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SHORT COMMUNICATION

DIFFERENTIAL HEAT-STABILITY OF PHOSPHATASE ACTION ON PHYTIC ACID AND β-GLYCEROPHOSPHATE

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Abstract—Phosphatase activity of homogenates of parasitic angiosperms was more heat-labile towards phytic acid, at 50° or 60° during 5–10 min, than it was towards β-glycerophosphate.

INTRODUCTION

INOSITOL has been reported to occur in mistletoe, *Orobanche* and *Cuscuta.*[1] Investigations in this laboratory revealed that accumulation of phytic acid is a distinctive feature of these tissues (unpublished results); other experiments showed that the parasite tissues possess powerful acid phosphatase activity, tested with β-glycerophosphate[2] or fructose-1,6-diphosphate (unpublished observations) as substrate. During a search by the present authors for a specific phytase in the tissues of these parasites, it was observed that the phosphatase activity towards phytic acid was more heat-labile than that towards β-glycerophosphate, as tested in homogenates. This observation was unexpected since phytase of plant tissues is routinely assayed at comparatively high temperatures and with long incubation periods.[3,4]

RESULTS AND DISCUSSION

The acid phosphatase activity of homogenates of parasite tissues towards phytic acid and β-glycerophosphate had optimum in the pH range 4·5–5·0. Added Mg^{2+} (0·001–0·01 M) had only marginal effect on either activity.

A number of tests were conducted to establish the identity of, or distinction between, the enzyme(s) acting on the two substrates. The results of some tests pointed to an identity of enzyme action, but others to a distinction. On centrifugation of a homogenate of *Orobanche aegyptiaca* in cysteine at 1600 *g* for 45 min, nearly the same proportion of the phosphatase activity towards phytic acid (85 per cent) and β-glycerophosphate (90 per cent) passed into the supernatant. The incorporation of Triton-X-100 (1 per cent) in the cysteine grinding medium did not alter the magnitude of the individual activities in the homogenate, nor their recovery in the supernatant and the ratio between the activity towards the two substrates.

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[2] M. SINGH, M. U. BEG, D. V. SINGH, K. K. TEWARI and P. S. KRISHNAN, *Indian J. Biochem.* **4**, 146 (1967).
[3] G. H. SLOANE-STANLEY, in *Biochemists' Handbook* (edited by C. LONG), p. 259, E. & F. N. Spon, London (1961).
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TABLE 1. RELATIVE HEAT-STABILITY OF PHOSPHATASE ACTION TOWARDS β-GLYCEROPHOSPHATE AND PHYTIC ACID

Tissue	Conditions of heat treatment		Phosphatase activity, units/g fresh weight or equivalent, towards: β-Glycero-phosphate	Phosphatase activity, units/g fresh weight or equivalent, towards: Phytic acid	Loss in activity, %, due to heat treatment, towards: β-Glycero phosphate	Loss in activity, %, due to heat treatment, towards: Phytic acid	Ratio β-glycerophosphatase / phytase
Cuscuta reflexa	Untreated,	Control	22·1	1·80			12·3
	50°,	3 min	22·1	1·59	Nil	11·5	13·9
	50°,	6 min	22·1	1·38	Nil	23·2	16·0
C. indecora	Untreated,	Control	65·0	9·95			6·53
Dendrophthoe falcata	Untreated,	Control	40·0	6·30			6·34
	50°,	10 min	28·7	4·20	28·2	33·3	6·76
	60°,	5 min	16·3	1·10	59·8	82·5	14·8
Orobanche aegyptiaca	Untreated,	Control	29·3	5·95			4·93
	50°,	10 min	27·7	3·73	5·5	37·3	7·41
	60°,	5 min	23·5	3·87	19·8	37·0	6·07
O. cernua	Untreated,	Control	27·1	4·56			5·94
	50°	5 min	26·0	2·76	4·1	39·4	9·42
Petunia hybrida							
Shoot	Untreated,	Control	28·8	8·29			3·47
	50°,	5 min	35·1	6·50	21·5⁻	21·7	5·40
	60°,	5 min	29·0	3·87	0·70⁻	53·0	7·50
Root	Untreated,	Control	11·9	2·07			5·74
	50°,	5 min	10·2	1·38	14·0	33·3	7·40
	60°,	5 min	5·81	0·553	51·2	73·3	10·5
Sorghum vulgare	Untreated,	Control	20·2	3·73			5·41
	50°,	10 min	19·6	2·21	2·73	40·7	8·87
	60°,	5 min	15·8	1·80	21·9	51·9	8·77
Wheat bran	Untreated,	Control	9·68	15·5			0·625
	50°,	10 min	6·63	16·0	31·4	3·5⁻	0·414
	60°,	5 min	6·08	9·68	37·2	37·5	0·629

The filaments of *Cuscuta*, the whole growth of *Orobanche* and the leaves of mistletoe were used in the experiments. The sample of *S. vulgare* was 66-hr-old etiolated seedling. The assay system was as described in text. The homogenates of *C. reflexa* and wheat bran were dialysed prior to assay but the others were used without dialysis. A ⁻ sign indicates an actual gain in activity.

