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EFFECT OF POLYETHYLENE GLYCOL ON PROTEIN EXTRACTION AND ENZYME ACTIVITIES IN POTATO CELL CULTURES

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Key Word Index—*Solanum tuberosum*, Solanaceae, polyethylene glycol, glucose-6-P dehydrogenase; glutamate dehydrogenase; pyrroline-5-carboxylate reductase, water stress.

Abstract—Polyethylene glycol (PEG) is often used to produce water stress in plant cell suspension cultures. The amount of soluble protein extracted from potato cell suspension cultures treated with PEG-6000 (10% w/v) decreased to 50%. In addition, the activities of glucose-6-P dehydrogenase, glutamate dehydrogenase and pyrroline-5-carboxylate reductase in these extracts were differentially reduced. High concentrations of PEG (> 5%) in the enzyme assay systems were required to significantly affect the activities of the three enzymes extracted from PEG untreated cells. PEG must be removed from cells before proteins are extracted or enzymes assayed. A rapid procedure to harvest cells and subsequently to remove PEG from cells stressed with this compound is described.

INTRODUCTION

Polyethylene glycol 6000 (PEG) has been used to produce water stress in plant cell suspension cultures [1]. This compound lowers the water potential of solutions [2] and it is believed to not penetrate the cells [3]. PEG is known to precipitate proteins [4, 5] and it affects enzyme activities [6]. The presence of PEG in extracts of cells from water stressed suspension cultures may therefore produce undesirable effects when performing metabolic or enzymatic studies with these cells. In this paper we describe the effect of PEG on extraction of protein from potato cell suspension cultures and on the activities of glutamate dehydrogenase, pyrroline-5-carboxylate reductase, and glucose-6-P dehydrogenase. These enzymes are related to proline biosynthesis, which is increased as a response of many plants to water stress [1]. A procedure to remove PEG thoroughly from the harvested cells is also described.

RESULTS AND DISCUSSION

PEG and extraction of potato protein

Buffer containing various concentrations of PEG was used to extract soluble protein from frozen potato cells. The amount of total soluble protein in the extracts decreased in the extracts with higher concentrations of PEG (Fig. 1). In extracts made with buffer containing 10% PEG, only 55% of the soluble protein was found. A small but already measurable effect was observed with 2% PEG.

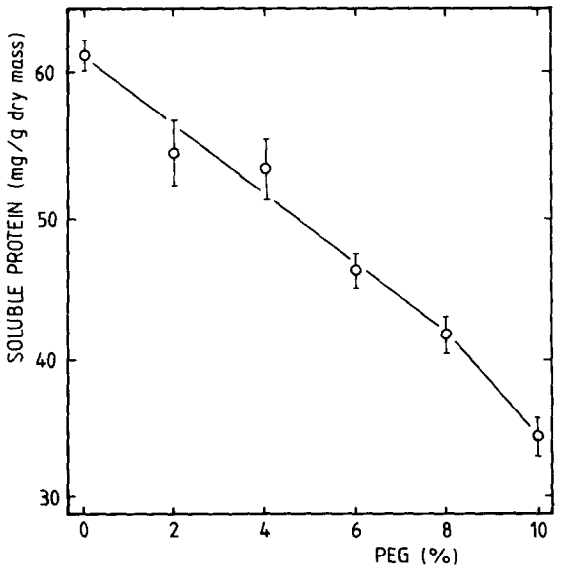

Fig 1 Effect of PEG-6000 on extraction of protein from potato cell suspension cultures PEG was added to the extraction buffer (see Experimental). Values are means of three samples ± s.d.

PEG and enzyme activities

The effect of PEG in the extraction buffer on enzyme activities was also measured. Activities of the enzymes found in the extracts (units/g dry mass) decreased substantially, even with 5% PEG (Table 1). Specific activity changes were enzyme dependent, indicating that a differential precipitation occurred. The absolute amount of protein in samples of Table 1 is higher than in Fig. 1 because Triton X-100 was used to prepare the extracts, facilitating extraction of membrane-associated proteins.

