AMINO ACID COMPOSITION AND TERMINAL AMINO ACIDS OF THE PHENOL OXIDASE OF THE COTTON PLANT

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We have studied the amino acid composition and terminal amino acids of the phenol oxidases of young leaves of the cotton plant isolated by the method described previously [1].

A sample (5-6 mg) was dissolved in 0.5 ml of 6 N HCl and heated under vacuum at 105°C. The hydrolyzate was analyzed on a type AAA-881 amino acid analyzer. The following substances were obtained (moles of amino acid per mole of protein): lysine 3; histidine 1; arginine 2; aspartic acid 5; threonine 5; serine 7; glutamic acid 3; glycine 4; alanine 4; valine 2; methionine 2; isoleucine 1; leucine 1; tyrosine 1; phenylalanine 1. The protein contained proline, but because of its low concentration its amount was not calculated. The results of an analysis of the protein oxidized with performic acid showed that it contained no cysteic acid. This indicates the absence of cysteine from the protein. The amount of tryptophan was determined by two methods. On hydrolysis of the protein with 14% $Ba(OH)_2$ solution (125°C, 20 h), no tryptophan was detected. The absence of tryptophan was confirmed by the reaction with p-dimethylaminobenzaldehyde.

The N-terminal amino acids were determined by the DNP and DNS methods. The DNP- and DNS-proteins were hydrolyzed with 6 N HCl in vacuum at 105°C for 16 h. The DNP- and DNS-(amino acid)s were identified in a thin layer of type KSK silica gel in various systems both by one-dimensional and by two-dimensional chromatography. Only one N-terminal amino acid was found — histidine.

The C-terminal amino acid was determined by hydrazinolysis and by the action of carboxypeptidase A. A solution of 4 mg of the freeze-dried protein in 0.5 ml of anhydrous hydrazine was heated at 105°C for 15 h. The excess of hydrazine was eliminated in a vacuum desiccator over H_2SO_4, and the residue was analyzed on an amino acid analyzer. Only one amino acid was found — serine. For the carboxypeptidase cleavage, 8 mg of protein was dissolved in 6 ml of bicarbonate buffer (pH 7.8), and 1 ml of enzyme solution (D_{280} = 0.5) was added. The mixture was incubated at 27°C. Samples were taken after 0.5, 1, 2, and 4 h and were analyzed in an amino acid analyzer. The following results were obtained, μmole: 0.5 h — Ser 0.02, Ala 0.017; 1 h — Ser 0.027, Ala 0.024; 2 h — Ser 0.027, Ala 0.024; 4 h — Ser 0.027, Ala 0.024. Consequently, the C-terminal amino acid is serine.

When the protein was hydrolyzed with 3 N HCl at 105°C, arabinose, xylose, galactose and glucose were detected in the hydrolyzate by the GLC method, their total amount coming to 26%.

Thus, the phenyl oxidase of the cotton plant contains a large number of hydroxy amino acids and dicarboxylic acids and a small amount of aromatic amino acids, and also sugars.

The N-terminal amino acid is histidine and the C-terminal acid is serine.

LITERATURE CITED

1. T. S. Yunusov and P. Kh. Yuldashev, Khim. Prirodn. Soedin., 122 (1974).

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