Occurrence of Alkaline Pyrophosphatase in Vegetable Tissues

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In the previous paper (Naganna, Raman, Venugopal & Sripathi, 1955; see also Naganna, 1951) the occurrence and properties of an alkaline pyrophosphatase in potato extracts have been reported. As this alkaline enzyme is the first of its kind to be demonstrated in a vegetable tissue, studies have been made with several plant tissues in order to establish the ubiquitous occurrence of this enzyme in the vegetable kingdom.

EXPERIMENTAL

Several plant products (cereals and pulses, roots, shoots, grass blades, tubers and leaves) were used. The cereals and pulses were obtained from the market and hence were not fresh products but stored ones. The rest were obtained fresh from the plants. The materials were washed free of dust with tap water followed by distilled water, dried in folds of blotting paper and then weighed. They were ground with 10 times their weight of ice-cold double-distilled water, unless otherwise stated. The suspensions were cooled in ice for 30 min., centrifuged and the clear supernatants used for determining the enzyme activities.

Pyrophosphatase activity was determined at pH values 5·3 and 8·7 by the methods given earlier for potato pyrophosphatases (Naganna *et al.* 1955) using 0·5 ml. of the vegetable extract and an incubation period of 15 min. at 38°. Phosphatase activity was also determined at pH 5·3 and 8·7 in the same manner, but using 0·1 M sodium β-glycerophosphate adjusted to the respective pH instead of 0·01 M pyrophosphate.

The results are shown in Table 1.

Table 1. *Pyrophosphatase and phosphatase activities of plant tissues*

Pyrophosphatase activity is given in μg. inorganic P liberated in 15 min. at 38° in 5 ml. reaction mixture containing 0·5 ml. of a plant extract (1 part of plant tissue + 10 parts water) and 0·5 ml. 0·01 M-$Na_4P_2O_7$. Phosphatase activity was determined similarly, using 0·5 ml. 0·1 M sodium β-glycerophosphate. Where used, the $MgCl_2$ concn. was 0·02 M.

	Pyrophosphatase activity at				Phosphatase activity at			
	pH 5·3		pH 8·7		pH 5·3		pH 8·7	
Plant tissue	No Mg^{2+} added	Mg^{2+} added	No Mg^{2+} added	Mg^{2+} added	No Mg^{2+} added	Mg^{2+} added	No Mg^{2+} added	Mg^{2+} added
Rice (*Oryza sativa*)	53	16	Trace	47	38	36	Trace	Trace
Brown wheat (*Triticum dicocum*)	139	125	39	60	71	—	Trace	Trace
White wheat (*T. vulgaris*)	150	70	12	40	37	32	Trace	Trace
Ragi (*Eleusina coracana*)	42	23	Trace	47	6	Trace	Trace	Trace
Green gram (*Phaseolus radiatus*)	137	67	10	109	85	76	Trace	Trace
Black gram (*P. mungo*)	90	47	Trace	74	42	38	Trace	Trace
Red gram (*Cajanus indicus*)	49	15	Trace	21	20	15	Trace	Trace
Bengal gram (*Cicer arietinum*)	49	35	Trace	29	15	14	Trace	Trace
Cow pea (*Vigna catiang*)	74	37	Trace	49	45	38	Trace	Trace
French bean (*Phaseolus vulgaris* L)	180	70	40	156	—	—	—	—
Field bean (*Dolichos lablab*)	50	15	Trace	65	25	26	Trace	Trace
Field bean leaves	50	16	Trace	57	44	24	Trace	Trace
Tapioca leaves (*Manihot utilissima*)	92	62	Trace	195	56	52	Trace	Trace
Croton leaves	39	19	Trace	242	56	48	Trace	Trace
Cabbage (1 in 4 dilution)	97	52	Trace	92	10	8	Trace	Trace
Grass (1 in 20 dilution)	Trace	Trace	Trace	114	Trace	Trace	Trace	Trace
Plantain leaves (*Musa paradisiaca*)	6	Trace	Trace	267	Trace	Trace	Trace	Trace
Onion shoots (*Allium cepa*)	31	7	Trace	49	31	28	Trace	Trace
Onion bulb	108	40	Trace	40	31	28	Trace	Trace
Radish leaves (*Raphanus sativus*)	76	47	Trace	251	36	28	Trace	Trace
Radish roots	7	6	Trace	25	5	5	Trace	Trace
Carrots (*Daucus carota*)	45	12	Trace	42	20	16	Trace	Trace
Coconut pulp (*Cocos nucefera*)	52	21	Trace	87	22	18	Trace	Trace

DISCUSSION

Several vegetable tissues were analysed for their phosphatase and pyrophosphatase activities, with and without added Mg^{2+}. Besides the already

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known acid phosphatase and acid pyrophosphatase, a potent alkaline pyrophosphatase, active only in the presence of added Mg^{2+}, was found to be present in all vegetable tissues tested. Fresh plant products showed more alkaline pyrophosphatase activity than the stored products. The richest sources are green leaves, which also contain large amounts of acid pyrophosphatase. However, some leaves like plantain leaves and grass blades, even though possessing large amounts of alkaline pyrophosphatase, were found to contain only negligible amounts of acid pyrophosphatase. Perhaps the alkaline pyrophosphatase is more essential for plant tissues where photosynthesis is taking place. Plant tissues, unlike animal tissues, do not contain any alkaline phosphatase.

