physiologic activities of *trans*- and *cis*-zeatin could be explained by the hypothesis proposed by Chen and co-workers (Chen et al. 1993, Korszun et al. 1989). Their modeling results, based on X-ray crystallography data, showed that in the *cis*-zeatin molecule, a hydrogen bond can be formed between the OH group of the side chain and N^1 of the adenine ring. Such bonding is conformationally impossible for *trans*-zeatin. Since analysis of structure-activity relationships demonstrated that an intact purine ring is necessary for cytokinin activity, the reactive N^1-position must be free (Kulaeva 1973, Kuraishi 1959, Matsubara 1990, Skoog et al. 1967). The existence of a hydrogen bond between the OH group and N^1 of the adenine ring in the *cis*-zeatin molecule should block its interaction with a cytokinin target in a plant cell and as a result would inhibit the hormonal activity of this substance.

It is not possible to verify this suggestion by means of bioassays because it has been shown that *cis*-zeatin can be converted to its *trans*-isomer in plant tissues in the light (Mok et al. 1992). More recently, the isomerase responsible for this conversion was isolated and partially purified from immature seeds of *Phaseolus vulgaris* L. (Bassil et al. 1993). It is therefore very important to compare the functional activities of *trans*- and *cis*-zeatin in a model system, preferably in vitro, containing one of the molecular targets for cytokinin and in which

Abbreviations: BAP, N^6-benzylaminopurine; *R*-NEPA, *R*(−)-N^6-1-(1-naphthyl)ethyl-1H-purine-6-amine; *S*-NEPA, *S*-(+)-N^6-1-(1-naphthyl)ethyl-1H-purine-6-amine; CBP, cytokinin-binding protein.

*Author for correspondence.

there is little possibility for isomerization. In this connection, protein kinase, associated with chromatin or with RNA polymerase I from barley leaves, is of interest because it is activated *in vitro* by the cytokinins N^6-benzylaminopurine (BAP) or kinetin (Kulaeva 1990, Selivankina et al. 1987, 1988). Adenine, as an inactive analogue of BAP did not affect chromatin- or RNA polymerase-associated protein kinase (Kulaeva 1990, Selivankina et al. 1988).

The aim of this work was to compare the direct effects of *trans*- and *cis*-zeatin on protein kinase activity associated with chromatin isolated from barley leaves.

The second goal of the work was to determine whether the described model system can distinguish optical cytokinin isomers. Differences in biologic activities of synthetic and natural optical isomers of cytokinins have been demonstrated in several bioassays (Corse et al. 1992, Koshimizu et al. 1968, Kulaeva 1973, Matsubara 1980, 1990). We have compared the in vitro effects of two optical isomers *R*-(−)-N^6-1-(1-naphthyl)ethyl-1H-purine-6-amine (*R*-NEPA) and *S*-(+)-N^6-1-(1-naphthyl)ethyl-1H-purine-6-amine (*S*-NEPA). These substances were highly different in stimulation of *Glycine max* L. callus growth (Corse et al. 1992).

Materials and Methods

Plants

Barley plants (*Hordeum vulgare* L. cv. Viner) were grown in soil in a growth chamber under the following conditions: light intensity of 50 W/m^2, 16-h day, day temperature of 22–23 °C, night temperature of 18 °C, and relative humidity of 80%.

Chromatin Isolation and Purification

The first leaves of 8- to 10-day-old barley seedlings were used for chromatin isolation. Chromatin was isolated according to the protocol described previously (Selivankina et al. 1982). All procedures were carried out at 4°C. Leaves were homogenized in 100 mM Tris-HCl, pH 8.0, containing 250 mM sucrose, 100 mM $MgCl_2$, and 20 mM β-mercaptoethanol. The homogenate was filtered through several layers of gauze and then through a layer of Miracloth followed by centrifugation (1,000 × g, 10 min). The chromatin-containing pellet was washed with 100 mM Tris-HCl, pH 8.0, containing 350 mM sucrose, 10 mM β-mercaptoethanol, and 2% Triton X-100 followed by centrifugation (7,500 × g, 5 min). The procedure was repeated six times. The pellet was then washed twice with the same buffer but without Triton X-100. Chromatin was suspended in a buffer containing 50 mM Tris-HCl, pH 8.0, 20 mM $MgCl_2$, 1 mM β-mercaptoethanol, and 10% glycerol and used for protein kinase activity estimation.