In assays with a mixture of the two substrates, orthophosphate liberated was less than the sum of that from the individual substrates and somewhat less than the mineralization from the more reactive substrate, β-glycerophosphate. Molybdate (0·02 M) and fluoride (0·01 M–0·03 M) inhibited acid phosphatase activity, but without a significant difference using the two substrates.

As tested with *O. cernua*, and with incubation period of 30 min, the activity of phytase assayed at 50° was 165 per cent of that at 37°, while that of glycerophosphatase was 123 per cent, suggesting a higher temperature coefficient for phytase. A more significant distinction between the two types of activity was the effect of heating of the homogenates at 50° and 60° for 5–10 min on the phosphatase activity towards the two substrates, as reported in Table 1. For comparison, the data for wheat bran have also been included.

As reported by earlier workers,[5] wheat bran preparation responded to heat treatment at 50° with a greater loss of activity towards β-glycerophosphate than towards phytic acid. However, at 60° both were affected to a similar extent. The hydrolysis of phytic acid by homogenate of parasite was more heat-labile than that of β-glycerophosphate, tested at 50 and 60°. When the acid phosphatase activity towards β-glycerophosphate and phytic acid was expressed as a ratio, an increase in the ratio resulted on heat treatment.

The ratio of phosphatase activity towards the two substrates varied from parasite to parasite (the control values for *Cuscuta indecora* are also reported, Table 1), among species of a parasite (Table 1) and in the same parasite in different parts or regions (data not reported). Although the phytase activity of some of the parasites was highly significant in comparison with that of wheat bran, on a *fresh weight* basis, the latter source was characterized by the fact that the activity towards phytic acid was 1·6-fold that towards β-glycerophosphate contrasting with the former tissues which had several-fold higher activity towards β-glycerophosphate than phytic acid (Table 1).

That the above pattern of heat-stability was not specific for the parasite tissues was shown by the fact that homogenates of the etiolated seedlings of *Sorghum vulgare* and the shoot and root of *Petunia hybrida* infected by *O. cernua* also suffered a greater loss in the phosphatase activity towards phytic acid than β-glycerophosphate on heating at 50 and 60°.

EXPERIMENTAL

The raising of the parasitic plants (*Cuscuta indecora* Choisy on *Petunia hybrida* X. Hort ex. Vilm; *C. reflexa* Roxb. on *Lantana camara* L.; *Dendrophthoe falcata* (L.F.) Ettingsh on *Mangifera indica* L.; *Orobanche aegyptiaca* Pers. on *Brassica campestris* L. and *O. cernua* Loefl. on *Solanum melongena* L.) and the samplings of tissues were as reported earlier.[6–8] The seeds of *Sorghum vulgare* L. were sterilized with 0·2 per cent $HgCl_2$ in 50 per cent ethanol and allowed to germinate on moistened cotton and filter paper at 30° for 66 hr. Wheat bran was obtained from a local flour mill and was used after washing with water. It was kept soaked in contact with five parts of cold water for 4 hr and then ground in a glass mortar with the addition of freshly neutralized cysteine solution (0·01 M, final). The slurry was transferred to a Waring blendor and ground for 5 min at maximum speed. After filtering through two layers of muslin it was diluted to 20 per cent (w/v) concentration. 20 per cent (w/v) homogenates of other tissues were prepared in cysteine media of appropriate concentration using Waring blendor for tissue dispersion, but reducing the operation time to 3 min.

The assay was according to Peers,[9] with minor modifications. The standard incubation mixture in a total volume of 2·0 ml comprised, unless otherwise mentioned, of: 0·2 M acetate buffer, pH 5·0, 1·0 ml; 0·02 M

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ethylenediamine tetraacetate, pH 7·0, 0·10 ml; enzyme preparation (20 or 10 per cent, w/v, or equivalent) 0·50 ml; water or any supplements, 0·10 ml and 0·20 ml sodium phytate solution (prepared according to Peers[9]), pH 5·0, containing 600 μg acid-stable phosphate or 0·20 ml of 0·025 M sodium β-glycerophosphate, pH 7·0. After 30 min incubation at 37° the reaction was stopped by adding 1 ml 10 per cent trichloroacetic acid. The orthophosphate in the centrifuged supernatant, after removal of interfering phenolics by adsorption on Norit, was estimated according to Fiske and Subbarow.[10] The control was devoid of substrate during incubation, but received it following inactivation. One unit of the enzyme activity was the equivalent to the splitting of 1 μmole of orthophosphate under the standard assay conditions.

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