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# The influence of gibberellin on the level of soluble protein and activities of some hydrolases in corn seedlings

P. MASŁOWSKI and H. MASŁOWSKA

Institute of Biology, Department of Biochemistry, N. Copernikus University, Toruń, Poland

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Abstract:

The effect of gibberellin on the qualitative and quantitative composition of soluble proteins and the activity of $Mg^{++}$ dependent and $Na^{+}$-$K^{+}$-stimulated ATPase, RNase and peptidase in five days old maize seedlings were investigated.
The chromatography of soluble proteins on DEAE cellulose gave seven fractions. All these fractions had ATPase and RNase activities.
Gibberellin stimulated most strongly ATPase, to a smaller degree RNase and it did not effect the activity of peptidase.

## INTRODUCTION

In recent years hormones have been assigned mediating functions, at the level of informational transcription or translation processes, linking environmental and genetic control mechanism (Overbeek 1966; Key 1969). It is not excluded, however, that GA effects directly on the activity of various enzymes, bringing about consequently the changes in the cell metabolism (Paleg 1965, Jacobsen and Varner 1967; Clifford and Pollard 1969).

The aim of this work was to investigate the influence of $GA_3$ on qualitative and quantitative changes of soluble proteins in maize seedlings, with the special regard to the $Mg^{++}$ dependent and $Na^{+}$, $K^{+}$-stimulated ATPase, RNase and peptidase.

## MATERIAL AND METHODS

*Material.* The maize seeds var. 'Wigor' from the Scientific Station IHAR in Bąków were used for experiments. The seeds were surface sterilised by treatment with 0,1% solution of mercuric chloride and then one part was soaked for 24 h in distilled water, the other one — in water solution of gibberellin ($GA_3$) 100 mg/l.

Then the seeds germinated in the dark in water at 25° for 4 days. From the moment of seedlings occurence, they were sprayed twice a day with the above solution of $GA_3$.

The shoots of similar height were taken for the analysis, washed throughly with distilled water and dried between two sheets of filter paper.

*Extraction of soluble proteins.* 30 g of seedlings were homogenised with a mortar and pestle in 50 mM Tris-HCl buffer, pH 7,6, containing 5 mM of cysteine. (3 ml/g tissue). The homogenate was centrifuged at 1500 × g for 15 min. and then at 20000 × g for 20 min. Supernatant was dialysed for 30 h against the same buffer changed four times. The dialysed extract was centrifuged again (20000 × g for 30 min) and lyophilised to 6 ml. Protein was determined by the micro-method of Kjeldahl.

*DEAE — chromatography.* 3 ml of the protein solution were loaded on the column (2,5 × 35 cm) with DEAE-cellulose, equilibrated with 50 ml Tris-HCl buffer, pH 7,6. The chromatography was done using a linear gradient of NaCl. Flow rate was 10 drops/min.

The optical density (E) of fractions was measured in 1 cm light path at 280 nm.

*Assay of the ATPase activity.* The activity $Mg^{++}$ dependent and $Na^+$, $K^+$ stimulated-ATPases was assayed by the method of Abdel-Latif at al. (1967). As a basal medium the 50 mM Tris-HCl buffer, pH 7,6 with the addition of 2 mM ATP was used. Three kinds of incubation medium were used: a) without ions, b) with 2 mM $MgSO_4$, c) with 2 mM $MgSO_4$, 25 mM KCl, 25 mM NaCl.

1 ml of incubation medium was put into test-tubes, kept at 37° in order to level the temperature, and then every 30 sec 1 ml of the enzyme solution was added. The reaction was stopped by the addition of 3 ml of ice-cold 5% TCA.

The $P_i$ released from ATP was assayed by the method of Lowry and Lopez (1960).

As an activity unit of ATPase 1 nM of liberated $P_i$/1 ml/1 min. was taken.

*Determination of RNase activity.* The method Kessler and Engelberg (1962), with our slight modifications, were used.