Protein Kinase Activity Estimation

Protein kinase activity was determined in the medium (volume of 100 μl) containing 50 mM Tris-HCl, pH 8.0, 10 mM $MgCl_2$, 0.1% Triton X-100, 10 mM NaF, 20 nM [γ-^{32}P]ATP (148 PBq/mol, Izotop, St. Petersburg, FL), and chromatin (30–50 μg of chromatin protein). The reaction conditions were optimized in pilot experiments (Selivankina et al. 1988). Cytokinins were added directly to the reaction medium in concentrations designated in the figures. The reaction was carried out for 15 min at 37 °C and stopped by the addition of a cold solution (25 μl) of 50 mM ATP and 250 mM EDTA, pH 7.5. Samples were kept in the cold, collected on Whatman 3MM paper discs, and washed with a 5% solution of trichloroacetic acid and with ethanol at 0 °C. The discs were then dried, and radioactivity was measured under standard conditions in a scintillation counter (Tracor Europe, The Netherlands, model 6892). Nonenzymatic label association with chromatin due to absorption of radioactive ATP was measured in control probes in which the reaction was stopped at zero time. Phosphorus incorporation into protein was calculated in cpm/50 μg of chromatin protein. The data on cytokinin effects on protein kinase activity were calculated as a percentage of results in control reaction (without cytokinin in the medium). Experiments were repeated three times with three estimations in each of them. Means and their standard errors are shown in the figures.

Preparation of Cytokinin Compounds

trans-Zeatin was prepared according to Corse and Kuhnle (1978). *cis*-Zeatin was synthesized by the method described earlier (Leonard et al. 1971). *R*-NEPA was synthesized by heating a solution of 3 g (17.5 mmol) of 99% *R*-(+)-(1-naphthyl)-ethylamine (Aldrich Chemical Co., Milwaukee, WI, $[\alpha]_D$ + 60 °), 2.16 g (14 mmol) of 6-chloropurine, 3 ml of *N,N*-diisopropylethylamine, and 30 ml of 1-butanol overnight at 100–105 °C. The volatiles were removed in vacuo, water was added, and the oil was extracted several times with warm ethyl acetate. The extracts were combined, decolorized with carbon, and the ethyl acetate allowed to evaporate. The resulting crude *R*-NEPA (2.40 g) was purified by chromatographing on silicic acid using ethyl acetate and a gradient up to 50% methanol in ethyl acetate as eluants. The resulting 1.75 g (61%) of *R*-NEPA was recrystallized twice from ethyl acetate-petroleum ether; m.p. 197–199 °C; $[\alpha]_D^{27}$ − 178 ° (c = 6.4, MeOH); *m/z* 289.13260 (calculated for $C_{17}H_{15}N_5$: 289.13274); ^{1}H NMR (400 MHz, $CDCl_3$-ethyl acetate) δ 8.44 (1H, s, purine), 8.22 (1H, d, aromatic), 7.90 (1H, s, aromatic), 7.84 (1H, d,d, aromatic), 7.64 (1H, d, aromatic), 7.42—7.54 (3H, m, complex, aromatic), 6.40 (1H, s, anomeric H), 1.82 (3H, d, methyl); 200 MHz (dimethyl sulfoxide-d6) δ 12.76 (1H, s, purine).

