Effect of kinetin on nucleic acid synthesis in senescing and young leaves in *Brassica oleracea* L. var. *gongylodes* L.

J. LEGOCKA AND A. SZWEYKOWSKA

Laboratory of General Botany, Institute of Biology, Adam Mickiewicz University, Poznań, Poland

(Received: May 26, 1973)

Abstract

In detached kohlrabi leaves senescing in the dark, the decrease in chlorophyll b was more pronounced than in chlorophyll a. The retardation by kinetin of the chlorophyll loss was also markedly stronger in the case of chlorophyll b. Using the fractionation of nucleic acids on polyacrylamide gels it has been shown that during leaf senescence the level of all RNA species decreased, whereas the amount of DNA was more or less constant. In the presence of kinetin, the loss of RNA was inhibited and the incorporation of precursor into the cytoplasmic rRNA as well as into low molecular weight RNA species was supported. Chloroplast rRNA synthesis has not been detected in mature leaves and kinetin showed no effect in this respect.

In young expanding leaves detached and kept in light, the synthesis of cytoplasmic rRNA was strongly stimulated by kinetin, whereas in the case of chloroplast rRNA only an inhibitory effect of kinetin could be found. The results suggest that the cytokinins are primarily involved in processes of the synthesis of cytoplasmic rRNA and low molecular RNA fractions, and in this way affect the development of plastids, in particular the course of their senescence.

INTRODUCTION

In senescing leaves (attached or detached from plant), a decrease in the rate of photosynthesis, a gradual loss of chlorophyll, and eventually a disintegration of the chloroplast structure occurs (Shaw and Manocha 1965, Barton 1966, Dennis et al. 1967, De Vecchi 1971, Tettley and Thimann 1974). This is accompanied by a decrease in the levels of nucleic acids and of proteins (Mothes 1960, Kulaeva et al. 1967, 1969, Srivastava and Atkin 1968, Lynn and Pillay 1971). Kinetin inhibits the process of leaf senescence and simultaneously pre-
vents the loss of protein and RNA (Richmond and Lang 1957, Mothes et al. 1959, Wollgibehn 1961, Osborne 1962, Srivastava and Ware 1965). Investigations by several authors (Kulaeva 1967, Fletcher 1969, Zwar 1973) have shown, that the prevention of the RNA loss at least partially results from a stimulation by cytokinins of RNA synthesis. The problem of cytokinin action in the processes of senescence is particularly interesting in the cases when these growth regulators not only delay but even reverse the ageing processes, increasing the chlorophyll and protein contents above the initial level. Besides tobacco and broad bean, the kohlrabi belongs to plants showing this kind of response to the cytokinins (Mlodzianowski and Kwintkiewicz 1973).

Some investigations have shown that in the case of young organs cytokinins accelerate their development and next senescence. This effect of cytokinins had been demonstrated with respect to chloroplast ultrastructure in young leaves (Dennis et al. 1967) and to the activity of some catabolic enzymes (Gaspar et al. 1961, Schneider and Szweykowska 1974).

The purpose of this study was to investigate the differentiation of nucleic acids and the effect of cytokinin on the pattern of their synthesis in kohlrabi leaves, both young and senescing.

MATERIAL AND METHODS

Brassica oleracea L. var. gongylodes L. cv. Dworskiego plants were grown in a greenhouse under natural light and temperature of 20—25°C. For studying the effect of cytokinins on the senescence process, mature leaves were harvested, placed with their petioles in water (control) or in cytokinin solution, and kept in darkness for 5 days. In preliminary experiments, 6-Δ²-izopentenyl-aminopurine (2iP) and kinetin at concentrations of 25—200 μM were used. In further experiments, kinetin at 100 μM was used, as in this case highest degree of inhibition of chlorophyll degradation was shown. The levels of chlorophyll and nucleic acid synthesis ([³²P] orthophosphate incorporation) were determined after 5 days.

