Chemistry of Natural Compounds, Vol. 30, No. 1, 1994

ENZYME PEROXIDASE IN CALLUSES OF COTTON PLANTS OF THE SPECIES *Gossypium hirsutum* AND *G. herbaceum*

A. A. Akhunov, Z. Golubenko, S.A. Dzhataev, and N. Cherkashina

UDC 577.15.158

A comparative study has been made of the activity of the enzyme peroxidase and its isozyme spectrum from two species of cotton plant. It has been found that, as calluses grow, new isoforms of peroxidase are produced and the protein content increases.

At the present time great attention is being devoted to genetically determined multiple forms of enzymes (isoenzymes), and these are giving information on various properties of biological systems at different levels — from the molecular level to the population level. The use of quantitative methods in the determination of the relative amounts of protein components or isoenzymes separated electrophoretically enables valuable information to be obtained on plant material.

We have investigated callus tissues of cotton plants of the species *Gossypium hirsutum* and *G. herbaceum* grown from hypocotyles. After various intervals of time (1-2 weeks) samples were taken from the calluses for the study of their isoenzyme compositions and peroxidase activities.

The extraction and isolation of the peroxidase were achieved with the aid of an alkaline Tris–glycine buffer, pH 8. 3, and in this process it was found by isoelectric focusing that it was mainly acid peroxidases that were extracted, with a very small amount of alkaline isoforms. We investigated the peroxidase isoenzymes isolated with the aid of an acidic ammonium acetate buffer, pH 5.4. We initially isolated and determined the peroxidase from seven-day cottonplant shoots grown on an agar medium and found that in the shoots, as compared with mature plants, the peroxidase activity was fairly low. At the same time, the amount of peroxidase and its activity were different in different parts of the shoots. The peroxidase of the leaves was the most active (115.8 nkat).

Callus tissues were grown from the hypocotyles of these shoots on a hormonal nutrient medium with agar. After predetermined intervals of time, samples were taken from the calluses and their activity and isoenzyme compositions were studied. In calluses taken 17 days after transfer the specific activity had risen 20-fold in comparison with the green cottonplant shoots. Electrophoresis showed the presence in the calluses of, in addition to the main isoform, giving an intense band (R_f 0.3), another component, with a low molecular mass (Fig. 1, *A*, *D*).

With the growth of the calluses, the peroxidase activity increased, the protein content changed (Fig. 2), and the isoenzyme composition of the peroxidase became more complicated. It is obvious that in the earlier calluses (up to the 70th or 80th day) low-molecular-mass isoforms (below the main band with R_f 0.3) were produced, in contrast to mature cotton plants in which high-molecular-mass forms predominated (Fig. 1, *B, C*).

We also investigated callus tissue from a cotton plant of the Indian variety *G. herbaceum* grown in a suspension nutrient medium. Six forms of the calluses, depending on the stage of development, were taken from the suspension: 1) proembryos; 2) globular embryos; 3) cordiform embryos; 4) bipolar embryos; 5) torpedo-shaped embryos; and 6) undifferentiated massive tissues. In the early stages of development, the peroxidase activities of these forms proved to be low, and this also applied to the calluses from the cottonplant species *G. hirsutum*. At the same time, with the aid of electrophoresis in an alkaline gel it was possible to detect that the intense band from calluses of the species *G. hirsutum* (R_f 0.32) was more mobile than that from calluses of the cotton plant of the other species (Fig. 1, *D, E*)

A. S. Sadykov Institute of Bioorganic Chemistry, Academy of Sciences of the Republic of Uzbekistan, fax 627071. Institute of Genetics, Academy of Sciences of the Republic of Uzbekistan, fax 642230. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 90-92, January-February, 1994. Original article submitted July 13, 1993.

0009-3130/94/3001-0081$12.50

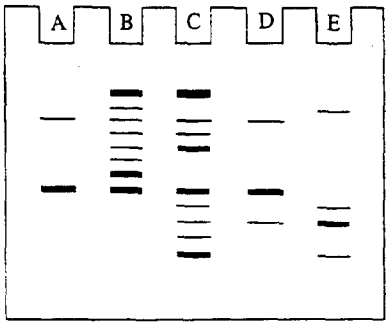

Fig. 1. Scheme of an electrophoretogram of cottonplant peroxidase: *A*) cottonplant shoots; *B*) peroxidase from the leaves of mature cotton plants; *C*) peroxidase from calluses (105th day of development); *D*) peroxidase from calluses (17th day of development); *E*) peroxidase from calluses of the species *G. herbaceum* (17th day of development).

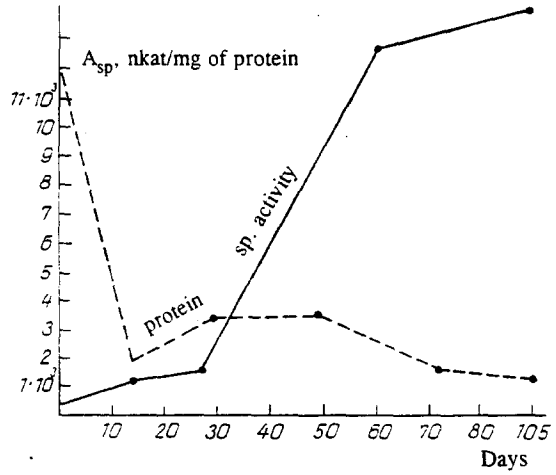

Fig. 2. Dependence of peroxidase activity on the time of development of calluses.

On the basis of the results given, it is possible to draw the conclusion that the isoperoxidase compositions of cotton plants vary considerably; with the growth of calluses the peroxidase activity and the protein content rise, and in cottonplant callus tissue in the early stage of development not more than 3-4 isoforms can be detected, while in mature plants grown under natural conditions the number of isoforms amounts to 7-8.

EXPERIMENTAL

Seeds of cotton plants of the species *Gossypium hirsutum* and *G. herbaceum* were obtained by the method of Trolinder and Goodin [1]. Pieces of hypocotyles of three-day shoots were sown onto a nutrient medium prepared from MS salts with the addition of 0.1 mg/liter of 2,4-D, 0.1 mg/liter of kinetin, and 30 g[/liter (?)] of glucose. After 17 days, part of the calluses was transferred to a hormone-free medium.

The peroxidase was extracted with alkaline Tris-glycine buffer, pH 8. 3. The calluses were ground in a mortar with cold buffer solution in a ratio of 0.5 g:2 ml of buffer. The homogenate was centrifuged at 7000 rpm for 20 min, and the peroxidase activity in the supernatant was determined by Boyarkin's method [2].

The electrophoretic separation of the peroxidases was carried out in alkaline 10% PAAG according to Davis [3], with revelation by benzidine.

Protein was determined by Lowry's method [4].

REFERENCES

1. N. L. Trolinder and S. R. Goodin, Plant Cell Reports, No. 6, 231 (1987).
2. A. N. Boyarkin, Biokhimiya, **16**, No. 4, 352 (1951).
3. L. Ornstein and B. J. Davis, Ann. N. Y. Acad. Sci., **121**, 132 (1964).
4. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, J. Biol. Chem., **193**, No. 1, 265 (1951).