S-NEPA was prepared and purified in exactly the same manner as the *R*-isomer, except that *S*-(−)-1-(1-naphthyl)ethylamine $[\alpha]_D$ −59 ° was used. The yield of *S*-(+)-NEPA was 50%; m.p. 196–198 °C; $[\alpha]_D^{27}$ +164 ° (c = 5.56, CH_3OH); *m/z* 289.13232 (calculated for $C_{17}H_{15}N_5$: 289.13274); the ^{1}H NMR spectrum was identical to that of *R*-NEPA.

It is interesting to note that both isomers of 1-(1-naphthyl)-ethylamine gave purine derivatives with opposite rotations, although the *R*- and *S*-conformations remained unchanged. This is true for other aromatic rings in our experience, namely, where the aromatic ring is either phenyl or 2,4,6-trimethylphenyl.

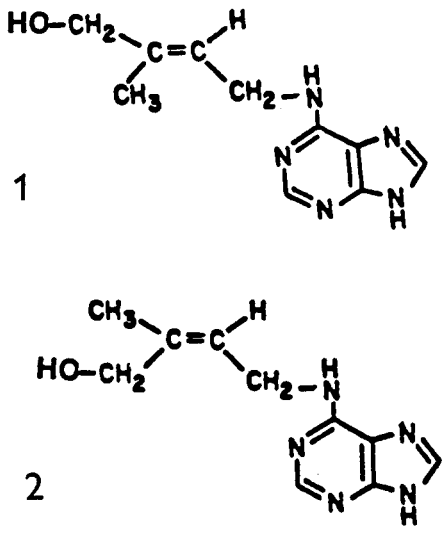

Fig. 1. Structural formulas of *trans*- (1) and *cis*-zeatin (2).

Results

The effects of *trans*- and *cis*-zeatin (Fig. 1) on the activity of the protein kinase associated with barley leaf chromatin were studied. Compounds tested were added directly into the reaction medium for enzyme activity estimation. *trans*-zeatin was examined in a range of concentrations of 10^{-10} to 10^{-6} M (Fig. 2). It was highly effective in protein kinase activation. The maximum increase in the enzyme activity was detected at a *trans*-zeatin concentration of 10^{-9} M. These data indicate high affinity of the compound to its molecular target in the system used. A further increase in the *trans*-zeatin concentration diminished the cytokinin effect on the enzyme activity. No influence was registered for *cis*-zeatin in a concentration range of 10^{-10} to 10^{-5} M. These results demonstrated that only *trans*-zeatin possessed a capacity to interact with a molecular target for cytokinin followed by an increase in the protein kinase activity. The data agree with the results obtained in a barley leaf bioassay. Only *trans*-zeatin prevented senescence of detached barley leaves. *cis*-Zeatin had no effect on this process (data not shown).

In other experiments, we compared the direct effect of two optical isomers *R*-NEPA and *S*-NEPA on the protein kinase activity. Structures of these isomers are presented in Figure 3. As Figure 4 shows, only *S*-NEPA induced activation of the chromatin-associated protein kinase. *R*-NEPA did not stimulate the enzyme activity and even decreased it at low *R*-NEPA concentrations (10^{-8}–10^{-7} M).