5 g of cooled to 2° seedlings were ground with 0,1 M sodium phosphate-citrate buffer, pH 6. The homogenate was strained through 2 layers of cheese cloth and centrifuged at 20000 × g for 20 min. To 1 ml of supernatant 1 ml of 0,5% RNA solution in 0,1 M sodium phosphate-citrate buffer pH 6 was added and kept for 60 min. at 37°. The reaction was stopped by the addition of 1 ml of 0,3% uranyl acetate in 0,2 M $HClO_4$. The samples were left overnight in the refrigerator and then centrifuged at 7000 × g.

The O.D. of supernatant was measured in Spectrophotometer MOM in 1 cm light path at 260 nm.

One enzyme unit corresponds to an increase of $E_{260}$ of 1.0 per ml and 60 min incubation.

*Determination of peptidase activity.* Peptidase activity was determined by Anderson and Rowan (1965) method.

5 g of seedlings were ground in mortar with 0,2 M sodium phosphate-citrate buffer, pH 5,5. The resulting precipitate was removed by centrifugation at 20000 × g

for 30 min. The supernatant was dialysed against 2000 ml of buffer at 1° and recentrifuged at 20000 × g for 20 min. 2 ml of investigated solution was incubated with 2 ml of 0,4% gelatin in sodium-acetate buffer pH 5,5 for 6 h at 37°. Peptidase activity was determined by measuring the released α-amino nitrogen (Cocking, Yemm 1954). One enzyme unit corresponds to an increase of $E_{570}$ of 0,1/ml/60 min.

Four series in triplicate were performed in each experiment.

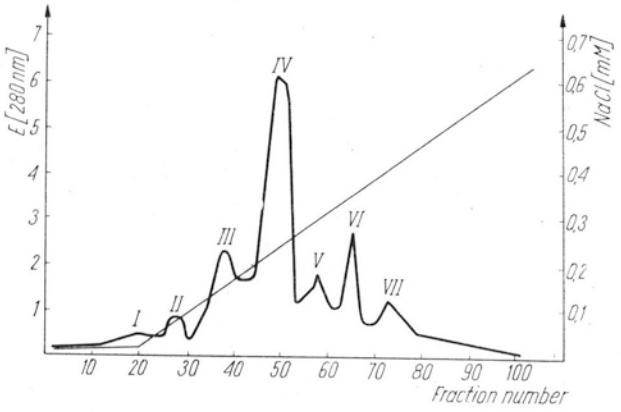


Fig. 1. DEAE-cellulose column chromatogram of soluble protein components in 5 days old maize seedlings — without $GA_3$

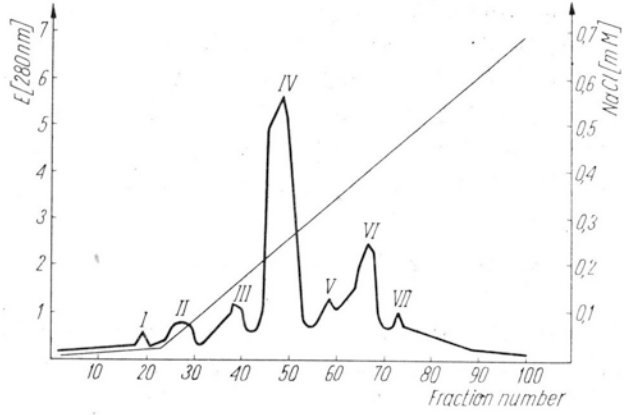


Fig. 2. DEAE-cellulose column chromatogram of soluble protein components $GA_3$ treated 5 days old maize seedlings

## RESULTS and DISCUSSION

The results of investigations presented in table 1 show, that seedlings treated with gibberellin contain 27% of soluble proteins more as compared with control samples.

Column chromatography on DEAE cellulose resolved the protein containing supernatant extract into 7 fractions in the case of control and gibberellin treated samples (Figs. 1 and, 2).

Table 1

Effect of gibberellin on the content ot soluble proteins in maize seedlings

| Sample | Proteins in mg/100 seedlings | Proteins in mg/g fresh weight |
|---|---|---|
| Not treated $GA_3$ | 143 | 3.26±0.05 |
| Treated $GA_3$ | 178 | 4.13±0.08 |
| % in relation to controls | | 126.7 |

The data presented in table 2 show, that the absolute increase of the protein level under the influence of $GA_3$ was the result of the increase of fractions I, III, IV and V. (193%, 251,5%, 117,4%, 178,4%, respectively).

