Rapid Kinetin Effects on Lipid Synthesis in Isolated Mesophyll Protoplasts of *Petunia*

U. Kull and U. Ultes
Biologisches Institut der Universität, D-7000 Stuttgart

Cytokinins show effects on the lipid fatty acid composition of isolated green leaves within 2 to 4 h [1]. These effects follow a dose-response curve with an optimum dose. Furthermore, lipid synthesis in leaf tissue is stimulated by cytokinins within a few hours [2]. Using isolated mesophyll protoplasts from *Petunia*, we have now shown that kinetin influences lipid and fatty acid synthesis respectively, within 45 min. The effects of phytohormones which can be recognized in a period shorter than 1 h are usually classified as rapid effects. Thus, for the first time a rapid cytokinin effect on lipid metabolism can be established.

Mesophyll protoplasts were isolated from leaves of *Petunia hybrida* by simultaneous treatment with pectinase and cellulase after removing the lower epidermis with carborundum [3]. The viability of the protoplasts was tested using Larkin's method [4]. Protoplasts were then incubated in mannitol-containing kinetin solutions together with ^{14}C-acetate for 45 min or 1 to 3 h. Controls were run in the same manner without kinetin. After the incubation period, total lipids were extracted; following a thorough washing of the lipids, the relative specific activity was determined by a windowless CH_4-flow counter and by scintillation counting [5].

The total lipid content and fatty acid composition of the protoplasts were not significantly altered by kinetin. Since different amounts of ^{14}C-acetate were applied, or different incubation periods took place, the absolute values of specific activity for controls range from 600 to 12 000 cpm/mg lipid. Table 1 shows that the labeling activity is enhanced most considerably by 1 µg kinetin/ml. Application of 10 µg/ml always led to a decrease in the ^{14}C incorporation, results comparable to the negative effects of the too high concentrations already described [1]. The activity of the different fatty acids was measured by radio-gas chromatography [5] (Table 2). Even after the very short incubation period of 45 min, a significant rise in the activity of unsaturated C_{18} acids could be measured. Extension of the application period of kinetin to 2 and 3 h led to an increase in percentage activity in C_{16} acids.

From these results, together with some earlier findings [1, 2], it may be concluded that kinetin stimulates fatty acid synthesis in mesophyll protoplasts, and that this stimulation is a rapid hormone effect, indicating an early stage in the network of effects caused by the cytokinin. Moreover, current investigations show that a similar rapid effect is also found in intact leaf tissues, which suggests that this effect is not the special process of a perhaps disturbed metabolism of protoplasts.

Table 1. Alterations of relative specific activity of total protoplast lipids, expressed as percentage difference against controls (=100%) after application of kinetin

Period of incubation with kinetin and ^{14}C-acetate [h]	µg kinetin/ml		
	0.1	1	10
3	+13	+46	−90
2	+10	+30	−97
1	+26	+88	− 2
0.75	+19	+65	−15

Table 2. Alterations of specific activity of the fatty acids as percentage difference against controls (=100%) after application of 1 µg kinetin/ml

Fatty acid	Application period of kinetin + ^{14}C-acetate			
	1 h		45 min	
	exp. 1	exp. 2	exp. 1	exp. 2
14:0+14:1	± 0	+ 3	+ 5	+ 2
16:0	+ 18	+ 10	+ 21	+ 17
16:1	+ 64	+ 73	+ 80	+ 66
16:3	+ 10	+105	+ 74	+ 51
18:0	+ 95	+ 10	+ 21	+ 41
18:1	+153	+190	+ 93	+ 87
18:2	+146	+178	+104	+123
18:3	+ 3	+ 50	+ 10	+ 19

The authors are grateful to Miss B. Kühn and Miss E.-M. Moser for valuable technical assistance, and to Mrs. A. Noller for correction of the English text. The investigations were kindly supported by a grant from the DFG to U. Kull.

Received September 7, 1979

1. Kull, U., et al.: Plant Cell Physiol. *19*, 801 (1978)
2. Schweizer, J., et al.: Plant Physiol. *59*, Suppl., 75 (1977)
3. Beier, H., Bruening, G.: Virology *64*, 272 (1975)
4. Larkin, P.J.: Planta *128*, 213 (1976)
5. Wiedmaier, J., Kull, U.: Biochem. Physiol. Pflanzen *172*, 421 (1978)