Therefore, the stereochemical properties of the

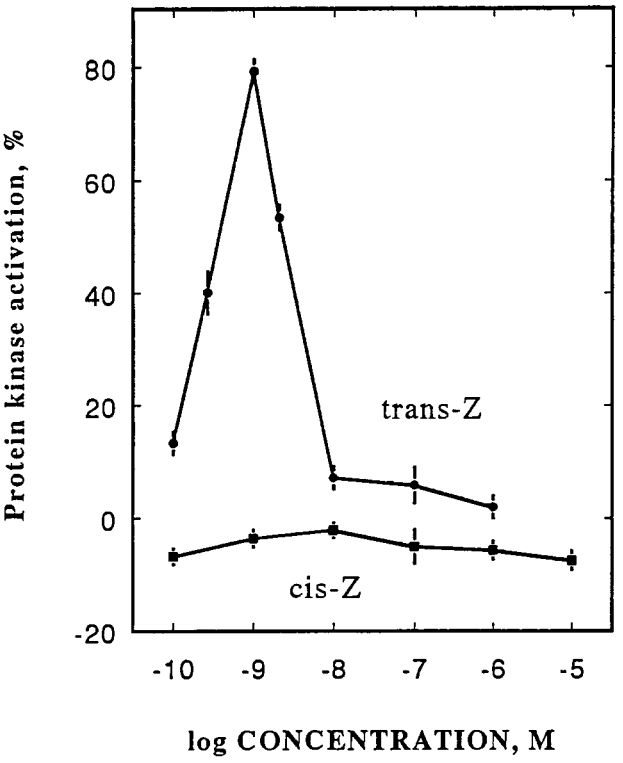

Fig. 2. In vitro effects of *trans*- and *cis*-zeatin on protein kinase associated with barley leaf chromatin. The results are expressed as the percentage activation of the control protein kinase activity measured in the absence of zeatin (0 point on OY). *Bars* denote SE.

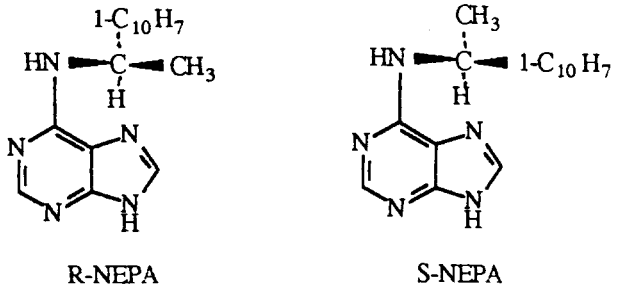

Fig. 3. Structural formulas of *R*-NEPA (*left*) and *S*-NEPA (*right*). 1-$C_{10}H_7$ refers to 1-naphthyl in the drawings.

side chain configuration in this cytokinin molecule are important for in vitro regulation of the protein kinase activity.

We studied the in vitro effect of various synthetic analogues of cytokinin on the protein kinase activity. The results of these experiments are summarized in Table 1. Only physiologically active cytokinins (*trans*-zeatin, BAP, kinetin) stimulated in vitro protein kinase associated with chromatin from barley leaves. Inactive analogues, namely, *cis*-

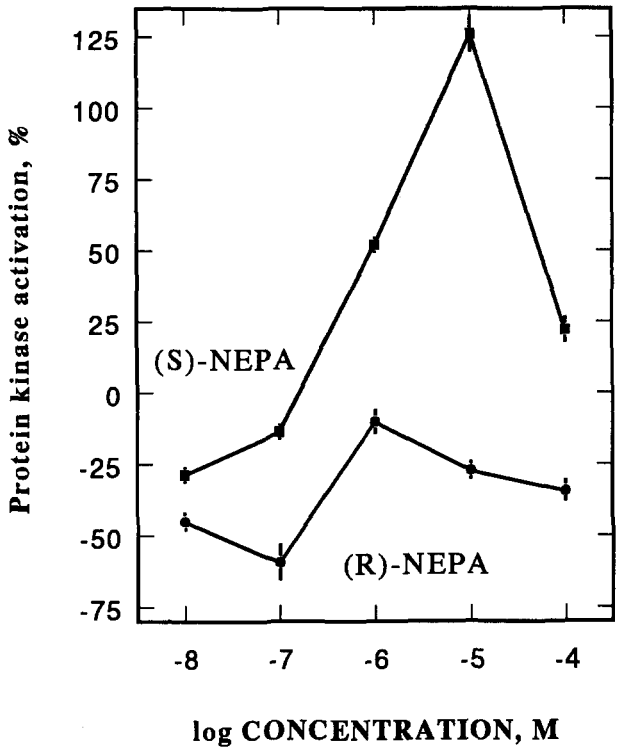

Fig. 4. In vitro effects of *R*-NEPA and *S*-NEPA on the activity of the chromatin-associated protein kinase isolated from barley leaves. The results were calculated as in Figure 2.