For investigation of cytokinin action on young leaves, leaves from two-week-old seedlings were used with leaf blades 3—7 cm long. Leaves were placed with their petioles in water or kinetin solution, kept in fluorescent light (1000 lux) for 24 or 72 hours and next used for the determination of nucleic acid synthesis (incorporation of [³²P] orthophosphate).

Chlorophyll estimation

Chlorophyll was extracted and determined according to Kirk (1968). Disks of 15 mm diameter, consisting of the midribs and of the neighbouring leaf tissue, were excised with cork-borer from the region 5 cm below
the leaf apex. The 250 mg samples of disks were homogenized in cold mortar with 5 ml of cold 80% acetone. The extracts were clarified by centrifugation at 1000 \( \times g \) for 3 min., made up to 5 ml and their absorption was determined in the "Spectromom 202" spectrophotometer at 645 and 663 nm. The concentrations of chlorophyll a and b were estimated using Kirk nomogram.

Incubation with \([^{32}P]\) orthophosphate

Five hours before the end of experiment, the leaves were surface sterilized with 0.1% HgCl\(_2\) and disks of 15 mm diameter were excised (midrib omitted) with a flame-sterilized cork bored. Leaf disks were incubated for 4 h in Petri dishes in 10 ml of kinetin solution or in water (control), with the addition of 300 \( \mu \)Ci of \([^{32}P]\) KH\(_2\)PO\(_4\). The labelling was carried out in similar conditions as the previous leaf incubations, i.e. the disks of senescing leaves were kept in the dark and those of young leaves in the light. The disks were then washed with 0.05 M phosphate buffer, pH 7.0, and water, and used for nucleic acid extraction.

Extraction of nucleic acids

Nucleic acids were extracted by the phenol-detergent method, according to Parish and Kirby (1966). In the case of mature leaves, the nucleic acid preparations were additionally purified from polysaccharides according to the method of Trewavas (1970). The deproteinization grade, \( E_{260}/E_{280} \), amounted to 0.5

Electrophoretic fractionation of nucleic acids

Nucleic acids were fractionated in 2.4% polyacrylamide gels as described by Loening (1967). The samples (80 \( \mu \)g, in a solution of a concentration 1 mg/ml) were separated electrophoretically at 9 V/cm and 5 mA/gel for 2.5 h. The gels were stained with 2% toluidine blue and scanned at 542 nm in the Vitatron densitometer. For radioactivity measurement the gels were frozen in solid CO\(_2\), sliced in 0.8 mm sections, dried on filter paper and counted in toluene scintillator in Packard Scintillation Counter. The relative amounts of respective RNA species were calculated by the comparison of areas (determined by the weighing method) of corresponding regions of densitometric and radiometric profiles. The results were expressed as per cent values.
RESULTS

A. Effect of kinetin on the level and synthesis of nucleic acids in senescing leaves

For a better characterization of the plant material under investigation, the contents of chlorophyll $a$ and $b$ in mature, freshly harvested leaves (initial material) and after their incubation in the dark in the absence and presence of kinetin were determined. As can be seen from Table 1, the levels of the chlorophylls $a$ and $b$ in detached and kept in the dark kohlrabi leaves markedly decreased. The ratio of chlorophyll $a/b$ increased indicating a more intensive loss of chlorophyll $b$. Kinetin prevented the loss of chlorophylls and its effect was more significant in the case of chlorophyll $b$.

<table>
<thead>
<tr>
<th>Table 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effect of kinetin (100 $\mu$M) on the level of chlorophyll $a$ and $b$ in detached kohlrabi leaves. Leaves were placed with petioles in water or kinetin solution and kept in darkness for 5 days. Chlorophylls were determined by the method of Kirk (1968)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Leaf samples</th>
<th>Chlorophyll $a$</th>
<th>Chlorophyll $b$</th>
<th>$a/b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\mu$g/g fresh weight</td>
<td>$%$</td>
<td>$\mu$g/g fresh weight</td>
</tr>
<tr>
<td>Initial material</td>
<td>236</td>
<td>100</td>
<td>216</td>
</tr>
<tr>
<td>Control</td>
<td>64</td>
<td>29</td>
<td>44</td>
</tr>
<tr>
<td>Kinetin</td>
<td>172</td>
<td>73</td>
<td>186</td>
</tr>
</tbody>
</table>

