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# Influence of gibberellic acid on nucleic acids synthesis in resting spring barley seeds

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The possibility of breaking the dormancy of seeds by means of gibberellic acid ($GA_3$) has already been repeatedly demonstrated (Frankland, 1961; Curtis and Cantlon 1963; Lipe and Crane 1966; Bradbeer and Pinfield 1967; Bradbeer 1968; Schachl 1968).

The accumulation of endogenous gibberellins in tree seeds (Frankland and Wareing 1965) and potato tubers (Hemberg 1967) as dormancy recedes also points to the important role of these compounds in the regulation of after-harvest ripening.

The mechanism of gibberellin action consists probably in the depression of the genome, and this in turn enhances the synthesis of enzyme-specific RNA and at the same time of hydrolytic enzymes which make possible the utilization of reserve substances (Paleg 1965; Chrispeels and Varner 1967; Amen 1967).

The authors' earlier studies have demonstrated that in resting barley seed resynthesis of RNA is inhibited by a substance occurring in large amounts, whereas after recession of dormancy the RNA and DNA synthesis intensity and that of phospholipids greatly increase (Rejowski and Kulka 1967; Kulka and Rejowski 1970). The present paper is a continuation of the previous investigations in which the influence of $GA_3$ on the synthesis of the particular RNA and phospholipid fractions was studied in the embryos of resting barley seed.

## METHODS

1. The investigations were carried out on spring barley seeds (Browarny PZHR) which is characterized by a long after-harvest period of rest. The harvested grain was stored in the laboratory in air-dried state at room temperature. Ten days after the harvest, part of the seed was soaked in Petri dishes in an aqueous $Na_2H^{32}PO_4$ solution, specific activity 0.5 μCi/ml with gibberellic acid added to a final concentration of 100 mg/l. The other part of the seeds were soaked in similar conditions but without $GA_3$. The dishes were kept in the dark in a thermostat set at 20°C. After 48 and 96 hr samples of the swollen grain were taken, washed thoroughly in water and then the embryos with the germinal disk were isolated for analysis.

The RNA P content was determined in intact embryos, mitochondria and in the supernatant (cytoplasm fraction deprived of nuclei, plastids and mitochondria),

and phospholipids content was determined in intact embryos. Moreover, the radioactivity of the compounds under study was measured by a Geiger-Müller counter.

2. Isolation of the mitochondrial fraction. Freshly isolated embryos were homogenized in a porcelain mortar with a smooth inner surface in 0.5 M saccharose prepared with 0.01 M tris buffer (pH 7.6). After homogenization the pH of the suspension decreased to 7.2—7.3. The homogenate was then pressed through 8 layers of cheesecloth. The filtrate practically deprived of intact cells (microscopic control) was differentially centrifuged (Cunningham 1964): first at 1500 g for 10 min; the precipitate (nuclei, plastids, fragments of cellulose membranes etc.) was discarded, and the supernatant was further centrifuged at 12 000 g for 10 min. The mitochondrial sediment obtained was washed once with 0.5 M saccharose and centrifuged again at 12 000 g.

In the mitochondrial precipitate thus obtained, and in the supernatant containing the ribosomal fraction RNA and phospholipid content were determined and the radioactivity of these substances was measured.

All the proceedings connected with isolation of the mitochondrial fraction were performed at 0—4°C.

3. Phospholipids and RNA determination. Acid-soluble phosphorus was removed from the material under study (intact embryos, mitochondria, supernatant) by thorough homogenization in 7 percent trichloroacetic acid (TCA) for 20 min. Then the suspension was centrifuged at 6000 g, the supernatant was discarded, the precipitate was twice washed with 1 percent TCA and then with distilled water until no reaction to acid and phosphorus could be detected. The processing was done at 0—4°C.