Table 1. Effect of cytokinins and their synthetic analogues on the activity of the protein kinase associated with barley leaf chromatin

Compound	Concentrations studied (M)	Enzyme activity (% of control)	Optimal concentration (M)
trans-Zeatin	10^{-10}–10^{-6}	183	10^{-9}
BAP	10^{-9}–10^{-5}	196	2.5×10^{-5}
Kinetin	10^{-9}–10^{-5}	130	10^{-5}
S-NEPA	10^{-8}–10^{-4}	327	10^{-5}
cis-Zeatin	10^{-10}–10^{-5}	No	
cis-Norzeatin	10^{-8}–10^{-4}	No	
9-α-Cyanoethyl-*trans*-zeatin	10^{-8}–10^{-4}	No	
Adenine	10^{-7}–5×10^{-3}	No	

norzeatin, 9-α-cyanoethyl-*trans*-zeatin, and adenine, had no promoting effect on the enzyme activity.

Discussion

Analysis of direct effects of *trans*- and *cis*-zeatin on the activity of the chromatin-associated protein kinase from barley leaves revealed that only *trans*-zeatin activated this enzyme in vitro whereas *cis*-zeatin had no effect. Hence, under conditions excluding isomerization, *cis*-zeatin was not active. Therefore the data obtained provide direct experimental evidence confirming the hypothesis of Chen et al. (1993) on intramolecular inactivation of *cis*-zeatin due to hydrogen bond formation between the OH group of the side chain and N^1 of the purine ring. This prediction was also confirmed in our work by computer modeling of *cis*- and *trans*-zeatin conformers with minimum energy using Alchemy III program (Tripos Associates Ltd., St. Louis, MO). Figure 5 shows the *cis*-zeatin conformer with minimum energy (35.6 kcal). It shows reasonable distance (2.6 Å) for hydrogen bond formation (Pauling 1945) between the OH group of the side chain and N^1 of the purine ring. The minimum energy conformer for *trans*-zeatin has no OH-N^1 distances that would permit hydrogen bonding (data not presented).

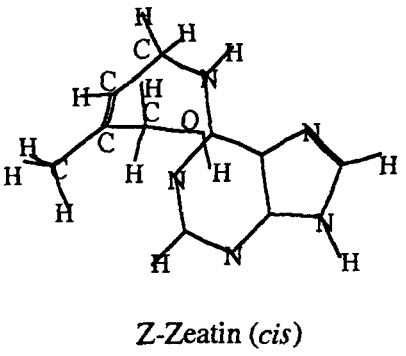

Fig. 5. Structure of *cis*-zeatin conformer with a minimum energy incorporating a hydrogen bond from the aliphatic hydroxyl to the 1-position of the purine ring.

Hence, the results of our study of *trans*- and *cis*-zeatin in vitro effects on the protein kinase activity agree with theoretical prediction.

Crucial for the success of our strategy seems to be the use of the in vitro assay for the direct measurement of cytokinin effect on the enzyme activity under conditions excluding *cis*-zeatin isomerization. The low cytokinin activity of *cis*-zeatin showed in bioassays (Schmitz et al. 1972, Skoog and Ghani 1981) could be the result of its partial isomerization in plant cells (Bassil et al. 1993, Mok et al. 1992).

It is also necessary to stress the fact that the absence of a *cis*-zeatin effect on the chromatin-associated protein kinase is the best control test for specificity of *trans*-zeatin action on the enzyme activity. Sensitivity of the chromatin-associated protein kinase to *trans*-zeatin was very high. Comparison of an optimum concentration of *trans*-zeatin

(10^{-9} M) with optimum concentrations of synthetic cytokinins presented in Table 1 showed that *trans*-zeatin affinity to a cytokinin molecular target in this system was drastically higher than to BAP and other synthetic analogues of cytokinin. In contrast to our data, *trans*-zeatin affinity to cytokinin-binding protein (CBP) from wheat embryos was very low compared with BAP, and that was one of the arguments against the receptor function of this protein (Keim et al. 1981). BAP possesses a higher activity than *trans*-zeatin in many bioassays because of its higher stability in plant cells (Letham and Palni, 1983). These data demonstrate the advantages of the test system for cytokinins based on their direct effect on protein kinase activity. It should be noted that we tested the *trans*-zeatin effect on the chromatin-associated protein kinase from barley leaves many times using different seed lots and plants grown under various conditions. In some experiments, an optimum *trans*-zeatin concentration was 10^{-8} M, but in all cases it was at least 100 times lower than an optimum BAP concentration.

