THE ACTION OF PHYTOTOXIC SUBSTANCES OF THE FUNGUS Verticillium dahliae ON THE PERMEABILITY OF A SYNTHETIC PHOSPHOLIPID MEMBRANE

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From the culture liquid of the fungus V. dahliae of the Yangiyul' population, by a method developed by workers in the Laboratory of the Chemistry of Natural Compounds of the V. I. Lenin State University two toxic substances of polypeptide and oligosaccharide nature have been isolated. The first of them is a red substance readily soluble in water, methanol, and acetone, and sparingly soluble in nonpolar solvents. It has the following elementary compositions (%): H 4.75; C 41.54; N 5.20 (weight of sample 5.580 mg; weight of residue 0.710 mg). By the method of Dodgson and Spenser in Nechaeva's modification [1] we have determined its sulfur content, which proved to be 2.15% and have qualitatively established the presence of iron. When the substance was hydrolyzed with 6 N HCl for 24 h, 15 amino acids were detected. When it was chromatographed on Silufol, the following Rf values were obtained: 0.86 [isopropanol-ammonia-water (10 : 1 : 1)] and 0.48 [butan-1-ol-acetic acid-water (4 : 1 : 5)].

The results of biological tests showed that this metabolite of polypeptide nature causes the formation of chlorotic zones and necrotic spots on the leaves of the cotton plant of variety 108-F when plants with cuts on the stems are immersed in a solution of it.

The characteristics of the substance of oligosaccharide nature have been described previously [2].



Fig. 1. Dependence of the conductivity of a APM on the concentration of the polypeptide factor (1) and the oligosaccharide factor (2).

To investigate the mechanism of the action of the phytotoxic substance of polypeptide nature - factor 1 and that of oligosaccharide nature - factor 2 - isolated from the culture liquid of the fungus V. dahliae, we studied their action on the permeability of an artificial phospholipid membrane (APM). The experiments were performed by a known method [3]. The factors 1 and 2 were dissolved in distilled water. The initial concentration was 10⁴ μ g/ml. Figure 1 shows the dependence of the conductivity of the APM on the concentrations of the factors 1 and 2 in an aqueous solution of K^+ and Na⁺ (1000 mM). As can be seen from Fig. 1, at a concentration of factor 1 of up to 50 μ g/ml the conductivity rises monotonically. A further increase in the concentration of factor 1 does not lead to a significant increase in conductivity. The gradient of factor 1 in the APM creates an additional potential from the side of high concentrations. The potential is close to theoretical in the region below 50 µg/ml. This shows that the increase in the conductivity of the APM in the presence of factor 1 in solution is due to the intrinsic transport of factor 1 in the form of a penetrating anion [3, 4].

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At a concentration below 100 μ g/ml, factor 2 decreases, and at a higher concentration it increases conductivity with a plus sign in a cell with a smaller concentration of factor 2. Thus, this substance is a penetrating cation. On this basis it is possible to explain the shape of the curve obtained with factor 2. The initial decrease in the conductivity of the APM in the presence of factor 2 (less than 100 μ g/ml) depends on the removal of the negative surface charge. The subsequent rise in conductivity (more than 100 μ g/ml) is created by the inherent transport of the factor through the APM.

On the basis of the results obtained it may be assumed that the phytotoxic substances of polypeptide and oligosaccharide nature of the fungus V. dahliae interact with plasmatic and intracellular membranes, change their functions, and, consequently, disturb the cell metabolism.

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