The effect of PEG on the enzyme activities was also studied by adding this compound directly to the reaction mixtures in the activity assay systems. A differential effect was observed, P-5-C-R being the most sensitive (Fig. 2). The differences between effects of PEG on enzymes

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Table 1 Effect of polyethylene glycol on extraction of enzyme activities from potato cells

	Enzyme activity (units/g dry wt)			(units/mg protein)		
PEG (%)	0	5	15	0	5	15
Enzymes						
GDH	125±3	83±4	10±1	1 38	1 65	0.25
G-6PDH	17±1	9±0	8±0	0 19	0.19	0.20
P-5-C-R	12±0 3	6±0.3	4±0 2	0 13	0 12	0 10

PEG-6000 was added to the extraction buffer (10 mM HEPES, pH 7, with 0 1% Triton X-100) Protein contents in extracts of cells made with 0, 5 and 15% PEG were 90, 50 and 39 mg/g dry mass, respectively Values are means of three samples ±s d Cells grown without PEG in the culture medium were used for these experiments

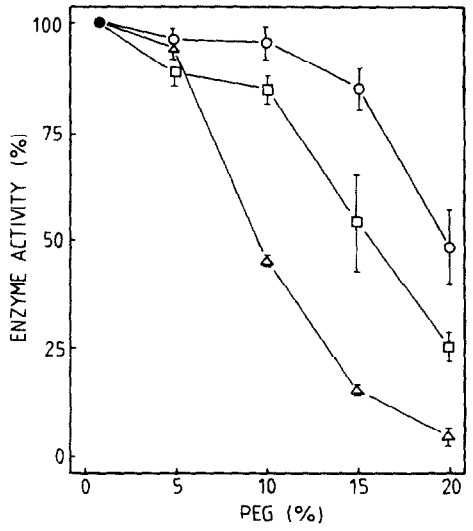

Fig 2. Effect of PEG-6000 on activity of various enzymes Enzyme extracts were made with buffer without PEG. This compound was added directly to the reaction mixture in the cuvette Values are means of three samples ±s.d. (○) glutamate dehydrogenase, (□) glucose-6-P dehydrogenase, (△) pyrroline-5-carboxylate reductase

Table 2 Removal of remaining culture medium from harvested potato cells

Sample	Dry wt (mg/sample) Non-washed	Washed	Filtrate
Non-stressed (0% PEG)	102±5	91±2	12±0.2
Stressed (10% PEG)	202±8	93±1	103±6

Cells were harvested by filtration (non-washed) and subsequently washed with water and filtered again (see Experimental) PEG-6000 was used in the stressed sample The dry weight of the filtrate of the washed cells was also determined. Numbers are means of five samples ±s d

observed in Table 1 and Fig 2 may be due to the length of time in which the extracts were in contact with PEG In the first case PEG was present during extraction and dialysis (several hr). In the second case, PEG was added at the beginning of the enzyme activity assays which lasted only a few min. It has been proposed that phase separation of proteins from PEG-water systems can be partially attributed to an unfavorable interaction of PEG with charges residing on the surface of the protein, thus producing differential precipitation of different proteins [7].

Removal of PEG from harvested cells

PEG from the growth medium of stressed cells may also affect extraction of protein and enzyme activities, as described above For this reason, the harvested cells were washed with water to remove excess medium (see Experimental). The harvest and subsequent wash of the cells could be performed in about 1 5 min. The dry masses of the control (non-stressed) cells and of stressed cells (treated with PEG) after washing were the same (Table 2) This and the differences of dry mass of filtrates indicated that nearly all PEG remaining with the growth medium in the harvested cells was removed The activities of enzyme were higher in thoroughly washed stressed cells than in non-washed stressed cells (Table 3). This difference could be attributed to PEG present in the remaining culture medium in the non-washed stressed cells