Comparison of the *R*-NEPA and *S*-NEPA effects on the chromatin-associated protein kinase activity also showed a high specificity of the enzyme response to cytokinins. Only the (+)isomer (*S*-NEPA) activated the protein kinase, whereas another one (*R*-NEPA) did not. Hence, the absolute configuration around the asymmetric carbon is important for cytokinin interaction with its target molecule in the test system. This conclusion agrees with the results of bioassays (Corse et al. 1992, Koshimizu et al. 1968, Kulaeva 1973, Matsubara 1980, 1990). In particular, Corse et al. (1992) showed that *S*-NEPA in a concentration of 10^{-6} M activated *G. max* L. callus growth six-fold. At the same time, *R*-NEPA induced such an effect at a concentration 10 times higher. It is also necessary to point out that the synthetic analogue of cytokinin containing the naphthyl ring in the N^6 radical (*S*-NEPA) was 10,000 times less active than natural cytokinin (*trans*-zeatin). Optimal concentrations of *S*-NEPA and *trans*-zeatin were 10^{-5} and 10^{-9} M, respectively.

As we know, the chromatin-associated protein kinase from barley leaves is the first enzyme shown to be activated *in vitro* by cytokinins (Kulaeva 1990, Selivankina et al. 1987, 1988). Our results demonstrated that this enzyme feature could be used successfully for the study of cytokinin structure-activity relationships. Thus, the data in Table 1 demonstrate that only physiologically active cytokinins stimulated the protein kinase from barley leaf chromatin. Physiologically inactive analogues had no effect. Because cytokinin activity is determined in vitro, results do not depend on two main sources of mistakes which can exist in bioassays: cytokinin metabolism and different cell permeability for compared compounds. At the same time, this system cannot be used for the cytokinin activity analysis of unknown substances because, as our previous data showed (Selivankina et al. 1988), barley leaf chromatin contains some other protein kinases that are sensitive to other phytohormones.

The cytokinin-responsive protein kinase was coisolated with RNA polymerase I (Selivankina et al. 1987, 1988) and associated with this enzyme over all steps of its purification on phosphocellulose, heparin-Sepharose, DEAE-cellulose, and Sephacryl S-300 (Kulaeva et al. 1992, 1994). After such a procedure RNA polymerase I was purified up to electophoretic homogeneity, but it was associated with protein kinase activity. This protein kinase phosphorylated RNA polymerase I subunits (Kulaeva et al. 1994) and did not phosphorylate kasein and histone H5 (Selivankina et al. 1988). The protein kinase was not sensitive to other phytohormones.

Since RNA polymerase is a highly complicated multipeptide complex, we do not know yet whether the protein kinase is closely associated with this enzyme complex or if one of the RNA polymerase subunits possesses a protein kinase activity. It is also unknown whether cytokinin interacts directly with the protein kinase or its action is mediated by another protein. Nevertheless, it is obvious that the system used contains a molecular target for cytokinin and the cytokinin-regulated protein kinase which makes it possible to analyze cytokinin activity in vitro.

It is well known that protein kinases play an important role in hormone signal transduction in animal cells. Currently, protein kinase involvement in the plant cell response to phytohormones attracts increasing attention (Kulaeva 1990, Verhey and Lomax 1993).

