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STUDY OF THE FUNCTIONAL ROLE OF THE EXTENSIN-LIKE PROTEINS OF THE COTTON PLANT

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The action on myeloma X Ad8. 653 of extensin-like proteins isolated from a suspension culture and from two-day shoots of the cotton plant has been studied. A suppression of the inclusion of [^{3}H]thymidine with an increase in the concentration of protein was found. The presence of extensin-like proteins in cotton protoplasts has been revealed by cross-immunochemical analysis.

A characteristic feature of the membrane-bound glycoproteins of plants is their polyfunctionality due to to the diversity of the spatial structures of the carbohydrate components. It is assumed that it is precisely these components that carry the signal for recognition and may function as markers in intercellular interactions directed, in particular, to the protective strategy of plants.

The greatest attention of researchers has been attracted by extensins because of the uniqueness of their structure [1]. This group of proteins is highly glycosylated — 2/3 of the molecule consists of the carbohydrate components —, while the protein moiety is enriched with a rarely encountered amino acid — hydroxyproline [2]. It is known that extensins take part in the formation of the primary cell wall [3]; moreover, the level of these proteins rises sharply when plants are attacked by a pathogen [4] and under the action of low temperatures [5] and also in various stress states of plants [6]. Suggestions that glycoproteins of lectin nature cause the specific agglutination of cancer cells or retard the development of these cells while suppressing the synthesis of protein are extremely interesting [7].

In this connection, it appeared of interest to study the biological activity and the mechanism of the action of the extensin-like proteins of the cotton plant in the formation of the cell wall or the protective strategy of plants and the role of the glyco moiety in this with the use of monoclonal antibodies.

A convenient model for answering these questions is provided by plant protoplasts. Their lack of a cell wall and its formation during the process of cultivation permits an estimate of the roles of various agents and factors, and other effects.

With the aid of mcABs we have studied the presence of extensin-like proteins in cottonplant protoplasts. We had previously obtained a set of mcABs to the total membrane proteins of the cotton plant. Among them, the antibodies of clone 2C8.2 exhibited affinity both to the membranes and to the extensin-like proteins of the cotton plant in ELISA cross-immunochemical analysis [8]. The presence of these proteins in the plant protoplasts was evaluated by the same method. The cottonplant protoplasts were obtained enzymatically from a cultivated 5- to 6-month callus [9] and were used as the antigenic material (Table 1). The extensin-like proteins were isolated from the suspension culture and two-day cottonplant shoots by extraction with 0.2 M $CaCl_2$ using Lamport's method, with some modification [8]. The cross-reactivity of the mcABs of clone 2C8.2 both with the extensin-like proteins and with the protoplasts gave grounds for assuming the presence of these proteins on the surface of the protoplasts.

As another system for studying the biological action of the extensin-like proteins we selected a rapidly dividing culture of myeloma X Ag8.653 myeloma cells. It appeared of interest to determine what type of biological activity the glycoproteins isolated from the cotton plant possessed, namely: mitogenic, antimitogenic, or agglutinating capacity.

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TABLE 1. Immunochemical Analysis of mcABs*

mcABs	ΣMP	MPgr	Extensin-like proteins	Protoplasts
2C8.2	+	+	+	+

*Antigen–antibody (AT—AB) affinity was determined by ELISA solid-phase immunoenzyme analysis. Σ MP stands for the total membrane proteins, and MPgr the total membrane proteins fractionated in a 34/45 sucrose density gradient.

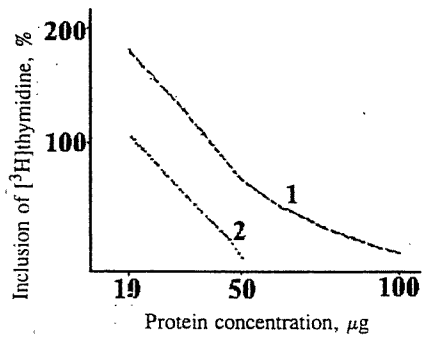

Fig. 1. Change in the proliferative activity of cells under the action of the extensin-like proteins: *1*) from two-day shoots; *2*) from a suspension culture.

One of the widely used methods for determining the activity of cells, in addition to morphological evaluation, is measurement of the inclusion of thymidine in the DNA being synthesized. Myeloma cells were cultivated in growth medium in 96-well plastic plates in a final volume of 500 μl in the presence of various concentrations of the extensin-like proteins for a day. The proliferative activity of the cells under the action of the protein was evaluated from the inclusion of [^{3}H]thymidine in the DNA. The results are shown in Fig. 1.

As can be seen from Fig. 1, with a rise in the protein concentration the inclusion of [^{3}H]thymidine in the DNA being synthesized decreased, and this process had a pronounced nature in the case of the proteins isolated from the suspension culture. At the same time, the proteins isolated from two-day shoots acted as a mitogen at a concentration of 10 μg , greatly increasing the inclusion of [^{3}H]thymidine in DNA (170%), while with an increase in the protein concentration the level of biosynthesis of DNA in the cells was depressed.

Thus, we have shown the presence of extensin-like proteins in plant protoplasts and also the influence of these proteins on the level of biosynthesis of DNA in animal cells.

Using the monoclonal antibodies, we shall study the role of the extensin-like proteins, especially the glyco part, for the fine morphogenetic control of the assembly of the cell wall during cell differentiation in the system of regulating the biosynthesis of cotton fiber and the protective strategy.

EXPERIMENTAL

Isolation of the Protoplasts. Cottonplant protoplasts were obtained enzymatically from a 5- to 6-month cultivated callus by treatment with an enzyme mixture containing 0.5% of cellulase (Sigma) and 0.6% of maserase (Calbiochem). The protoplasts were purified by the usual method [9].

Determination of the Proliferative Activity of Cells. Cells of myeloma XAg8.653 were sown in 96-well plastic plates at a density of 100 thousand cells per well (500 μl) in RPMI-1640 medium with 10% of serum and antibiotics.

The proteins were dissolved in the same growth medium without the serum. Cells with the preparation (50 μl) were incubated in an absolutely humid atmosphere with 5% CO_2 in air for 24 h. All the experiments, including the controls, were

set up in three parallel wells. [^{3}H]Thymidine in an amount of 10 mCi per well in a volume of 10 μl was added 4 h before the end of cultivation. Control cells were incubated in the same growth medium without the preparation.

Taking and Evaluation of Samples. A 300-μl sample of the resuspended cell suspension was transferred to GFC filters, fixed with 5% TCA, and washed with distilled water, and it was then dried with alcohol, transferred into 5 ml of scintillator (PPO—POPOP—toluene), and counted in a liquid scintillation spectrometer.

The antigenic properties of the protoplasts were studied by the ELISA solid-phase immunoenzyme method, as described previously [8].

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