PEG decreased the amount of extractable protein and affected enzyme activities differentially. It is therefore recommended, in order to make metabolic studies with cells from suspension cultures stressed with PEG, that the remaining culture medium is removed thoroughly before cell extracts are made

EXPERIMENTAL

Cell cultures Solanum tuberosum L. c v H H. 258 cell suspension cultures were maintained in a modified M-240 medium [8, 9] One-week-old cultures (13% cell packed volume, middle log phase of growth) were used for the experiments To remove the medium, the cells were centrifuged at 800 *g* for 10 min A portion of cells was resuspended in the same medium (referred to in the text as non-stressed, −5 5 bar) Another portion of cells was suspended in medium with 10% (w/v) PEG-6000 (stressed, −9 5 bar). The cultures were kept for 20 hr in darkness and under continuous shaking before harvest The cells were harvested by filtering 10 ml of cell suspension on 3 layers of a 80 μm nylon cloth A group of cells was frozen in liquid N_2 immediately

Table 3 Enzyme activities in washed and non-washed potato cells grown in culture medium with PEG

	Enzyme activity Units/g dry wt		Units/mg protein	
	Washed	Non-washed	Washed	Non-washed
GDH	83±2	51±1	1.17	0 95
G-6-P-DH	13±1	7±0 2	0 19	0 13
P-5-C-R	10±0 3	5±0 2	0 14	0 09

Potato cell suspension cultures were water-stressed with 10% (w/v) PEG-6000 in the culture medium Cells were harvested by filtering the suspension (non-washed) and subsequently washed to remove remaining medium with PEG Values are means of three samples ±s.d

after filtering (non-washed cells). A second group of cells was also harvested by the same procedure but it was subsequently washed with 10 ml H_2O and filtered again on the 80 μm nylon cloth under slight vacuum. These cells were also frozen in liquid N_2. The entire procedure took *ca* 1 5 min until the cells were frozen.

Enzyme extracts. Frozen cells (ca 1 g) were ground in 2.5 ml of 10 mM HEPES buffer (pH 7) with 2 mM β-mercaptoethanol and 5 mg/ml insoluble polyvinyl-pyrrorolidone (Polyclar AT). The extracts were centrifuged at 30000 *g* for 10 min at 4°. The supernatant fluid (*ca* 3 ml) was dialysed overnight against 3 l. of 50 mM Tris–HCl buffer with 2 mM β-mercaptoethanol (pH 8). This extract was used fresh to measure enzyme activities. To measure the effect of PEG-6000 on extraction of soluble protein or on extraction of enzyme activities, PEG was added to the extraction buffer.

The amount of protein in the crude extract was measured by the dye binding method of Bradford, as modified in ref. [10], using bovine serum albumin as a standard.

Enzyme assays. Enzyme activities were measured spectrophotometrically at 28° in 50 mM Tris–HCl buffer (pH 8) as follows: (a) GDH (L-glutamate: NAD oxidoreductase, deaminating, EC 1 4.1 2.) was measured as previously described [11]. The reaction mixture contained 0.3 mM NADH, 200 mM NH_4OAc, 6.7 mM α-ketoglutarate, and 0.05 ml enzyme extract, in a total vol. of 3 ml. (b) G-6-PDH (D-glucose-6-phosphate: NADP oxidoreductase EC 1.1.1 49) activity was measured as described [12]. The reaction mixture contained 0 33 mM NADP, 1 5 mM glucose-6-P, 10 mM $MgSO_4$ and 0 1 ml enzyme extract in a total vol. of 3 ml (c) P-5-CR (L-proline: NAD(P)-5-oxidoreductase, EC 1.5.1.2.) [13]. The reaction mixture contained 0.8 mM NADH and 0.8 mM P-5-C (pyrroline-5-carboxylate) and 0.1 ml of enzyme extract, in a total vol. of 1 ml.

One unit is the enzyme activity catalysing the reduction or oxidation of 1 μmol of pyridine nucleotide in 1 min at 28°. The reactions were started by addition of substrates.

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