After removal of the acid-soluble fraction the sediment was washed once with cold ethanol and twice extracted with ether—ethanol (1:1), the suspension being maintained at boiling point for 10 min. Then the precipitate was once washed with ethanol and finally with ether and dried at 37°C. The extracts were combined and phosphorus content and phospholipid radioactivity were determined in the mixture.

RNA was determined in the particular sediments after Ogur and Rosen (1950), and phosphorus according to Fiske and Subbarow.

## RESULTS AND DISCUSSION

Enhanced incorporation of radioactive precursors into RNA was noted under the influence of $GA_3$ in the aleurone layers of barley (Chandra and Varner 1965), of *Avena fatua* (Naylor 1966) and of isolated nuclei of pea germs (Johri and Varner 1968). Flether and Osborn (1966) report that $GA_3$ stimulates RNA and protein synthesis in the leaves of dandelion (*Taraxacum officinale*), thus preventing their ageing. According to Nitson and Lang (1966) the elongation of the epicotyl in lentil (*Lens culinaris* Med.) is caused by $GA_3$ and associated with an increased DNA and RNA synthesis. Similar results have been obtained by Breughton (1968) who treated with $GA_3$ the internodes of dwarf pea.

The data quoted indicate that gibberellins stimulate RNA and DNA synthesis in various tissues and cell structures. The universality of action of these compounds on nucleic acids metabolism is also evident when they are applied to resting seeds which respond by a different physiological reaction to stimulators.

It was found previously by the authors that in the embryos of resting barley seed (incapable of germination) nucleic acid (NA) synthesis is not completely arrested only greatly reduced. After recession of dormancy, already in the initial period of grain swelling, $^{32}P$ incorporation into RNA and DNA is enhanced and so is the total content of these compounds in the embryos (Kulka and Rejowski 1970). The factor controlling NA synthesis in this case are presumably endogenous gibberellin compounds which have been detected in the embryo of germinating barley grain (Yomo and Iinuma 1966; Radley 1967; MacLeod and Palmer 1967).

The present investigations demonstrated that the resting barley seeds treated with $GA_3$ germinated after 4 days in 75 percent, whereas grain soaked without the stimulator did not germinate at all. During incubation with $GA_3$ the dry weight of the embryos also increased (Table 1).

Breaking of dormancy was associated with an enhancement of total RNA synthesis which was pronounced already after 48 hr of incubation. After 96 hr $^{32}P$ incorporation into the embryonal RNA increased under the influence of $GA_3$ more than twofold as compared with that in resting seed, and when calculated to 100 embryos (total activity) almost threefold (Table 1).

A similar increase in RNA synthesis was observed in previous studies during normal germination of seed after emergence from dormancy (Kulka and Rejowski 1970). A several times more intensive RNA synthesis has also been observed by Jarvis et al. (1968) when studying the influence of $GA_3$ on the information--transcription system of resting embryos of hazel seeds.

Changes in the total RNA content due to $GA_3$ were, on the other hand, only slight (Table 1). A certain rise in the RNA level was only noted after recalculation to 100 embryos. A slight increase of the absolute RNA amount observed simultaneously with the intensive synthesis of this compound indicates the occurrence of continuous breakdown owing to the action of nucleolytic enzymes.

Ribonuclease activity in embryos of resting barley seed is low. However, with receding dormancy and advancing physiological ripeness this activity increases considerably (Kulka 1969). A similar enhancement of the activity of the enzyme probably occurs when dormancy is broken by gibberellin compounds. This is confirmed by the data of Chrispeels and Varner (1967a) who describe a rapid increase in RNase activity in the aleurone layers of barley grain incubated with $GA_3$. $^{32}P$ incorporation into the ribosomal and mitochondrial RNA fraction in embryos of resting seeds was much more intensive than that into the total RNA (see tables 1, 2 and 3). As the grain swells, not only does the RNA synthesis become more intensive in the ribosomes and mitochondria, but also its absolute, particularly mitochondrial amount increases, (tables 2 and 3). These results agree with those of earlier investigations in which active NA synthesis was revealed in the mitochondria

