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# Effect of $GA_3$ on ribonuclease activity in the embryos and endosperm of spring barley grain in the post-harvest dormancy period

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Abstract:

The effect of $GA_3$ on ribonuclease activity in the embryos and endosperm of barley seeds was investigated during dormancy and after its end. Incubation of dormancy grain with $GA_3$ stimulated its germination, increased the dry weight of the embryos and their protein content. Ribonuclease activity in seeds treated with $GA_3$ was considerably enhanced. The increase was more rapid and intensive in the embryos than in the endosperm. The action of $GA_3$ was most effective, particularly as regards the endosperm, on grain which had already passed through the stage of dormancy and had reached physiological maturity. This is evidence that the internal factor regulating the process of dormancy exerts a stronger influence on the activity of ribonuclease than does exogenic giberrellic acid.

## INTRODUCTION

It has been demonstrated in earlier papers (Rejowski and Kulka, 1967; Kulka and Rejowski, 1970) that barley resting grain embryos have but a low ability of RNA synthesis. When the state of rest of the grain is over, the rate of $^{32}P$ incorporation into the embryonal RNA greatly increases and so does its absolute content.

Breaking of grain dormancy by the action of $GA_3$ enhances RNA synthesis in the embryos, particularly in the ribosomal fraction and in the mitochondria. Total RNA content, however, does not undergo significant changes (Rejowski and Kulka, 1970). The slight increase of the RNA value observed simultaneously with the intensive synthesis of this compound seems to indicate its continuous breakdown owing to the enhanced nucleolytic enzymes activity.

The present study was undertaken in order to confirm this sugestion. RN-ase activity was investigated in the embryos and endosperm of $GA_3$-treated barley in the period of dormancy and after its breaking.

## METHODS

Morphologically ripe spring barley grain (Browarny PZHR) was used for the investigations. The grain was stored at room temperature in air-dry state. Samples for examination were collected 8, 24 and 68 days after the harvest. The dates of sampling for analysis were established on the basis of the results of earlier experiments (Rejowski and Kulka, 1967), and after testing the germination power of the grain in the present work. On the 8th day after the harvest the grain was in a state of deep dormancy, after 24 days rest was partly broken and on the 64th day it had receded completely. The grain taken for analysis was soaked in $GA_3$ solution (100 mg/l.). As control served grain swelling in water. After 48 and 96 h the quantitative changes in dry weight, protein content and RN-ase activity were determined in the embryos and in the endosperm separately. All analyses were replicated four times.

Protein. The material was homogenised (embryos and endosperm separately) in phosphate buffer, pH 6.2. Protein was precipitated from the homogenate with 8 per cent trichloroacetic acid. The sample was then cooled in a refrigerator for half an hour and centrifuged at 4 000 g. The supernatant was discarded and the precipitate was washed with acetone. The latter was then dissolved in 1 N NaOH in the course of 12 h at 37°C, and protein was determined colorimetrically by Lowry's method.

Ribonuclease was determined in 100 embryos and in the endosperm of 100 grains. The material was ground in 12 ml of 0.1 M phosphate buffer, pH 6.2. at 0°C and the homogenate was passed through four cheesecloth layers. The filtrate was made up with buffer to 15 ml.

Ribonuclease activity was determined after Anfinsen (according to Szarkowski, 1965) but with different proportions of the reactants. For analysis 0,5 ml of homogenate and 2 ml of 0.2 per cent RNA were taken in 0.1 M phosphate buffer and incubated for 1 h at 37°C. The reaction was interrupted with uranyl acetate (0,5 ml of 0.75 per cent uranyl solution in 25 per cent perchloric acid). The sample was then cooled for 15 min in the refrigerator and centrifuged at 4 000 g. Extinction was measured in the supernatant at 260 nm in a 1-cm layer. The control sample to which extinction was referred contained the enzyme and was incubated without RNA which was added together with uranyl acetate after incubation.

The quantity of enzyme which under the above described conditions and in the given dilutions increased the extinction in the supernatant $E^{1\,cm}_{260\,nm}=0.01$ was adopted as unit of ribonuclease activity (R.u.). Specific activity was expressed as the number of ribonuclease units calculated to 1 mg of protein (Szarkowski, 1965).

## RESULTS AND DISCUSSION

The possibility of breaking the dormancy of barley seed by the action of $GA_3$ has been demonstrated in the previous paper (Rejowski and Kulka, 1970). The present experiments confirmed the earlier data (Table 1). On the 8th day after the harvest the grain soaked in water germinated in 13 per cent, whereas the

Table 1

Germination power of barley seed (%) in the period of dormancy

| Medium | No. of days after harvest | | |
|---|---|---|---|
| | 8 | 24 | 68 |
| Water | 13 | 59 | 98 |
| $GA_3$ | 63 | 90 | 100 |

germination power of that incubated with $GA_3$ increased to 63 per cent. This considerable difference in germination was still observed on the 24th day. Only as late as the 64th day did the germination power of both samples become practically equal.