The separation of nucleic acids on polyacrylamide gels showed that DNA content was relatively stable during senescence in the dark, whereas the levels of the remaining nucleic acid fractions comprising low molecular weight RNA and four species of rRNA (25, 23, 18 and 16 S) decreased markedly (Fig. 1 A—C). A comparison of densitometric profiles indicates that cytoplasmic rRNAs were less stable than plastid rRNAs (Table 2). The level of low molecular weight RNA also markedly decreased which indicates that degradation of rRNA was almost complete and the main reaction products were not polynucleotide RNA fragments, but rather free nucleotides. The presence of kinetin during the incubation strongly prevented the RNA loss. The analysis of the densitometric profiles (Fig. 1 A—C and Table 2) showed that kinetin was particularly effective in the case of 23, 18 and 16 S RNAs.

A synthesis of DNA or plastid rRNAs could not be detected either in dark-incubated or in freshly harvested mature kohlrabi leaves. On the other hand, $^{32}$P orthophosphate was incorporated into both cytoplasmic rRNA and low molecular weight RNA fractions. In leaves treated with
Fig. 1 A—C. Electrophoretic fractionation in 2.4% polyacrylamide gel of nucleic acids from mature kohlrabi leaves, freshly harvested (initial material, A), kept in darkness for 5 days in water (control, B) or in kinetin solution (100 μM, C). Nucleic acids were labelled with $^{32}$P orthophosphate during the last 4 h of experiment. Smooth curve, absorbance at 542 nm; point curve, radioactivity
Table 2

<table>
<thead>
<tr>
<th>Fraction of RNA</th>
<th>Area on densitometric profile</th>
<th></th>
<th></th>
<th>Area on radiometric profile</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>initial</td>
<td>control</td>
<td>kinetin</td>
<td>initial</td>
<td>control</td>
<td>kinetin</td>
</tr>
<tr>
<td>25 S</td>
<td>cm²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>37.8</td>
<td>24.5</td>
<td>26.8</td>
<td>26.0</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>23 S</td>
<td>cm²</td>
<td>8.5</td>
<td>7.5</td>
<td>10.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>100</td>
<td>88.2</td>
<td>124.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 S</td>
<td>cm²</td>
<td>18.6</td>
<td>6.9</td>
<td>19.4</td>
<td>16.2</td>
<td>6.2</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>100</td>
<td>37.1</td>
<td>104.3</td>
<td>100</td>
<td>41.3</td>
</tr>
<tr>
<td>16 S</td>
<td>cm²</td>
<td>5.7</td>
<td>4.1</td>
<td>10.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>100</td>
<td>71.1</td>
<td>178.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low molecular</td>
<td>cm²</td>
<td>22.5</td>
<td>13.7</td>
<td>17</td>
<td>11.9</td>
<td>10</td>
</tr>
<tr>
<td>weight</td>
<td>%</td>
<td>100</td>
<td>60.9</td>
<td>175.1</td>
<td>100</td>
<td>84</td>
</tr>
</tbody>
</table>

Kinetin during dark incubation, incorporation of precursor into cytoplasmic rRNA was twice as high as in the water control and amounted to 70—80% of the incorporation in the freshly harvested material under light conditions. Kinetin also markedly stimulated incorporation into low molecular weight RNA fraction which was even somewhat higher than in the initial material and about 1.7 times higher when compared with the dark water control.

B. Effect of kinetin on nucleic acid synthesis in young leaves

In contrast to the mature leaves, in young kohlrabi leaves $[^{32}P]$ orthophosphate was incorporated into all nucleic acid species, i.e. DNA, 25, 23, 18 and 16 S rRNAs and low molecular weight RNA (Fig. 2). This is in agreement with the earlier data of Treha ne et al. (1970) and of P r a n j o t hy and W a r e i n g (1971) who also showed that synthesis of plastid rRNA occurs in leaves only at early stages of development.