Table 1 - Tabela 1

$^{32}P$ incorporation into RNA of barley seed embryos during soaking

Włączanie $^{32}P$ w RNA zarodków pęczniejącego ziarna jęczmienia

| Soaking hr<br>Czas pęcznienia w godz. | Embryos of dormant seeds<br>Zarodki ziarna spoczynkowego | | | | | | Embryos of seeds treated with $GA_3$<br>Zarodki ziarna traktowanego $GA_3$ | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | dry weight of preparation of 100 embryos mg<br>sucha masa preparatu 100 zarodków w mg | RNA content<br>zawartość RNA | | radioactivity of RNA<br>radioaktywność RNA | | | dry weight of preparation of 100 embryos mg<br>sucha masa preparatu 100 zarodków w mg | RNA content<br>zawartość RNA | | radioactivity of RNA<br>radioaktywność RNA | | |
| | | P µg/1 g of preparation<br>P µg/1 g preparatu | P µg/100 of embryos<br>P µg/100 zarodków | cpm/1 g of preparation<br>imp/min/1g preparatu | cpm/100 embryos<br>imp/min/100 zarodków | cpm/1 mg of P<br>imp/min/1 mg P | | P µg/1 g of preparation<br>P µg/1 g preparatu | P µg/100 embryos<br>P µg/100 zarodków | cpm/1 g of preparation<br>imp/min/1g preparatu | cpm/100 embryos<br>imp/min/100 zarodków | cpm/1 mg of P<br>imp/min/1 mg P |
| 48 | 80.10 | 2940 | 236 | 5990 | 480 | 2033 | 100.00 | 2700 | 270 | 8900 | 890 | 3300 |
| 96 | 98.00 | 2550 | 250 | 6370 | 624 | 2500 | 125.00 | 2320 | 290 | 14080 | 1760 | 6700 |

Table 2 - Tabela 2

$^{32}P$ incorporation into cytoplasmic /ribosomal/ RNA

Włączanie $^{32}P$ w RNA cytoplazmatyczny /rybosomalny/

| Soaking hr<br>Czas pęcznienia w godz. | Embryos of dormant seeds<br>Zarodki ziarna spoczynkowego | | | | | | Embryos of seeds treated with $GA_3$<br>Zarodki ziarna traktowanego $GA_3$ | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | dry weight of supernatant of 100 embryos, mg<br>sucha masa supernatantu 100 zarodków w mg | RNA content<br>zawartość RNA | | radioactivity of RNA<br>radioaktywność RNA | | | dry weight of supernatant of 100 embryos, mg<br>sucha masa supernatantu | RNA content<br>zawartość RNA | | radioactivity of RNA<br>radioaktywność RNA | | |
| | | P µg/1 g of preparation<br>P µg/1 g preparatu | P µg/100 embryos<br>P µg/100 zarodków | cpm/1 g of preparation<br>imp/min/1g preparatu | cpm/100 embryos<br>imp/min/100 zarodków | cpm/1 mg of P<br>imp/min/1 mg P | | P µg/1 g of preparation<br>P µg/1 g preparatu | P µg/100 embryos<br>P µg/100 zarodków | cpm/1 g of preparation<br>imp/min/1g preparatu | cpm/100 embryos<br>imp/min/100 zarodków | cpm/1 mg of P<br>imp/min/1 mg P |
| 48 | 40.0 | 1500 | 60 | 9025 | 361.5 | 6000 | 45.0 | 1657 | 74 | 10066 | 452.9 | 6121 |
| 96 | 38.8 | 2318 | 90 | 18222 | 704.4 | 7860 | 58.8 | 1836 | 108 | 17990 | 2822.0 | 26129 |

