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ISOLATION OF PYROPHOSPHATASE FROM COTTON-PLANT SHOOTS

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Inorganic pyrophosphatase (EC 3.6.1.1) catalyzes the reversible hydrolysis-synthesis reaction of PP_i. This enzyme is assigned a fundamental role in the phosphorus metabolism of plants but the methods for its isolation and purification, particularly from the tissues of higher plants, are imperfect [1-3]. The aim of the present work was to isolate pyrophosphatase from the cotton plant and to investigate some of its properties.

The pyrophosphatase was isolated from three-day shoots of a cotton plant of variety 108-F. The protein extract was obtained by homogenizing the cotyledons of the shoots in a cooled mortar with a glass pestle in a small volume of Tris-HCl buffer, pH 7.2. Then the homogenate was centrifuged at 10,000g for 30 min in the cold (2-4°C). The proteins were precipitated with the aid of cooled acetone. They were collected by centrifugation and the precipitate was resuspended in the initial buffer and was recentrifuged under the conditions described above. The supernatant obtained was dialyzed against distilled water for 16-18 h.

The protein extract was concentrated by ultrafiltration through Amicon membranes, which pass proteins with a molecular weight of up to 1 kD under a pressure of gaseous nitrogen (3-4 atm). The protein extract was subjected to gel filtration on a 1.9 × 70 cm column filled with Sephadex C-100 (Sweden), and equilibrated with the initial buffer, rate of elution was 6.6 ml/h. The proteins were chromatographed on a 1.3 × 30 cm column filled with DEAE-cellulose (Serva) which had been suspended in and equilibrated with the initial buffer in an NaCl gradient at a rate of elution of 6 ml/h. Rechromatography of the enzyme extract was carried out on a column of DEAE-cellulose DE-52 (Whatman, United Kingdom) under the same conditions. The yield of protein was recorded with the aid of a Uvicord-III instrument (LKB) at 280 nm.

In parallel, the protein in the samples was determined by the Lowry method [4]. The substrate used was $Na_4P_2O_7 \cdot 10H_2O$ (Sigma). The pyrophosphatase activity was measured from the formation of organic phosphate, the amount of which was determined by the method of Weil-Malherbe and Gree [5] with the aid of Spectromom-195 and 402 instrument (Hungary). The stages of the purification of the enzyme are shown below:

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Fraction	Volume, ml	Protein, mg	Total activity, mU	Specific activity, mU/mg	Degree of purification	Yield, %
Cell-free extract	250	3885	62500	16	1	100
Extract from acetone powders	42	242	30500	126	7,8	49
Gel filtration on Sephadex-G-100	25	28,9	10100	350	21,8	16,3
Ion-exchange chromatography on DEAE-cellulose						
form 1	9	4,9	3800	800	50,0	
form 2	6	2,5	1214	480	30,1	
Rechromatography on DEAE-cellulose DE-52						
form 1	6	2,6	2100	830	52,7	3,3

Thus, after the use of the methods of isolation described above, an enzyme preparation was obtained that had been purified more than 50-fold relative to the cell-free extract. It was established that this enzyme exhibited its activity maximum in the alkaline pH region (8.6) and required the presence of bivalent activator metals such as Mg^{2+}, Zn^{2+}, Co^{2+}, and Mn^{2+}.

On chromatography, another iso form of the enzyme was obtained the degree of purification of which amounted to almost 30-fold. Investigations of the properties of this enzyme showed that it differed from the first preparation by the fact that it was active in the acid pH range of 4.5-5.0 and metal ions were not necessary as activators for the manifestation of its activity.

Thus, we have isolated pyrophosphatase isoenzymes from the cotton plant which differ with respect to their pH optima and sensitivity of the action of bivalent metal ion.

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