In table 3 there are data, concerning the changes of ATPase, RNase and peptidase activities under the influence of $GA_3$. These results indicate the considerable effect of this hormone on the activity of determined hydrolases in maize seedlings. The greatest effect is obtained in case of basal ATPase (31,4%). The addition of $Mg^{++}$, $Na^{+}$ and $K^{+}$ decreases the effect of $GA_3$ on ATPase by about 5%.

Comparing also the ribonuclease activity in extracts from gibberellin treated and control seedlings, the increased activity of preparations with $GA_3$ by 23% is observed. (Tab. 3). No greater influence of this hormone on peptidase activity was detected, This influence was rather negative.

It should be noted that ATPase and RNase activity was present in all peaks. These facts suggest both a wide distribution of enzyme activity in the elution profile and also the heterogenous composition of the individual peaks.

No detailed analysis of individual fractions was done, therefore it is difficult to say whether the increase of particular proteins was connected with the synthesis de novo.

There are known cases, that the enzymes activity was increasing even if the level of proteins was decreasing (Anderson, Rowan 1965; Kawashima at al. 1967a; Atkin and Srivastava 1969).

The evident lack of newly formed fractions of proteins suggests, that gibberellin-induced changes are quantitative rather than qualitative.

The limited distributive ability of DEAE cellulose might not show small changes in the composition of these proteins .

Table 2

Changes in amount of soluble protein components in 5 days old maize seedlings

| Sample | Soluble protein components mg/g seedlings | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Fraction I 15—22 | Fraction II 22—30 | Fraction III 30—43 | Fraction IV 43—55 | Fraction V 55—60 | Fraction VI 60—68 | Fraction VII 68—75 | Total protein components |
| Not treated | 0.043±0.004 | 0.238±0.013 | 0.338±0.038 | 1.348±0.049 | 0.255±0.019 | 0.751±0.023 | 0.149±0.007 | 3,3 |
| Treated $GA_3$ | 0.083±0.007 | 0.187±0.008 | 0.850±0.222 | 1.583±0.055 | 0.455±0.015 | 0.397±0.014 | 0.116±0.012 | 4.19 |
| % in relation to controls | 193±3,91 | 76.4±2.18 | 251.5±2.49 | 117.4±2.89 | 178.4±3.04 | 55.5±2.01 | 78.1±2.2 | 127 |

Table 3

Effect of $GA_3$ on the RNase, peptidase and ATPase activities in maize seedlings (Enzyme unites per 1 g of fresh tissue)

| Enzyme | Control | $+GA_3$ | % of control |
|---|---|---|---|
| ATPase $-Mg^{++}$ | 5.4±0.13 | 7.1±0.19 | 131.4±2.2 (10) |
| ATPase $+Mg^{++}$ | 9.4±0.14 | 11.9±0.21 | 126.5±1.09 (10) |
| ATPase $+Mg^{++}, Na^{+}, K^{+}$ | 11.8±0.15 | 14.9±0.17 | 126.2±1.36 (10) |
| RNase | 412.00±2.26 | 510.00±3.12 | 123.7±5.3 (8) |
| Peptidase | 10.7±0.54 | 9.8±0.28 | 91.5±2.7 (10) |

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## *Wpływ gibereliny na poziom białek rozpuszczalnych i aktywność niektórych hydrolaz w kiełkach kukurydzy*

Streszczenie

Badano wpływ gibereliny na skład jakościowy i ilościowy białek rozpuszczalnych oraz aktywność $Mg^{++}$ i $Na^{+}$ -$K^{+}$-ATPazy, RNazy i peptydazy w pięciodniowych kiełkach kukurydzy.

Białka rozpuszczalne rozdzielano na DEAE celulozie. Uzyskano 7 frakcji zarówno w próbie kontrolnej, jak i z dodatkiem $GA_3$, różniących się tylko ilościowo. Wszystkie te frakcje wykazywały aktywność ATPazową i RNazową.

Giberelina najsilniej stmulowała ATPazy, w mniejszym stopniu RNazę, a nie wpływała na aktywność peptydazy.