REFERENCES

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Metabolism of Ethers in the Rabbit

2. NUCLEAR-SUBSTITUTED ANISOLES

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In a previous paper (Bray, James, Thorpe & Wasdell, 1953) it was shown that when anisole was given to a rabbit, the main product excreted in urine was *p*-methoxyphenol; no phenol could be detected, so that it seemed improbable that demethylation was a significant metabolic pathway. Kossel (1880, 1883) and Lehmann (1889) made similar observations for phenetole in the dog, *p*-ethoxyphenol being the chief metabolite. Smith & Williams (1949*a*, *b*), however, isolated *p*-acetamidophenylglucuronide from the urine of rabbits which had received either phenacetin or *p*-phenetidine, showing that in the presence of an amino group in the *para* position, the ethoxy group could be hydrolysed. Huggins, Jensen & Cleveland (1948) also observed cleavage of *p*-nitrophenyl ethers in the rat. It was, therefore, of interest to investigate the effect of other substituents on the stability of alkoxy groups *in vivo*. The present study has been confined to methoxy compounds, which were *m*- and *p*-nitro-, *p*-chloro-, *p*-methyl-, *p*-methoxy-, *p*-hydroxy-, *p*-carboxy- and *p*-cyano-anisole. The effect of each of these compounds on the excretion by the rabbit of ethereal sulphates, ether glucuronides (glucosiduronic acids), free phenols and of other appropriate metabolites has been determined and certain metabolites have been isolated and characterized. The kinetics of some of the reactions have also been investigated. Some preliminary experiments showing that substituted aromatic ethers can be demethylated by rabbit tissue slices have also been carried out. Huggins *et al.* (1948) have shown that some *p*-nitrophenyl ethers can be demethylated by homogenates of rat tissues.

MATERIALS AND METHODS

Materials. *m*- and *p*-Nitroanisoles, *p*-chloroanisole, *p*-bromoanisole, *p*-methoxyanisole (*p*-dimethoxybenzene), *p*-methoxyphenol (*p*-hydroxyanisole) and anisic acid (*p*-carboxyanisole) were purchased. *p*-Methylanisole (*p*-methoxytoluene), b.p. 176°, was prepared by methylation of *p*-cresol with dimethyl sulphate and NaOH. *p*-Cyanoanisole was obtained by heating *p*-methoxybenzamide (anisamide) with PCl_5 (cf. Henry, 1869). 3- and 4-Nitrocatechols were prepared according to Weselsky & Benedikt (1882) and 4-chlorocatechol by the method of Frejka, Šefránek & Zika (1937). For the preparation of 4-cyanocatechol (cf. Hoesch & Zarzecki, 1917), 3:4-dihydroxybenzaldoxime was heated in boiling acetic anhydride for 1 hr. After removal of excess acetic anhydride by distillation *in vacuo*, the product was deacetylated by keeping overnight in 2·5 N-NaOH. Cyanocatechol was then extracted with ether from the acidified solution. Recrystallization from water of the residue left after evaporation of the ether gave colourless plates of 4-cyanocatechol dihydrate, m.p. 83°. (Found: loss at 105°, 19·9. Calc. for $C_7H_5O_2N, 2H_2O$: water, 21·0%.) On heating at 105° the dihydrate gave 4-cyanocatechol, m.p. 152° (Hoesch & Zarzecki give 156°). The nitrile was unchanged by boiling in 5 N-H_2SO_4 for 1 hr.

All melting points agreed with those given in the literature. Melting points recorded in this paper are uncorrected.

Preparation of derivatives. For characterization or isolation of the phenols formed as metabolites, derivatives were prepared as described by Johnson, Shennan & Reed (1950). Quinol ditoluene-*p*-sulphonate was, however, obtained by the method of Porteous & Williams (1949). p-*Cyanophenyl* p-*nitrobenzyl ether* forms pale yellow elongated platelets, m.p. 164°. (Found: C, 66·1; H, 3·8; N, 11·4. $C_{14}H_{10}O_3N_2$ requires C, 66·1; H, 3·9; N, 11·0%.) Since the isolation of derivatives of the phenols provided the main evidence for demethylation, some indication of the quantitative efficiency of the preparation of these derivatives was required.