When detached leaves were kept under light conditions in water (control) for 72 h, incorporation of radioactivity into all nucleic acid species still continued. Kinetin (100 μM) strongly stimulated incorporation of $[^{32}P]$ orthophosphate into cytoplasmic rRNA (Fig. 3 A—B), the incorporation into plastid rRNAs, however, was suppressed. To eliminate a possi-
Fig. 2. Electrophoretic fractionation in 2.4% polyacrylamide gel of nucleic acids from young kohlrabi leaves. Nucleic acids were labelled with $^{32}$P orthophosphate for 4 h. Smooth curve, absorbance at 542 nm; point curve, radioactivity.

Fig. 3 A—B. Electrophoretic fractionation in 2.4% polyacrylamide gel of nucleic acids from young kohlrabi leaves. Leaves were placed with petioles in water (A) or kinetin, 100 μM (B) and kept in light for 72 h. Nucleic acids were labelled with $^{32}$P orthophosphate during the last 4 h of experiment. Smooth curve, absorbance at 542 nm; point curve, radioactivity.
Fig. 4 A—C. Electrophoretic fractionation in 2.4% polyacrylamide gel of nucleic acids from young kohlrabi leaves. Leaves were placed with petioles in water (A) or kinetin, 2.5 μM (B), 5 μM (C) and kept in light for 24 h. Nucleic acids were labelled with $^{32}$P orthophosphate during the last 4 h of experiment. Smooth curve, absorbance at 542 nm; point curve, radioactivity.
bility that the kinetin concentration used (inhibitory to the leaf senescence) was too high for young leaves, in a next experiment the concentration of the regulator was lowered to 5 μM and 2.5 μM, resp., and the time of treatment was shortened to 24 h. Both kinetin concentrations used markedly stimulated (2—3 times when compared with the control) cytoplasmic and low molecular weight RNA synthesis (Fig. 4 A—C). At 2.5 μM kinetin concentration and with 24 h treatment, the synthesis of plastid rRNA was on the control level, and at the twice as high (5 μM) kinetin concentration the process was inhibited. In another experiment, the time

Fig. 5 A—B. Electrophoretic fractionation in 2.4% polyacrylamide gel of nucleic acids from young kohlrabi leaves. Leaves were placed with petioles in water (A) or kinetin, 2.5 μM (B), and kept in light for 72 h. Nucleic acids were labelled with [32P] orthophosphate during the last 4 h of experiment. Smooth curve, absorbance at 542 nm; point curve, radioactivity.
of treatment with 2.5 μM kinetin solution was prolonged to 72 hours. However, this prolonged treatment resulted only in a further enhancement of cytoplasmic rRNA synthesis and in an inhibition of the synthesis of plastid rRNA (Fig. 5 A—B).

DISCUSSION

Młodzianowski and Kwintkiewicz (1973) showed that the first symptom of chloroplast degradation in detached kohlrabi leaves senescing in the dark was the breakdown of grana, and the most striking effect of kinetin was their preservation or even promotion of development. Our investigations showed that during dark-induced senescence of these leaves, the loss of chlorophyll b was more pronounced than of chlorophyll a, and the inhibitory effect of kinetin was also higher in the case of chlorophyll b. Similar results were obtained by Romanko et al. (1968) who found that 6-benzylaminopurine more effectively inhibited degradation of chlorophyll b than of chlorophyll a in senescing tabacco leaves. Our results concerning kohlrabi leaves, together with those obtained by Młodzianowski and Kwintkiewicz (1973) seem to support data that chlorophyll b is mainly bound up with the grana thylakoids (Govindjee and Rabinowith 1960, Park and Sane 1971).

