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ISOLATION AND GENERAL PROPERTIES OF COTTON EXTENSIN-LIKE PROTEINS

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Extensin-like proteins (ELP) from 2-day sprouts and suspended cotton culture were isolated and characterized.

Key words: extensin-like proteins, cotton.

At least three classes of proteins containing hydroxyproline have been found in plants. These are extensins [1], arabinogalactans [2], and hemagglutinating proteins [3]. Extensin is the principal hydroxyproline-containing glycoproteide of the plant primary cell wall. The carbohydrate content in extensin-like proteins (ELP) is about 2/3 of the protein molecule whereas it reaches 95% in arabinogalactans. It has been found that extensin is a very important structural component of the cell wall. It functions by imparting strength and flexibility to the cell wall. Extensins are also involved in protecting the plant and in intercellular interactions connected with recognition processes [4, 5].

ELP were isolated from a suspended culture and 2-day cotton sprouts by a modified Lamport method [6].

The cell suspension and 2-day sprouts were held for 1 d at 4°C before ELP isolation in order to increase their content. It is known that the plant cell wall contains a rather low level of hydroxyproline-containing proteins. Thus, the hydroxyproline (Hyp) content in melon-seed cell wall is 0.1% whereas it is 2-3% in the callus [7]. The ELP content increases under stress, namely, cell culture, pathogenic infection, plant growth and development, low temperatures, etc. [8, 9]. Extensin mRNA was found to increase three-fold at low temperatures.

Water-soluble proteins were removed in the first isolation step. For this, sprouts or precipitated cells were treated with distilled water, homogenized, and centrifuged. The supernatant, which contained the water-soluble proteins, was discarded. The precipitate was treated several times in the same manner. Then ELP were extracted with $CaCl_2$ solution (0.2 M) for more complete protein extraction because extensins from cell walls typically are very poorly soluble. This is obviously due to the fact that the extensin monomer is secreted into the cell wall and is bonded covalently to another monomer through isodityrosine linkages to form an insoluble complex with cell-wall polysaccharides [10, 11]. Various extraction agents such as detergents, HCl:ethanol mixture, HF, and salts were used to solubilize and isolate extensin from cell walls of plants of the nightshade family. Thus, $CaCl_2$ solution (50 mM) was used to isolate extensin from the cell wall of a suspended tomato culture [6]. Under these conditions, soluble protein was extracted completely after 2-5 min. Higher ionic strength ($CaCl_2$ 0.2 M) was used to isolate extensin from carrot cell wall [12]. Various selective hydrolysis methods were used to isolate extensin or extensin fragments from cell walls of suspended cotton culture [11]. The results show that the solubilization of extensin depends on the type and strength of the cell walls. Therefore, young callus (about 2-3-month, young) was selected, converted to a suspension, shaken for 1 d at 4°C, and used in experiments to isolate cotton ELP. The cell wall of 2-day sprouts was destroyed by freezing and grinding in liquid N_2. ELP were isolated as described in the Experimental section. However, peroxidase is also solubilized from the cell wall during ELP extraction with $CaCl_2$ (0.2 M). It has been demonstrated [6] that peroxidase is eluted by 30 mM $CaCl_2$; extensin, 50 mM. Therefore, peroxidase was separated by precipitation with trichloroacetic acid (TCA). We used this same procedure to isolate cotton ELP, i.e., protein extract in 0.2 M $CaCl_2$ was treated with TCA to a final concentration of 10% and held for 24 h at 4°C. Under these conditions, ELP are solubilized whereas peroxidase remains in the precipitate. The acid-soluble fraction was collected, dialyzed against water, transferred into sodium-phosphate buffer (pH 7.8), and purified over KM-cellulose. Figure 1 shows the elution profile and separation conditions.

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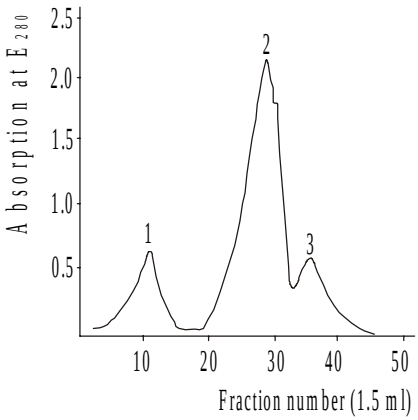

Fig. 1

Fig. 2

Fig. 1. Ion-exchange chromatography of ELP over a KM-cellulose column (1.0 × 10) equilibrated with Tris-HCl buffer (25 mM, pH 8.0) with stepwise elution of protein from the column (25 and 90 mM NaCl) in the same buffer.
Fig. 2. Electrophoregram of ELP in polyacrylamide slab-gel: total ELP extracted with $CaCl_2$ (0.2 M) (1) and ELP fraction after purification over KM-cellulose (2).