Breaking of dormancy and initiation of growth processes were associated with an increase in dry weight of the embryos and of the protein content in them, together with corresponding losses in the endosperm (Table 2).

Total and specific ribonuclease activity in the embryos and in the endosperm of grain soaked in water distinctly increased as the state of dormancy receded (Table 3). This increase was noticeable in the embryos as early as after 48 h of soaking, whereas in the endosperm it could be revealed after 96 h. In the endosperm of dormant seeds (8th day after harvest) the activity of the enzyme was unchanged.

The results concerning the increased ribonuclease activity in embryos agree with those obtained by Kulka (1969).

Under the influence of $GA_3$, the ribonuclease activity in dormant seed increased significantly, and like in the case of natural breaking of dormancy, this enhancement of activity occurred more rapidly in the embryos than in the endosperm (table 3). After 48 h of incubation with $GA_3$, the ribonuclease activity in the embryos was doubled, whereas in the endosperm a similar enhancement was noted as late as after 96 h.

Treatment of seeds with gibberellin after partial recession of dormancy (24 days after the harvest) produced a further increase of ribonuclease activity. $GA_3$ proved most effective, particularly as regards the endosperm which has already passed through the period of dormancy and reached physiological maturity (64 days after harvest). Ribonuclease activity in the embryos of these seeds as compared with that in dormant seeds treated with $GA_3$ increased more than two times, and in the endosperm as much as several times.

The enhanced activity of hydrolytic enzymes in the endosperm of cereals under the influence of exogenic gibberellic acid has been demonstrated in many papers (Chrispeels and Varner, 1967; Jacobsen and Varner, 1967, Jones, 1969; Pollard, 1969).

All these investigations aimed at the elucidation of the mechanism of $GA_3$ action were performed in isolated aleurone layers. The purpose of the present study like that of earlier ones (Rejowski and Kulka, 1967 and 1970; Rejowski, 1970) was the elucidation of the biochemical aspects of dormancy in barley under natural

Table 2

Effect of $GA_3$ on dry weight and protein content (mg) in embryos and endospern of barley in the period of dormancy (calculated to 100 seeds)

| Spaking | | Material | No. of days after harvest | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 8 | | 24 | | 68 | |
| Time, hrs | Medium | | dry weight | protein | dry weight | protein | dry weight | protein |
| 48 | Water | embryos | 142,0 | 30,47 | 174,8 | 35,63 | 188,3 | 37,50 |
| | | endosperm | 3579,6 | 210,00 | 3701,2 | 192,19 | 3708,8 | 202,81 |
| | $GA_3$ | embryos | 171,0 | 38,75 | 207,8 | 41,56 | 246,4 | 43,59 |
| | | endosperm | 3529,1 | 204,43 | 3689,8 | 182,87 | 3568,2 | 189,25 |
| 96 | Water | embryos | 168,1 | 32,47 | 264,4 | 48,21 | 489,5 | 72,12 |
| | | endosperm | 3587,4 | 198,12 | 3542,4 | 175,31 | 3463,4 | 175,69 |
| | $GA_3$ | embryos | 373,9 | 62,81 | 491,0 | 77,56 | 649,2 | 83,75 |
| | | endosperm | 3265,8 | 182,50 | 2149,9 | 161,76 | 3157,4 | 152,56 |

Table 3

Changes in total RN-ase activity and specific activity mg protein) calculated to 100 seeds

| Soaking | | Material | No. of days after harvest | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 8 | | 24 | | 68 | |
| Time, hrs | Medium | | total activity | specific activity | total activity | specific activity | total activity | specific activity |
| 48 | water | embryos | 21 | 0,69 | 35 | 0,99 | 38 | 1,02 |
| | | endosperm | 85 | 0,40 | 82 | 0,43 | 85 | 0,42 |
| | $GA_3$ | embryos | 43 | 1,11 | 65 | 1,56 | 112 | 2,57 |
| | | endosperm | 85 | 0,42 | 102 | 0,55 | 113 | 0,60 |
| 96 | water | embryos | 35 | 0,89 | 91 | 1,88 | 163 | 2,62 |
| | | endosperm | 82 | 0,42 | 145 | 0,79 | 315 | 1,79 |
| | $GA_3$ | embryos | 145 | 2,31 | 187 | 2,41 | 342 | 4,08 |
| | | endosperm | 150 | 0,82 | 195 | 1,20 | 734 | 4,81 |

conditions, with special reference to the interaction of the embryo with the endosperm. The ribonuclease activity in the embryo and endosperm is influenced in this case not only by exogenic gibberellic acid, but also by endogenic changes in the complex inhibitor-promotor, regulating the process of dormancy.

