In vivo Peroxidase Inhibitor in Bush Bean (*Phaseolus vulgaris*) Leaves

Recent reports from this laboratory described a peroxidase enzyme from bush bean roots which catalysed the oxidative decarboxylation of oxaloacetate (OAA) to malonate[1–3]. Attempts to demonstrate this enzyme in crude extracts of bush bean leaves were unsuccessful. Using standard enzyme purification procedures, however, one of us (J. Y. L.) purified a peroxidase enzyme from bush bean leaves 240-fold and showed that the purified enzyme was indeed able to convert OAA to malonate, confirming the presence of the peroxidase enzyme in bush bean leaves. Subsequent studies showed that the crude enzyme preparation from bean leaves contained an inhibitor of peroxidase catalysed reactions. Inhibitors of peroxidase catalysed reactions have also been identified in pea epicotyl[4], pineapple stem tissue[5], and cotton leaf tissue[6]; but the extent to which these inhibitors exert their effect *in vivo* has not been determined. This communication presents evidence indicating that the peroxidase inhibitor in bush bean leaves inhibited the *in vivo* peroxidase catalysed conversion of OAA to malonate.

Evidence concerning *in vivo* inhibition of peroxidase was obtained by diminishing the supply of potassium to the plant, thereby decreasing pyruvate kinase activity[7,8]. Under conditions of decreased pyruvate kinase activity phosphoenolpyruvate (PEP) is shunted to OAA[9]. If the peroxidase inhibitor were absent or were inactive *in vivo*, this increased supply of OAA could serve as a substrate for peroxidase and thus cause an increase in malonate content of the tissue. If, however, the peroxidase inhibitor were active *in vivo*, the increased supply of OAA would be an ineffective substrate for peroxidase.

Bush bean plants (*Phaseolus vulgaris*, var. Tendergreen) were grown in sand culture using Hoagland's No. 2 nutrient solution, complete and minus potassium. Leaves and roots were harvested 44 days after planting, dried at 70° C for 48 h, ground through a 40-mesh screen, and stored at room temperature until chemically analysed. The organic acids were removed by three successive 10-min extractions with 80, 20, and 50 per cent ethanol (v/v) on a hot-water bath at 98° C. The extracts were combined and passed through 'Dowex-50 (H^+)' cation exchange resin and then through 'Dowex-1 ($HCOO^-$)' anion exchange resin. The organic acids were displaced from the anion exchange resin and separated by the silica-gel column chromatography method of DeKock and Morrison[10]. The identity of the acid peaks eluted from the silica-gel column were confirmed by co-chromatography on paper with authentic standards using a solvent system of ether/acetic acid/water (5 : 2 : 1)[11]. Potassium was determined from wet-ashed plant material using a flame photometer. The results of this experiment are presented in Table 1. When potassium was omitted from the nutrient solution, the potassium content of the tissue was greatly diminished, while the malate content was increased markedly. Since malate is in equilibrium with OAA, this observation confirms the contention that potassium deficiency may shunt PEP to OAA.

Table 1. Influence of Potassium Nutrition on Malate and Malonate in Leaves and Roots of Bush Bean

Treatment	Potassium % dry weight	Malate μequiv./g dry weight	Malonate
		Leaves	
+ K	1·40	186	153
− K	0·46	291	105
		Roots	
+ K	1·35	44	79
− K	0·62	85	120

In addition, when potassium was omitted from the nutrient solution the malonate content of root tissues was increased while that of leaf tissues was decreased. Since peroxidase inhibitors have not been detected in bean root tissues the increase in root malonate confirms the contention that the increased supply of OAA brought about by potassium deficiency may serve as a substrate for peroxidase and be converted to malonate. The failure to detect a comparable increase in leaf malonate suggests that the peroxidase inhibitor in leaf tissues may cause an *in vivo* inhibition of leaf peroxidase and thereby prevent the increased supply of OAA from serving as a substrate for malonate formation.

Under conditions which shunt PEP to OAA one would anticipate a concomitant decrease in acetyl coenzyme *A*. Hence, the observation that leaf malonate was actually diminished under conditions of potassium deficiency suggests that malonate biosynthesis in bean leaves proceeds via acetyl coenzyme *A* carboxylase. The predominance of the acetyl coenzyme *A* carboxylase pathway in leaf tissues is in agreement with the work of Hatch and Stumpf, who reported that bean leaf tissue contained acetyl coenzyme *A* carboxylase activity whereas root tissues were essentially devoid of this enzyme[12].

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[1] de Vellis, J., Shannon, L. M., and Lew, J. Y., *Plant Physiol.*, **38**, 686 (1963).
[2] Shannon, L. M., de Vellis, J., and Lew, J. Y., *Plant Physiol.*, **38**, 691 (1963).
[3] Shannon, L. M., and Lew, J. Y., *Plant Physiol.*, **38** (Supp.), 46 (1963).
[4] Furuya, M., Galston, A. W., and Stowe, B. B., *Nature*, **193**, 456 (1962).
[5] Gortner, W. A., and Kent, M. I., *J. Biol. Chem.*, **233**, 731 (1958).
[6] Morgan, P. W., and Hall, W. C., *Plant Physiol.*, **38**, 365 (1963).
[7] Miller, G., and Evans, H. J., *Plant Physiol.*, **32**, 346 (1957).
[8] Evans, H. J., *Plant Physiol.*, **38**, 397 (1963).
[9] Pattee, H. E., and Teel, M. R. (submitted to *Plant Physiol.*).
[10] DeKock, P. C., and Morrison, M. I., *Biochem. J.*, **70**, 272 (1958).
[11] Palmer, J. K., *Bull.* 589 (Conn. Agric. Exp. Sta., New Haven, Conn., 1955).
[12] Hatch, M. C., and Stumpf, P. K., *Plant Physiol.*, **37**, 121 (1962).

Hexosamines in the Seeds of Higher Plants (Spermatophytes)

Amino-sugars, important constituents of various mucopolysaccharides and glycoproteins, are widespread in Nature. Their occurrence, chemistry and biological role have formed the subject of several reviews in the past twenty years[1].

Rather interestingly, apart from the animal kingdom, it is only in the phylogenetically lower classes of the vegetable kingdom that the presence of hexosamines has been well established. Glucosamine or derivatives of it are well known to occur in several bacterial polysaccharides[2] and cell wall polysaccharides[3], and glucosamine is also thought to be present in a chitin-like material obtained from the cell membranes of certain algæ[4] and higher fungi[5].

In the seed-bearing or higher plants (spermatophytes) hexosamines have never been clearly recognized as cell constituents although aqueous extracts of plant tissues have been shown to react with the Elson–Morgan reagent[6]. Moreover, small amounts of UDP–*N*-acetyl hexosamine[7] have been shown to occur in the nucleotide-bound sugar fraction of certain seeds and hexosamine is thought to be a component in lipopolysaccharides obtained from bean and potato leaves[8], but these amino-sugars have not been isolated and crystallized.

In the work recorded here the hexosamine content of various seeds was investigated. In order to exclude the possibility of estimating artefacts known to be formed from amino-acids and sugars under certain conditions[9] the amino-sugars were first separated from other components of the seed by chromatography on an ion-exchange resin column[10] ('Amberlite *IR*–120', 50 × 1 cm, 0·38 N for Na^+ and *p*H 4·18) and the eluates were tested with the Elson–Morgan reagent[11].