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EFFECT OF DEGLYCOSYLATED EXTENSIN-LIKE PROTEIN ON CELL CULTURE

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Extensin-like proteins (ELP) participate in the construction of plant cell walls, are implicated in interactions between cells connected with molecular recognition, and exhibit protective functions similar to lectins [1, 2].

Therefore, it seemed interesting to study the role of ELP in cell culture with respect to structure—function relationships.

ELP were isolated from 2-day cotton sprouts by extraction with $CaCl_2$ (0.2 M). The resulting proteins were characterized by electrophoresis. The quantitative content of total sugars was determined by spectrophotometry using the anthrone-H_2SO_4 method [3]. The carbohydrate content in the isolated samples was 25%.

The role of oligosaccharide fragments in the ELP was elucidated by deglycosylating the ELP using anhydrous HF in pyridine as before [4]. Incubation was carried out at room temperature for 30, 60, and 90 min. The reaction was stopped by adding cold water. The precipitate after deglycosylation was separated from the supernatant by centrifugation. The supernatant containing deglycosylated protein was dialyzed to remove HF, methanol, and pyridine. Deglycosylation was performed under mild conditions without destroying peptide bonds [4]. The supernatant containing deglycosylated protein contained about 6% total sugars.

The biological activity of the protein for KML tumor cells grown from melanoma B-16 was determined by innoculating cells (40,000/mL) into RPMI-1640 medium (3 mL) containing fetal calf serum (10%), antibiotics, and glutamine. During the logarithmic growth phase 24 h later, ELP and deglycosylated ELP (dELP) were added at doses of 100 µg/mL. The action of protein on XAg8.653 myeloma cells was studied analogously. The protein activity was determined using a cytotoxic test for incorporation of ^{3}H-thymidine and by counting living cells.

Comparison of the results (Table 1) led to the conclusion that dELP had the greatest cytotoxic effect. dELP suppressed incorporation of marker into KML cells by 87.6% and suppressed cell growth by 82%. Analogous results were obtained for myeloma cells. Therefore, deglycosylation increased the cytotoxic activity of the protein. This was due to the carbohydrate content in the protein. We determined that about 6% of the carbohydrates remained bound to the protein after deglycosylation. The protein structure may have undergone conformational rearrangements, as a result of which the biological activity changed.

TABLE 1. Effect of Deglycosylated ELP on Cell Culture

Sample	Suppression of ^{3}H-thymidine incorporation, %	
	KML	XAg 8.653
ELP	55.5 (49.0)*	61.2 (57)
dELP	87.6 (82.0)	84.5 (80.0)
Control	100.0 (100.0)	

*Cell growth suppression determined by counting living cells is shown in parentheses.

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We propose that sugar antennae that recognize and then form cell-target linkages adopt a more favorable conformation, as a result of which the number of protein attachment sites on the cell surface increases. This may explain the distinct antiproliferation activity of deglycosylated ELP. It has been shown that oligosaccharides are definitely involved in suppression of tumor and metastasis growth [5].

Thus, cotton ELP exhibit cytotoxic activity that is higher for dELP.

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