When analysing the data in table 3, it is noteworthy that the activity of the enzyme in the endosperm of physiologically mature seed soaked in water is much higher than in that of dormant grain treated with $GA_3$. This difference, though somewhat smaller, is noted also in relation to seed incubated with $GA_3$ after partial breaking of dormancy, although the germination power of the samples compared and the increase in dry weight of the embryos are closely similar (cf. Tables 1 and 2). On the other hand, after complete recession of dormancy, the ribonuclease activity in the endosperm treated with $GA_3$ increases more than two times.

These data suggest that the internal factors regulating the process of dormancy exert a higher influence on the enzyme activity in the endosperm than exogenic gibberellic acid.

In contrast to ribonuclease activity in the endosperm, that in the embryo of dormant seed incubated with $GA_3$ is almost equal to, and after partial breaking of dormancy even exceeds the activity of the enzyme in embryos of physiologically mature grain soaked in water.

It may, therefore, be supposed that the mechanism of reaction of the embryo and the endosperm to $GA_3$, is different.

Similar conclusions result from the investigations of Pinfield and Stobart (1969) concerning the influence of $GA_3$ on nucleic acids metabolism in the embryos and endosperm of hazel seeds. According to these authors the embryo is the site of the primary reaction to $GA_3$ whereas the reaction of the endosperm is secondary.

The factor depressing the reaction of the endosperm to $GA_3$ is probably an inhibitor occurring in large quantities in dormant barley seed. This inhibitor has been identified on the basis of chromatographic and spectrophotometric analysis as abscisic acid (ABA) (Rejowski, 1967 and 1970). The physiological role of this inhibitor on the course of rest of barley seed and the influence of the former on nucleic acids synthesis have been discussed in an earlier paper (Rejowski and Kulka, 1967).

Chrispeels and Varner (1967b) report an antogonistic action of $GA_3$ and ABA on the synthesis of enzymes in the isolated aleurone layers of barley. These authors suggest that ABA inhibits specific m-RNA synthesis or else inhibits m-RNA incorporation into the complex synthesising the enzyme. Numerous data seem to indicate that $GA_3$ and ABA exert antagonistic influences on the process of dormancy (cf. Amen, 1968).

It is interesting that the introduction of $GA_3$ only partly reverses the inhibitory action of ABA (Villiers, 1968; Shih and Rappaport, 1970). This confirms the results of the present experiments, in which, after incubation of dormant seed with $GA_3$, ribonuclease activity in the endosperm was much lower than in the endosperm of physiologically mature grain soaked in water.

The intensive synthesis of RNA detected in the earlier studies in embryos of resting barley seed treated with $GA_3$ with a simultaneous only slight increase of its absolute amount is probably connected with the high activity of ribonuclease in these embryos.

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## *Wpływ $GA_3$ na aktywność rybonukleazy w zarodkach i bielmie ziarna jęczmienia jarego w okresie spoczynku pożniwnego*

Streszczenie

Zbadano wpływ $GA_3$ na aktywność rybonukleazy w zarodkach i bielmie ziarna jęczmienia podczas spoczynku. Wyniki badań pozwalają na sformułowanie niżej podanych stwierdzeń.

Przerwanie spoczynku ziarna jęczmienia przy pomocy $GA_3$ i inicjacja procesów wzrostowych

związane są ze zwiększeniem suchej masy zarodków i zawartości w nich białka. Pod wpływem $GA_3$ wzrasta również w spoczynkowym ziarnie jęczmienia aktywność rybonukleazy, przy czym wzrost ten w zarodkach jest szybszy i intensywniejszy niż w bielmie.

W miarę ustępowania spoczynku reakcja bielma na $GA_3$ wzmaga się. Czynnikiem obniżającym reakcję bielma na kwas giberelinowy jest prawdopodobnie inhibitor występujący w znacznych ilościach w spoczynkowym ziarnie.

Wykryta w poprzednich badaniach intensywna synteza RNA w zarodkach spoczynkowego ziarna jęczmienia traktowanego $GA_3$, przy jednoczesnym nieznacznym tylko wzroście jego absolutnej ilości, związana jest prawdopodobnie z wysoką aktywnością w nich rynobukleazy.

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