It can be seen that the main protein peak eluted from the column with 25 mM NaCl (2). A small amount of protein eluted from the column with 90 mM NaCl (3). Peak 1 represents the void volume. The corresponding peak fractions (2 and 3) were collected, dialyzed against water, dried by lyophilization, and used in biochemical studies. Analysis with phenylthiocarbomoyl- (PTC)-derivatives detected hydroxyproline-containing protein, ELP, in peak 2 and an insignificant amount in peak 3 [13]. The fraction obtained upon elution with 25 mM NaCl (2) was used in further experiments.

Proteins were characterized by electrophoresis. We used electrophoresis conditions from the literature [14] with certain modifications. The separating gel contained acrylamide (7%), methylene-*bis*-acrylamide (0.05%), and urea and potassium-acetate buffer (8 M) at pH 3.0; the concentrating gel, acrylamide (3.5%), *bis*-acrylamide (0.05%), and urea and potassium-acetate buffer (8 M) at pH 5.2.

Figure 2 shows the electrophoregrams.

Two principal protein bands are visible after purification over KM-cellulose. The proteins isolated by us could be soluble extensin precursors. This is consistent with the literature [6, 14] on the isolation from suspended tomato and carrot culture of two soluble extensin precursors.

The total sugar content was determined spectrophotometrically using anthrone—H_2SO_4 reagent. It was found that the total sugar content in the isolated cotton ELP samples was 20 and 60% in ELP_s (sprouts) and ELP_c (suspended culture), respectively.

The qualitative composition of carbohydrates in cotton ELP was determined by acid hdyrolysis of protein in H_2SO_4 (2 N) with subsequent paper chromatography using butanol:pyridine:water. Cotton ELP contains mainly arabinose and an insignficiant quantity of galactose. The results agree with those previously reported [6, 15] that showed that the principal carbohydrates in extensin are arabinose and galactose where arabinose binds to hydroxyproline and galactose to serine. Traces of glucose were observed in certain instances [16].

The amino-acid composition of ELP was determined for PTC-derivatives after acid hydrolysis. It has been found that the protein contains 17 amino acids, among which Hyp, Thr, Ala, Tyr, and Arg dominate.

KML cell culture (isolated from murine B-16 melanoma) aggregated 4-6 h after ELP treatment and broke into individual cells upon light agitation [17].

Hydroxyproline-containing proteins with hemagglutinating activity were isolated from cell walls [3, 18]. Therefore, the hemagglutinating activity of ELP was determined using a suspension of human erythrocytes (2%). It has been found that ELP do not cause agglutination of human erythrocytes.

Thus, we isolated and characterized ELP from a suspended culture and 2-day cotton sprouts.

EXPERIMENTAL

A cell suspension and sprouts were placed for 24 h in a refrigerator at 4°C. Then, the cell suspension was concentrated by centrifugation at 1000 g. Sprouts were homogenized in sodium-phosphate buffer at pH 7.0 and centrifuged. Precipitates obtained from the cell suspension and sprouts were treated with distilled water [12]. For this, a five-fold volume of distilled water was added to the cell precipitates. The mixture was homogenized and centrifuged. The supernatant was discarded. The precipitate was treated this way several times (3-5). Then, the resulting precipitate was treated with a five-fold volume of $CaCl_2$ solution (0.2 M). The mixture was extracted three times for 30 min with constant rocking and centrifuged. The supernatant was collected, concentrated to one third the volume, treated with TCA to a final concentration of 10%, left at 4°C for 24 h [6], and centrifuged at 15,000 rpm (TsLR, Kyrgyzstan). The supernatant was dialyzed against several changes of water and adjusted to 25 mM Tris-HCl at pH 8.0. A column (1.0 × 10) of KM-cellulose was equilibrated with Tris-HCl buffer (25 mM) at pH 8.0. This same buffer was passed through the column after protein was placed on it. Proteins were eluted from the column stepwise in the same buffer containing 25 and 90 mM NaCl. The hydroxyproline content was determined in each fraction using PTC-derivatives [13]. Proteins were dialyzed against water and dried by lyophilization.

Electrophoresis used standard conditions [14]. Samples were dissolved in glycine buffer at pH 4.5 containing urea (8 M). Proteins were detected using TCA (50%). The gel was colored with coomassie blue R-250 or silver to develop the protein bands.

The total sugar content was determined spectrophotometrically using anthrone—H_2SO_4 reagent.

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