Similarly as in leaves of tabacco (Wollgiehn 1961) and cocklebur (Dyer and Osborne 1970), also in senescing kohlrabi leaves the DNA was relatively stable and kinetin did not affect its synthesis. On the other hand, the level of RNA strongly decreased during the senescence, which could be due to an increase in nuclease activities or to a reduced RNA synthesis (Srivastava and Ware 1965, Srivastava 1968). Plastid 23 and 16 S rRNA synthesis was not detected in mature kohlrabi leaves, similarly as in fully expanded bean and radish leaves (Ingle 1968, Treharne et al. 1970, Paranjothy and Wareing 1971). This synthesis was also not induced by kinetin treatment. As the kinetin markedly inhibited the decrease in plastid rRNA, this must have resulted from a strong inhibition of the decomposition of these RNA species which is in agreement with the finding of Paranjothy and Wareing (1971) for radish leaves. On the other hand, the synthesis of cytoplasmic rRNA and of low molecular weight RNA, when compared with water control, was stimulated by kinetin and maintained at the level of the initial material (freshly harvested leaves). Paranjothy and Wareing (1971) think to be questionable that the stimulation of cytoplasmic rRNA synthesis is a primary effect of kinetin in the inhibition of leaf senescence and they rather connect this effect with the inhibition of the degradation of chloroplast rRNA. There are, however, evidences that ageing of chloroplasts is controlled by processes occurring in cytoplasm (Choe and Thimann 1974)
and that the action of cytokinins on cells is mainly connected with their participation in cytoplasmic processes controlled by nuclear DNA (Berridge et al. 1970, Wozny and Szweykowska 1975). In this regard, the coincidence between high level of cytoplasmic rRNA synthesis and maintenance of high chlorophyll content and intact chloroplast structure seems to be significant.

The different sensitivity and response of chloroplast and cytoplasmic rRNA synthesis to cytokinins is also noteworthy. In the case of young kohlrahi leaves, the treatment with kinetin resulted in a distinct inhibition of this synthesis, whereas the synthesis of cytoplasmic rRNA and of low molecular weight RNA were strongly stimulated. Thus the application of kinetin to young leaves gave a pattern of \(^{32}\text{P}\) orthophosphate incorporation similar to that obtained with mature leaves kept in the dark. These results are interesting in connection with the finding of Dennis et al. (1971) that 6-benzylaminopurine enhanced the senescence processes of chloroplasts in young leaves in brussels sprout as revealed by ultrastructural studies. On the other hand, the strong stimulation by kinetin of cytoplasmic rRNA synthesis indicates again an involvement of cytokinins in the nuclear-cytoplasmic control of the metabolism of leaves.

REFERENCES


Wpływ kinetyny na syntezę kwasów nukleinowych w starzejących się i młodych liściach *Brassica oleracea* L. var. gongylodes L.

**Streszczenie**

W starzejących się w cienności liściach kalarepy stwierdzono silniejszy ubytek chlorofilu b niż a, a hamujący wpływ kinetyny na rozkład chlorofilu również w większym stopniu dotyczył chlorofilu b. Stosując metodę rozdziału kwasów nukleinowych na żelu poliakrylamidowym wykazano, że podczas starzenia się liści rybosomale i niskocząsteczkowe RNA ulegają rozkładowi, natomiast DNA jest bardziej stabilny. Kinetyna hamuje spadek zawartości RNA oraz stymuluje włączenie prekursora do cytoplazmatycznych frakcji rRNA i niskocząsteczkowych RNA zarówno u liści starzejących się jak i młodych, nie wpływa natomiast na syntezę DNA.

Nie stwierdzono syntezy chloroplastowych rRNA w liściach dojrzałych, a kinetyna nie miała wpływu na ten fakt. W przypadku liści młodych kinetyna hamowała syntezę chloroplastowych rRNA. Otrzymane wyniki sugerują, że cytokininy prawdopodobnie nie uczestniczą w kontroli syntezy plastydowych rRNA, natomiast są zaangażowane w procesy syntezy cytoplazmatycznych rRNA oraz frakcji niskocząsteczkowych RNA i poprzez te procesy mogą wpływać na rozwój plastydów zwłaszcza zaś na opóźnianie ich starzenia się.