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Preliminary report

Changes in chloroplastic and cytoplasmic ribosomal protein after GA_3-treatment of *Zea mays* leaves

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Abstract

Protein was isolated from chloroplastic and cytoplasmic ribosomes of 14-day-old maize leaves subjected to the action of gibberellic acid. The proteins were separated electrophoretically on polyacrylamide gel. Fourteen fractions of ribosomal protein were obtained exhibiting wide electrophoretic differences. Qualitative differences were found between the chloroplastic and cytoplasmic ribosomes. Gibberellic acid caused the appearance of an additional protein fraction in cytoplasmic ribosomes. It did not, however, affect the qualitative composition of ribosome proteins from chloroplasts.

INTRODUCTION

There is some evidence that gibberellic acid (GA_3) affects de novo synthesis of α-amylase in isolated barley aleuron (V a r n e r and C h a n d r a 1964; F i l n e r and V a r n e r 1967; J a c o b s o n et al. 1970). Recently E v i n s and E v i n s et al. (1971) have reported that GA_3 affects synthesis by ribosomes and endoplasmic reticulum in barley aleuron.

Changes in quantitative content of ribosomal proteins in plants treated with GA_3 could also to be expected. The investigation of this phenomenon is the subject of the present paper.

MATERIALS AND METHODS

1. Materials

Fodder corn 'Wigor' from the Experimental IHAR Station in Bąków was used in these experiments. Seeds sterilized by sodium hypochlorite

were immersed in distilled water for 24 hours (control) or in AG_3 solutions (Gibrescol — Kutno Pharmaceutic Works "Polfa") at 10 mg% concentration. After the seeds had swollen they were placed in sterilized sawdust and cultured for 14 days at room temperature with glow-lamp light with an intensity of 5000 lux. During growth the experimental plants were sprinlled with a solution of gibberellic acid (same concentration as above) and control plants with water.

2. Isolation of ribosomes

200 g of corn leaves were washed several time with redistilled water and chloroplasts and chloroplastic ribosomes were isolated by the method of Ruppel (1969). Cytoplasmic ribosomes were isolated and purifed by the method of Lyttleton (1968). The obtained solutions of ribosomes were clear and light yellow in colour. All manipulations during isolation and purification of ribosomes were perfmored at 0-2°C.

3. Isolation of ribosomal proteins

Ribosomal proteins were extracted with lithium chloride (Spitnik-Elson 1965) in the following way. To 15-20 mg of ribosomes 1 ml 0.035 M β-alanine buffer pH 4.5 containing 0.3 g urea 0.1 g LiCl was added. The mixture was shaken for 48 hours at 0-2°C and the precipitated RNA was removed by centrifuging at 20 000 × g for 15 minutes. Protein was estimated by the method of Lowry et al. (1951).

Before electrophoresis the ribosomal proteins were dialyzed against 0.035 M β-alanine buffer pH 4.5 containing 6 M urea and were subsequently concentrated in dialysis lags in a flow of air at 4°C.

4. Electrophoresis on polyacrylamide gel

Electrophoretic separation of the ribosomal proteins was performed by the method of Williams and Reisfeld (1964) using 12% acrylamide and 6 M urea in 0.035 M β-alanine buffer pH 4.5. To each tube 100-200 μg of protein in 25% glicerol or 20% saccharose were applied.

The initial current intensity was 2 mA/tube; after the proteins had penetrated the gel the voltage was increased to obtain 4 mA/tube. The separation was performed for 110 minutes at a temperature not exceeding 10°C.

The gel after electrophoresis was stained with 1% amido black in 60% ethanol for 3 days. The excess of dye was removed electrophoretically in 7% acetic, acid, and the gel was additionally stained with 0.25% Coomassie blue in 7% acetic acid.

RESULTS AND DISCUSSION

Figures *A*, *B*, *C*, *D* present the separations of proteins from cytoplasmic and chloroplastic ribosomes of 14-day-old maize leaves of plants raised on water or water plus GA_3.

As can be seen above proteins from maize leaf ribosomes can be separated into 14 fractions of considerable electrophoretical diversity (Fig. 1 *A*, *C*). The electrophoretic appearance of proteins from cytoplasmic ribosomes is considerably different from that of proteins from chloroplastic ribosomes. These data suggest — in agreement with Sagan (1967) and Gualerzi and Cammarano (1970) — some genetic independence of chloroplasts the genome of which codes the synthesis of specific ribosomal proteins. The ribosomes of maize leaves subjected to gibberellic acid show an additional protein fraction "Y" (Fig. 1 *A*, *B*) of a considerable electrophoretic mobility which was not observed for chloroplastic ribosomes (Fig. 1 *C*, *D*). GA_3 thus probably causes de novo protein synthesis in cytoplasmic ribosomes. The excellent reproducibility of results of six series of experiments excludes the possibility of formation of artefacts during preparation or electrophoretic separation on polyacrylamide gel.

Further experiments are being performed to confirm this suggestion.

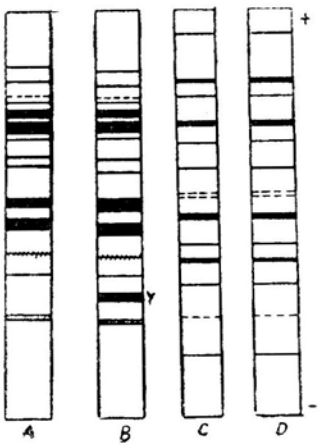

Fig. 1. Electrophoretic comparison of proteins extracted from chloroplastic and cytoplasmic ribosomes

A — cytoplasmic ribosomal protein (control), *B* — chloroplastic ribosomal protein + GA_3; *C* — chloroplastic ribosomal protein (control); *D* — chloroplastic ribosomal protein + GA_3

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Wpływ kwasu giberelowego na skład jakościowy białek rybosomów chloroplastowych i cytoplazmatycznych liści kukurydzy (*Zea mays*)

Streszczenie

Wyizolowano białka z rybosomów cytoplazmatycznych i chloroplastowych 14-dniowych liści kukurydzy, poddanych działaniu kwasu giberelowego.

Białka rozdzielano elektroforetycznie na żelu poliakrylamidowym. Uzyskano 14 frakcji białek rybosomalnych o dużym zróżnicowaniu elektroforetycznym. Stwierdzono różnice jakościowe między białkami rybosomów chloroplastowych i cytoplazmatycznych.

Kwas giberelowy indukował pojawienie się dodatkowej frakcji białkowej w rybosomach cytoplazmatycznych. Nie wpływał na skład jakościowy białek rybosomów z chloroplastów.