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PHOSPHATASES IN THE LATEX OF *PAPAVER SOMNIFERUM**

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Abstract—Phosphomonoesterases have been isolated from *P. somniferum* latex which show variability in their response to heavy metal ions and EDTA which are pH dependent, thus suggesting the presence of a group of enzymes. Acid phosphatase was found to be concentrated in the latex 25000 *g* fraction.

INTRODUCTION

Two types of phosphomonoesterase "acid" and "alkaline" are reported as occurring in living cells; however, the present literature suggests that plant cells, in contrast to animal cells, exhibit activity mainly at acid pH [2]. The occurrence of acid phosphatase in plants has been associated with both particulate and soluble fractions of cell free extracts and in general particulate phosphatase was found to be contained in particles with other hydrolases. It has therefore been used by some workers as one of a number of marker enzymes in the identification of lysosomes [3]. Recently, Matile [4], working with *Chelidonium majus* latex, isolated particles sedimenting at 600 *g* which contained high acid phosphatase activity and other hydrolytic lysosomal enzymes together with most of the alkaloid content of the latex. Work with poppy latex has shown that particles with similar sedimentation also contain most of the latex alkaloids [5,6] together with all the phenolase activity [7]. As part of a detailed study of the enzymic nature of poppy latex an investigation of phosphomonoesterases was therefore carried out, the results of which are described below.

* Part 4 in the series "Enzymic Studies with *Papaver somniferum* latex". This work forms part of a dissertation by M. D. Antoun. For Part 3 see Ref. [1].

RESULTS AND DISCUSSION

Phosphatase activity in the latex of *P. somniferum* was investigated using freshly collected latex suspended in an equal volume of ice cold 100 mM Tris–500 mM mannitol buffer pH 7·0. A 1000*g*/30 min fraction was separated from the main part of the latex which will subsequently be referred to as the "supernatant". Phosphatase activity using 40 mM citrate buffer pH 5·0 and *p*-nitrophenylphosphate as the substrate was found to be 6·4 units/ml latex in the "supernatant" compared with 1·0 unit/ml for the 1000 *g*/30 min fraction. Low activity (0·9 unit/ml latex) was also observed in the "supernatant" using 40 mM Tris pH 8·0.

As the main part of the phosphatase activity occurred in the "supernatant", the 1000 *g*/30 min fraction which also contained phenolase activity was discarded in subsequent experiments. The small but significant activity at pH 8·0 in the "supernatant" led to further investigation of the variation in phosphatase activity with pH. It is known that variations in phosphatase activity which are pH dependent occur in the presence of heavy metal ions and chelating agents [8–13] and therefore activity in poppy latex "supernatant" was further studied over a pH range from 4·0–7·0 using 40 mM citrate buffer, and from 7·0–9·0 using 40 mM Tris buffer. The results show maximal phosphatase activity at pH 6·0 with a small shoulder at pH 4·5–

Table 1. Effect of heavy metal salts, EDTA and sodium fluoride on the latex phosphatase

Complete system + the following additives mM		% Phosphatase activity pH 5·0 S.L.	pH 7·0 F.L.	pH 8·0 S.L.	F.L.
Control		100	100	100	100
$BaCl_2$	5×10^{-4}	90	131	80	90
	25×10^{-4}	96	—	120	—
$CaNO_3$	5×10^{-4}	100	97	60	51
	25×10^{-4}	105	—	60	—
$CoSO_4$	5×10^{-4}	102	1201	340	362
	25×10^{-4}	102	—	360	—
Zn acetate	5×10^{-4}	106	119	12	60
	25×10^{-4}	106	—	42	—
$CuSO_4$	5×10^{-4}	104	43	60	24
$MgCl_2$	25×10^{-4}	96	663	660	491
	50×10^{-4}	135	—	720	—
NaF	5×10^{-4}	65	—	120	—
EDTA	5×10^{-4}	101	89	0	48

S.L. = stored latex; F.L. = fresh latex.

Complete system contained *p*-nitrophenylphosphate 5·5 mM (0·1 ml), buffer 40 mM (4·0 ml) and latex (0·05 ml). Other substances were added in a volume of 0·1 ml. Total volume was made up to 5·0 ml using distilled water. 40 mM citrate buffer was used at pH 5·0 and 40 mM Tris buffer at pH 7·0 and pH 8·0.

5·0; a second maximum occurred at pH 7·4. Mg^{2+} found in many instances to enhance phosphatase activity particularly at alkaline pH [14] was also found to produce enhancement of poppy latex phosphatase. Small increases in activity were observed at acid pH whilst activity was enhanced ten-fold at pH 7·0 and fourfold at pH 8·0. However, we are at present unable to explain the low enhancement of activity with Mg^{2+} in citrate buffer at pH 7·0 (four-fold) compared with the activity in Tris buffer at pH 7·0 (ten-fold).

These results suggest the existence of a group of phosphatases in poppy latex which exhibit acid phosphatase activity and at pH 7·0 and above enhancement of activity with Mg^{2+} which is reminiscent of the effects observed with alkaline phosphatases isolated from animal cells [15]. Phosphatases from animal and plant sources have in general been characterized by their behaviour in the presence of heavy metals and chelating agents, and as the addition of Mg^{2+} yielded interesting results further studies on the response of poppy latex phosphatase to these agents were made. Since the response of phosphatase to heavy metals was expected to be pH dependent, the experiments shown in Table 1 were carried out at pH 5·0 (citrate buffer), pH 7·0 and 8·0 (Tris buffer). The results show that poppy latex phosphatase is little affected by metal ions and EDTA at pH 5·0, but shows extreme sensitivity to these agents at pH 7·0 and pH 8·0. The enhancement of activity at pH 7·0 with Mg^{2+} and Co^{2+} is similar to that found for animal phosphatase [14–16]. Similarly, Ni^{2+} was also found to enhance activity at pH 7·0 and pH 8·0. However, Ca^{2+} only inhibited activity at pH 8·0 and Ba^{2+} had little effect on activity.