It is important to discuss a possible role of the cytokinin-sensitive protein kinase, which was coisolated with RNA polymerase I and phosphorylated its subunits, in cytokinin signal transduction in leaf cells. In this connection it should be mentioned that protein kinase associated with RNA polymerase I was also found in rat hepatoma (Rose et al. 1981). It was demonstrated that phosphorylation of RNA polymerase I subunits by this protein kinase increased RNA polymerase activity in transcription elongation (Duceman et al. 1981). In our experiments the addition of cytokinin into the medium at the stage of leaf homogenization followed by chromatin isolation and purification induced a significant increase in both protein kinase and RNA poly-

merase I activities in chromatin preparations (Selvankina et al. 1988). On the other hand, direct cytokinin addition into the medium containing chromatin or RNA polymerase I promoted only protein kinase activity without effecting RNA polymerase I. For the activation of the enzyme, cytosolic CBP in concert with cytokinin were necessary (Kulaeva et al. 1990). By the analogy to RNA polymerase II phosphorylation (O'Brien et al. 1994), it could be assumed that cytokinin-induced stimulation of RNA polymerase I phosphorylation could increase the enzyme affinity to transfactor (CBP in concert with cytokinin) which promotes RNA synthesis. One of our research goals is to verify this assumption and to elucidate the role of the protein kinase described in this paper in cytokinin signal transduction in leaf cells.

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References

Bassil NV, Mok DWS, Mok MC (1993) Partial purification of a *cis-trans*-isomerase of zeatin from immature seed of *Phaseolus vulgaris*. Plant Physiol 102:867–872

Chen C-M, Jin G, Andersen BR, Ertl JR (1993) Modulation of plant gene expression by cytokinins. Aust J Plant Physiol 20:609–619

Corse J, Kuhnle J (1978) An improved synthesis of *trans*-zeatin. Synthesis 11:618–619

Corse J, Pacovsky RS, Brandon DL, McKeon TA (1992) Identification of cytokinin receptors by means of structure-activity responses. In: Kaminek M, Mok DWS, Zazimalova E (eds) Physiology and biochemistry of cytokinins in plants. SPB Academic Publishing, The Hague, pp 211–214

Duceman BW, Rose KM, Jacob ST (1981) Activation of purified hepatoma RNA polymerase I by homologous protein kinase NII. J Biol Chem 256:10755–10758

Edwards CA, Armstrong DJ, Kaiss-Chapman RW, Morris RO (1981) Cytokinin-active ribonucleosides in *Phaseolus* RNA. I. Identification in tRNA from etiolated *Phaseolus vulgaris* L. seedlings. Plant Physiol 67:1181–1184

Keim P, Erion J, Fox JE (1981) The current status of cytokinin-binding moieties. In: Guern J, Peaud-Lenoel C (eds) Metabolism and molecular activities of cytokinins. Springer Verlag, Berlin, pp 179–190

Korszun ZR, Knight C, Chen CM (1989) A stereochemical model for cytokinin activity. FEBS Lett 243:53–56

Koshimizu K, Kobayashi A, Fujita T, Mitsui T (1968) Structure-activity relationships in optically active cytokinins. Phytochemistry 7:1989–1994

Kulaeva ON (1973) Cytokinins, their structure and function. Nauka, Moscow (in Russian)

Kulaeva ON (1990) The possible role of protein kinases in the plant cell response to phytohormones. In: Pharis R (ed) Plant growth substances 1988. Springer Verlag, Berlin, pp 547–551

Kulaeva ON, Novikova GV, Selivankina SY, Karavaiko NN, Moshkov IE (1992) Protein kinases and cytokinin-binding proteins in plant response to cytokinin. In: Kaminek M, Mok DWS, Zazimalova E (eds) Physiology and biochemistry of cytokinins in plants. SPB Academic Publishing, The Hague, pp 121–125

Kulaeva ON, Karavaiko NN, Moshkov IE, Selivankina SY, Novikova GV, Pereverzeva IN, Yakovleva LA (1994) Cytokinin-binding protein(s) and protein kinases in cytokinin signal transduction. In: Smith CJ, Gallan J, Chiatante D, Zocchi G (eds) Biochemical mechanisms involved in plant growth regulation. Clarendon Press, Oxford, pp 123–138

