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DECARBOXYLATION OF L-TYROSINE AND L-DOPA BY IMMOBILIZED CELLS OF *PAPAVER SOMNIFERUM*

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Key Word Index—*Papaver somniferum*; Papaveraceae; immobilized cells; decarboxylation; L-tyrosine; L-DOPA.

Abstract—*Papaver somniferum* (opium poppy) cells, after permeabilization in Tween 80, were immobilized by glutaraldehyde without any insoluble carrier. Cells immobilized by cross-linking decarboxylated L-dopa and L-tyrosine for three months, without significant loss of either activity.

INTRODUCTION

Recently there has been much interest in the use of immobilized plant cells for the production or biotransformation of secondary metabolites [1–7]. The immobilization process facilitates continuous culture operation at high cell densities. A variety of matrices have been employed: calcium alginate [1, 2], carrageenan [3], polyacrylamide and agarose [3]. In this paper the decarboxylation of L-tyrosine to tyramine and L-DOPA to dopamine by cells of *Papaver somniferum* L. cv Amarín immobilized by glutaraldehyde cross-linking is described. Both amines have an important role in initial steps of isoquinoline alkaloid biosynthesis [8]. Activity of L-DOPA decarboxylase has been demonstrated in poppy latex [9, 10]. Decarboxylation of the aromatic amino acids L-phenylalanine, L-tyrosine and L-DOPA was also demonstrated in seedlings of poppy plants in experiments *in vitro* [11]. In tracer experiments with labelled L-tyrosine, Roberts *et al.* [12] have shown the preferential utilization of tyramine in the biosynthesis of morphinan alkaloids in *Papaver somniferum* plants. Consequently, the study of L-tyrosine decarboxylase (EC 4.1.1.25, TDC) and L-DOPA decarboxylase (EC 4.1.1.26, DOPADC) as the regulatory enzymes of the biosynthesis of the amines was founded [13, 14]. In this study, the preliminary characterization of the enzymes, from *Papaver somniferum* suspension cells was performed. Using an *in vitro* assay of TDC and DOPADC activities, we investigated the stability of the enzyme in immobilized cells.

RESULTS AND DISCUSSION

One of the most widely used techniques for cell immobilization is cell entrapment, in which cells are enclosed in a polymer matrix [1–7]. The multifunctional enzyme systems of viable cells might be useful for the synthesis of some plant secondary metabolites or their precursors. In the cells immobilized by cross-linking using bifunctional reagent (glutaraldehyde), α-galactosidase and invertase activities still reach high values (J. Stano, unpublished data). Catalytic properties of partially purified enzyme preparation from suspension cultured cells were investigated. The pH optimum was 8.4 for TDC and 7.2 for DOPADC, the apparent K_m value for L-tyrosine 0.25 mM and 0.21 mM for L-DOPA; also coenzyme (PLP) pyridoxal-5-phosphate dependence, and the inhibitory effect of hydroxylamine and $HgCl_2$ were demonstrated [14]. Attempts to purify the enzyme to homogeneity have been unsuccessful due to the apparent instability of the enzyme preparation. The comparison of TDC and DOPADC activity in living and immobilized cells is given in Table 1. The permeabilization led to slight loss of protein content while DOPADC and TDC activities showed no significant decrease. L-DOPA-decarboxylation by immobilized cells was linear for 30 min reaching 83% of conversion, and then stopped. Decarboxylation of L-tyrosine by immobilized cells is linear for 20 min, but reached a final conversion of 32%.

Cultivation of the cells entrapped in the beds [4] was similar to that used for the suspension cultures (temperature, pH, oxygen accessibility, etc). On the other hand, it was shown that the biotransformation ratio of the cells entrapped in the beds was correlated with the viability of the immobilized cells. The biotransformation ratio of codeinone to codeine by freshly entrapped cells of *Papaver somniferum* was 70% and after 30 days of function under optimal conditions the ratio decreased to 42%. Storage stability of immobilized cells was tested by taking samples twice during three months, without significant loss of activity of either enzyme. In cells immobilized by glutaraldehyde the DOPADC still reached 85 and TDC

Table 1. L-Tyrosine decarboxylase and L-DOPA decarboxylase activities in living and immobilized fresh cells

Cells	Protein (mg g^{-1} dry wt)	Activity (nkat g^{-1} dry wt)		Specific activity (pkat mg^{-1} protein)	
		DOPADC	TDC	DOPADC	TDC
Suspension	12.1	4.84	0.32	400	26.4
Immobilized	10.8	4.58	0.30	424	27.8

84% of original activities (compared with the cell suspensions). The storage of the cells immobilized by the glutaraldehyde in 0.15 M NaCl with 0.02% NaN_3 solution seems to be an acceptable method for the long-term preservation of the enzymes.

EXPERIMENTAL

Tissue cultures. Long-term callus culture was derived from seedlings of *Papaver somniferum* L. cv Amarín (Dr K. Erdelský, Department of Plant Physiology, Comenius University, Bratislava) and continuously subcultured every 3 weeks on Z agar medium [15]. *Ca* 2–3 g of callus tissue was transferred to Z liquid medium with 0.05 ppm α-napthaleneacetic acid and grown on rotatory shaker (100 rpm) in 500 ml flasks, containing 100 ml of medium at 26° in the dark. The suspension cultures were subcultured every 2 weeks.

Permeabilization of the cells. Suspension cultured cells were filtered through a nylon cloth (15 g wet wt) and suspended in 50 ml of 5% Tween 80 in 0.15 M NaCl. Permeabilization proceeded for 3 hr under moderate stirring at 20°. The cells were filtered off and washed with 3 l of H_2O and 1 l of 0.15 M NaCl.

Immobilization. The permeabilized cells were immediately suspended in 50 ml of 0.15 M NaCl, and 5 ml of 25% glutaraldehyde under mild stirring was added slowly at room temp.

Determination of fresh and dry wt. Fresh and dry wts of living and immobilized cells were determined gravimetrically. For determination of dry wt, samples were dried to the constant wt at 100°.

Enzyme assay. The activities of TDC and DOPADC were determined using L-[U-^{14}C]tyrosine and L-[1-^{14}C]DOPA, respectively. The reaction mixt. contained 200 mM Na-Pi buffer pH 7.2; 500 μM L-[1-^{14}C]DOPA (1.85 kBq); 50 μM pyridoxal-5-phosphate (PLP) and suitable amount of immobilized cells (0.1 g per ml), or 20 mM Na-Pi buffer pH 8.4, 250 μM L-[U-^{14}C]tyrosine (4 kBq), 50 μM PLP and immobilized cells (0.1 g ml^{-1}) in the shake flask. Controls contained boiled immobilized cells. Both mixts were kept for 3–60 min at 30° in a rotatory shaker at 80 rpm and the reaction was stopped by adding of $NH_2OH \cdot HCl$ (final conc. 1 mM). Aliquots of incubation mixts were analysed by PC on Whatman No 1 in *iso*-PrOH–NH_4OH–H_2O (7:1:2) mixt. and the components identified by detection with ninhydrin using the standards of L-tyrosine, tyramine, L-DOPA and dopamine, respectively. Visualized spots of products and substrates were cut-off, and the radioactivity was measured in a scintillation spectrometer Packard Tri-Carb. In the case of the reaction mixts incubated in the presence of L-[1-^{14}C]DOPA the content of undecarboxylated substrate was measured. The cells were sepd from the reaction mixt, dried and the enzyme activities were calcd to 1 g of dry wt. The enzyme activity is expressed in katals. Protein contents were determined by the method of ref. [16], using bovine serum albumin as the standard protein.

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