Zn^{2+}, a known inhibitor of alkaline phosphatase at pH 8·0, was found to be strongly inhibitory at pH 8·0 with poppy latex "supernatant" and wide variations were observed with concentration and between stored and fresh "supernatant" samples. These results with Zn^{2+} are difficult to explain although it is known that zinc, a prosthetic group of milk alkaline phosphatase added at 10^{-6} M, shows stimulation, whereas at 10^{-4} M inhibition of activity is observed [16]. Further, Brunel and Cathala [14] have also indicated that Zn^{2+} inhibition is pH-dependent. Cu^{2+} was of interest in that strong inhibition of activity was observed at pH 7·0 and showed significant variations in its effect with fresh and stored latex at pH 8·0. EDTA showed no inhibition at pH 5·0 but slight inhibition at pH 7·0 and 52% inhibition at pH 8·0. Fluoride as expected [2] showed inhibition of activity only at acid pH.

These results suggest that a distinction can be made between protein which exhibits maximum activity at pH 5·0–6·0 and protein which exhibits activity at pH 7·0 and above.

The effect of heavy metal ions, fluoride and

Table 2. The occurrence and distribution of phosphatase in *P. somniferum* latex using *p*-nitrophenylphosphate as substrate

Poppy latex fraction	Protein mg/ml latex	Phosphatase activity pH 5·0 U	R	pH 7·0 U	R	pH 8·0 U	R
Supernatant	11·36	0·29	2·1	0·31	5·3	0·17	2·5
25000 *g*	0·68	3·32	24·0	0·45	7·8	0·17	2·5
10000 *g*	0·80	0·15	1·1	0·55	9·4	0·17	2·5
1000 *g*	6·80	0·14	1·0	0·06	1·0	0·07	1·0

U = μmol *p*-nitrophenol/hr/mg latex protein. R = ratio of activity in the various fractions assuming the activity of the 1000 *g* fraction as equivalent to 1·0.

Used 40 mM citrate buffer for pH 5·0 and 40 mM Tris buffer for pH 7·0 and pH 8·0. Activity was estimated using a reaction mixture which contained *p*-nitrophenylphosphate 5·5 mM (0·1 ml), buffer 40 mM (4·0 ml), latex (0·05 ml) and $MgCl_2$ (5×10^{-3} mmol, 0·1 ml).

EDTA could be explained by changes in conformation of the protein with pH and/or the presence of a number of isoenzymes with sensitivity to metal ions and EDTA which is similar to that recorded for alkaline phosphatase in animals and yeast [2,16].

Acid phosphatase has frequently been used as one of a series of enzymes for the identification of lysosomes [3] and since Matile [4] has suggested that the 1000 *g* fraction of *Chelidonium* latex was lysomal the characterization of the particulate fractions of poppy latex was of interest. A series of experiments (Table 2) in which latex was separated into 1000 *g*/30 min, 10000 *g*/30 min and 25000 *g*/2 hr fractions showed that a high concentration of acid phosphatase occurred in the 25000 *g* fraction, whereas phosphatase activity at pH 7·0 and 8·0 was more evenly distributed between the 10000 *g*/30 min, 25000 *g*/2 hr and supernatant. The occurrence of a high concentration of acid phosphatase in the 25000 *g*/2 hr fraction rather than the 1000 *g*/30 min alkaloid-containing vesicles, together with the fact that in poppy latex phenolase activity occurs exclusively in the 1000 *g*/30 min fraction is contrary to the findings for *Chelidonium* latex [4].

EXPERIMENTAL

Preparation of P. somniferum *latex.* Latex (1–2 ml) was collected as previously described [7] in 500 mM mannitol–100 mM Tris buffer at pH 7·0 such that the latex–buffer ratio was 1:1.

Isolation of 1000 g *organelles.* Latex was centrifuged at 1000 *g* for 30 min and the resulting sediment was resuspended in buffer (1 ml) and designated 1000 *g*/30 min organelles. The rest of the latex was termed the "supernatant" and unless otherwise stated this term will be used to refer to the whole latex minus the 1000 *g* organelles.

Further fractionation of the latex. For some experiments the "supernatant" was further fractionated by centrifugation at 10000 *g* for 30 min to give a mitochondria rich sediment which was resuspended in buffer (1 ml). The supernatant after removal of the 10000 *g* fraction was further centrifuged at 25000 *g* for 2 hr and the sediment obtained resuspended in buffer (1 ml). The remainder of the latex was considered as supernatant.

The determination of phosphatase activity. Prior to the estimation of phosphatase latex samples were dialyzed overnight against distilled H_2O. The standard assay used in all expts involved the hydrolysis of *p*-nitrophenylphosphate and the assay mixture used was as follows: *p*-nitrophenylphosphate 5·5 mM (0·1 ml), buffer 40 mM (4·0 ml), latex (0·05 ml) and other additives where applicable. The total vol. was made up to 5 ml with distilled H_2O. Each tube was shaken vigorously and incubated at 37° for 1 hr. The reaction was stopped by the addition of NaOH 1 M (0·4 ml). Control samples were prepared in a similar manner except that the latex was added after the NaOH. The presence of phosphatase was determined by measurement of the formation of *p*-nitrophenol by determination of the absorbance at 400 nm. (The molar E.C. for *p*-nitrophenol is $18·8 \times 10^6$ in alkaline media.) Protein was determined using the method of Lowry *et al.* [17].

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