Table 3 - Tabela 3

$^{32}P$ incorporation into mitochondrial RNA

Włączanie $^{32}P$ w RNA mitochondriów

| Soaking, hr<br>Czas pęcznienia w godz. | Embryos of dormant seeds<br>Zarodki ziarna spoczynkowego | | | | | | Embryos of seeds treated with $GA_3$<br>Zarodki ziarna traktowanego $GA_3$ | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | dry weight of mitochondria of 100 embryos sucha masa mitochondriów 100 zarodków /mg/ | RNA content zawartość RNA | | radioactivity of RNA radioaktywność RNA | | | dry weight of supernatant of 100 embryos sucha masa supernatantu 100 zarodków /mg/ | RNA content zawartość RNA | | radioactivity of RNA radioaktywność RNA | | |
| | | P µg/1 g of preparation P µg/1 g preparatu | P µg/100 embryos P µg/100 zarodków | cpm/1 g of preparation imp/min/1 g preparatu | cpm/100 embryos imp/min/100 zarodków | cpm/1 mg of P imp/min/1 mg P | | P µg/1 g of preparation P µg/1 g preparatu | P µg/100 embryos P µg/100 zarodków | cpm/1 g of preparation imp/min/1 g preparatu | cpm/100 embryos imp/min/100 zarodków | cpm/1 mg of P imp/min/1 mg P |
| 48 | 1.5 | 4.5 | 6.7 | 30.1 | 45.2 | 6747 | 2.1 | 5.2 | 6.7 | 27.0 | 56.8 | 8477 |
| 96 | 3.0 | 8.3 | 25.0 | 66.0 | 198.0 | 7920 | 4.5 | 5.8 | 26.0 | 142.0 | 638.0 | 24538 |

Table 4 - Tabela 4

$^{32}P$ incorporation into phospholipids of barley seed embryos during soaking

Włączanie $^{32}P$ w fosfolipidy zarodków pęczniejącego ziarna jęczmienia

| Soaking, hr<br>Czas pęcznienia w godz. | Embryos of dormant seeds<br>Zarodki ziarna spoczynkowego | | | | | Emoryos of seeds treated with $GA_3$<br>Zarodki ziarna traktowanego $GA_3$ | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | phospholipids content expressed as zawartość fosfolipidów w | | radioactivity of phospholipids radioaktywnośc fosfolipidów | | | phospholipids content expressed as zawartość fosfolipidów w | | radioactivity of phospholipids radioaktywność fosfolipidów | | |
| | P µg/1 g of preparation P µg/1 g preparatu | P µg/100 embryos P µg/100 zarodków | cpm/1 g of preparation imp/min/1 g preparatu | cpm/100 embryos imp/min/100 zarodków | cpm/1 mg of P imp/min/1 mg P | P µg/1 g of preparation P µg/1 g preparatu | P µg/100 embryos P µg/100 zarodków | cpm/1 g of preparation imp/min/1 g preparatu | cpm/100 embryos imp/min/100 zarodków | cpm/1 mg of P imp/min/1 mg P |
| 48 | 385 | 29.0 | 1728 | 130 | 4484 | 490 | 49 | 2900 | 290 | 5918 |
| 96 | 308 | 30.0 | 510 | 50 | 1600 | 234 | 30 | 3015 | 380 | 12660 |

of embryos both during the period of rest of barley seed, and after its natural recession (Kulka and Rejowski 1970).

The influence of $GA_3$ on ribosomal and mitochondrial RNA synthesis was stronger than on total RNA synthesis. In contrast, however, to total RNA, a distinct increase in $^{32}P$ incorporation into the fractions studied occurred only as late as the 96th hour of incubation of the seed with $GA_3$. Specific activity (per 1 mg of P) of ribosomal RNA was three times higher, and total activity (per 100 embryos) even four times higher than when grain was soaked without the stimulator. The specific and total activity of the mitochondrial RNA fraction was also three times higher (Table 3).

These results confirm the suggestion of Chrispeels and Varner (1967b) that application of the hormone causes a general depression of RNA synthesis, and not a preferential enhancement of the synthesis of one fraction.