Kuraishi S (1959) Effect of kinetin analogues on leaf growth. Sci Pap Coll Gen Ed Univ Tokyo 9:67–104

Leonard NJ, Playtis AJ, Skoog F, Schmitz RY (1971) A stereospecific synthesis of *cis*-zeatin. J Am Chem Soc 93:3056–3058

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

Matsubara S (1980) Structure-activity relationships of cytokinins. Phytochemistry 19:2239–2253

Matsubara S (1990) Structure-activity relationships of cytokinins. Crit Rev Plant Sci 9:17–57

Miller CO (1961) Kinetin and related compounds in plant growth. Annu Rev Plant Physiol 12:395–408

Mok MC, Martin RC, Mok DWS, Shaw G (1992) Cytokinin activity, metabolism and function in *Phaseolus*. In: Kaminek M, Mok DWS, Zazimalova E (eds) Physiology and biochemistry of cytokinins in plants. SPB Academic Publishing, The Hague, pp 41–46

O'Brien T, Hardin S, Greenleaf A, Lis JT (1994) Phosphorylation of RNA polymerase II C-terminal domain and transcriptional elongation. Nature 370:75–77

Pauling L (1945) Nature of the chemical bond. Cornell University Press, Ithaca, New York

Rose KM, Stetler DA, Jacob ST (1981) Protein kinase activity of RNA polymerase I purified from a rat hepatoma: Probable function of M_r 42,000 and 24,600 polypeptides. Proc Natl Acad Sci USA 78:2833–2837

Schmitz RY, Skoog F, Playtis AJ, Leonard NJ (1972) Cytokinins: Synthesis and biological activity of geometric and position isomers of zeatin. Plant Physiol 50:702–705

Selivankina SY, Romanko EG, Ovcharov AK, Kharchenko VI (1982) Involvement of cytokinin-binding proteins from barley leaves in cytokinin activation of chromatin-bound RNA polymerase. Sov Plant Physiol 29:274–281

Selivankina SY, Romanko EG, Novikova GV, Kulaeva ON (1987) Cytokinin regulation of protein kinase associated with chromatin and RNA polymerase I in barley leaves. Dokl Akad Nauk SSSR 292:766–769

Selivankina SY, Romanko EG, Novikova GV, Muromtseva DG, Kulaeva ON (1988) Effect of cytokinin and other phytohormones on protein kinases associated with chromatin and RNA polymerase I in barley leaves. Sov Plant Physiol 35:205–212

Skoog F, Armstrong DJ (1970) Cytokinins. Annu Rev Plant Physiol 21:359–384

Skoog F, Ghani A (1981) Relative activities of cytokinins and

antagonists in releasing lateral buds of *Pisum* from apical dominance compared with their relative activities in the regulation of growth of tobacco callus. In: Guern J, Peaud-Lenoel C (eds) Metabolism and molecular activities of cytokinins. Springer Verlag, Berlin, pp 140–150

Skoog F, Miller CO (1957) Chemical regulation of growth and organ formation in plant tissues cultured *in vitro*. Symp Soc Exp Biol 11:118–231

Skoog F, Hamzi HQ, Szweykowska AM, Leonard NJ, Carraway KL, Fugii T, Helgeson JPL, Loeppky RN (1967) Cytokinins: Structure/activity relationships. Phytochemistry 6:1169–1192

Verhey SD, Lomax TL (1993) Signal transduction in vascular plants. J Plant Growth Regul 12:179–195

Vreman HJ, Skoog F, Frihart CR, Leonard NJ (1972) Cytokinins in *Pisum* transfer ribonucleic acid. Plant Physiol 49:848–851

Vreman HJ, Thomas R, Corse J, Swaminathan S, Murai N (1978) Cytokinins in tRNA obtained from *Spinacea oleraceae* L. leaves and isolated chloroplasts. Plant Physiol 61:296–306