$GA_3$ also had a stimulating effect on phospholipid synthesis. $^{32}P$ incorporation into the phospholipids of resting seed embryos after 96 hr of soaking became less intensive, whereas the absolute amount of these compounds did not change practically (Table 4).

During incubation of seeds with $GA_3$, the radioactivity of the embryonal phospholipids increased, this increase being most pronounced after 96 hr. The possibility of a *de novo* synthesis of phospholipids in embryos parallelly with their translocation to the endosperm has already been pointed out by Hall and Hodges (1966).

The phospholipids content in embryos of $GA_3$-treated seed after 48 hr was much higher as compared with that in resting seeds. Further incubation associated with breaking of dormancy reduced the phospholipids to their level in resting seed embryos, although their synthesis at this time was much more intensive. This was probably due to the rapid utilization of phospholipids by the developing embryo (Varner 1965; Hall and Hodges 1966).

The present investigations revealed that breaking of dormancy of barley seeds by $GA_3$ is associated with an enhanced RNA and phospholipids synthesis in the embryos. This may be interpreted as the result of direct action on them of this hormone. It cannot, however be excluded that, as shown by Amen's model (1968), this synthesis is stimulated by cytokinins formed in the endosperm under the influence of hydrolytic enzymes activated by $GA_3$.

## SUMMARY AND CONCLUSIONS

The influence of $GA_3$ on RNA and phospholipid synthesis was investigated in the embryos of resting spring barley seeds (Browarny PZHR) with the use of $^{32}P$. The content of RNA and phospholipids and the radioactivity of these substances were determined in intact embryos as well as in the ribosomal and mitochondrial fractions.

If was found that:

1. Gibberellic acid breaks the dormancy of spring barley seeds. After 4 days of incubation with $GA_3$ the seeds germinated in 75 percent, and the dry weight of the embryos increased.

2. Breaking of dormancy is associated with a general enhancement of RNA synthesis in the embryos. The absolute value of this compound in intact embryos does not undergo major changes.

3. RNA synthesis was particularly stimulated by $GA_3$ in the mitochondria and in the ribosomal fraction. The absolute RNA content also increased in these fractions.

4. Phospholipid synthesis in barley seed embryos increases in the course of the entire period of incubation with $GA_3$. The total content of these compounds decreases, however, this indicating a rapid phospholipid utilization by the developing embryo.

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## *Wpływ kwasu giberelowego na syntezę kwasów nukleinowych w spoczynkowym ziarnie jęczmienia jarego*

Streszczenie i wnioski

Zbadano wpływ $GA_3$ na syntezę RNA i fosfolipidów w zarodkach spoczynkowego ziarna jęczmienia jarego (Browarny PZHR), stosując $^{32}P$. Oznaczano zawartość i radioaktywność RNA i fosfolipidów w całych zarodkach oraz zawartość i radioaktywność RNA we frakcji rybosomalnej i mitochondrialnej.

Stwierdzono, że:

1. Kwas giberelinowy przerywa spoczynek głęboki ziarna jęczmienia jarego. Po 4 dniach inkubacji z $GA_3$ ziarno kiełkuje w 75%; zwiększa się również sucha masa zarodków.

2. Przerwaniu spoczynku towarzyszy ogólne wzmożenie syntezy RNA w zarodkach. Absolutna zawartość tego związku w całych zarodkach natomiast nie ulega zasadniczym zmianom.

3. Szczególnie dużą stymulację syntezy RNA pod wpływem $GA_3$ wykryto w mitochondriach oraz we frakcji rybosomalnej. We frakcjach tych miał również miejsce wzrost absolutnej zawartości RNA.

4. Synteza fosfolipidów w zarodkach ziarna jęczniemia wzrasta w ciągu całego okresu inkubacji z $GA_3$. Ogólna zawartość tych związków zmniejsza się jednak, co wskazuje na szybką utylizację fosfolipidów przez rozwijający się zarodek.