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METABOLISM OF PURINE NUCLEOTIDES IN THE TOMATO PLANT

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Key Word Index—*Lycopersicon esculentum*; Solanaceae; tomato; metabolism; purine nucleotides.

Abstract—The enzymes 5'-nucleotidase (EC 3.1.3.5) and adenine phosphoribosyltransferase (EC 2.4.2.7) from the roots and leaves of tomato (*Lycopersicon esculentum*) have been purified and characterized. Two forms (root 1 and root 2) of 5'-nucleotidase from tomato roots were separated by chromatography on DEAE-cellulose. These were further purified by affinity chromatography on Blue Sepharose CL-6B. The enzyme from leaves appeared in only one form (leaf) when purified by similar methods. Root 2 and leaf enzymes were very similar in all respects including M_r (*ca* 68 000) whilst root 1 appeared distinct with a M_r close to 18 000. Tomato 5'-nucleotidase catalysed hydrolysis of isopentenylAMP and its action on AMP was inhibited in the presence of nucleoside monophosphates including isopentenylAMP. Adenine phosphoribosyltransferase existed in one form in roots and leaves and these differed from one another in several respects, e.g. pH optimum, M_r. Both enzymes catalysed phosphoribosylation of benzyladenine and the conversion of adenine to AMP was inhibited by the presence of cytokinin bases. The enzymes from the two sources differed in their patterns of inhibition by cytokinin bases.

INTRODUCTION

Endogenous purines, including the cytokinins, have been shown to exist in a variety of forms including bases, ribosides and ribotides. Whilst many early studies of cytokinins concentrated on those which were soluble in organic solvents, it is now clear that a significant proportion of the total cytokinins in plant tissues exist in the form of ribotides [1, 2], which do not dissolve readily in these solvents and which may break down during extraction [3]. The physiological significance of these compounds is unknown but any consideration of the action of cytokinins must take their existence and metabolism into account.

The pathways for the formation of cytokinin ribotides and for their breakdown into ribosides and bases appear to parallel those involved in the metabolism of other purines and to be catalysed by the same enzymes which show a low degree of specificity for the structure of the base [4–7].

Two pathways for the formation of purine ribotides from base are known—one via the riboside, involving the sequential action of adenosine phosphorylase and adenosine kinase, and the other a one-step reaction catalysed by adenine phosphoribosyltransferase.

Purine phosphoribosyltransferases seem to be more widespread and active than purine riboside phosphorylases and evidence for their presence is often more convincing. The direct pathway thus seems to be more important than the two-step one although adenosine kinase may be of significance in the conversion of purine ribosides, produced in other ways, into ribotides.

Adenine phosphoribosyltransferase has been found in soybean callus and barely leaves [8], black gram seedlings [9], wheat embryos [10], *Acer pseudoplatanus* cells [11, 12], Jerusalem artichoke shoots [13], lupin seeds [14], tobacco leaf protoplasts [15], soybean embryos [16], *Catharanthus roseus* [17] and wheat germ [7].

The salvage of adenine in animals and micro-organisms often involves the action of adenosine phosphorylase. However, this enzyme is not common in plants and salvage of adenine in higher plants is likely to occur through the action of adenine phosphoribosyltransferase. The enzyme from wheat germ has also been shown to catalyse phosphoribosylation of a variety of cytokinin substrates [7] and a function for adenine phosphoribosyltransferase in cytokinin metabolism has been suggested [7, 18].

The hydrolysis of purine riboside monophosphates to purine ribosides and inorganic phosphate may be catalysed by 5'-nucleotidase or by non-specific phosphatases. 5'-Nucleotidase has been studied in a variety of animal tissues and micro-organisms, and the enzyme from wheat germ has been shown to catalyse dephosphorylation of N^6-(isopentenyl)-adenosine-5'-monophosphate as well as AMP.

Purine nucleoside monophosphates are poor substrates for non-specific phosphatases [19] and these enzymes are unlikely to contribute significantly to nucleotide hydrolysis in intact plants (L. R. Burch and T. Stuchbury, unpublished results).

Since adenine phosphoribosyltransferase and 5'-nucleotidase are probably of major importance in control of the levels of purine nucleotides, including those of the cytokinins, we have undertaken a study of the properties of these enzymes in the tomato plant.

RESULTS

Adenine phosphoribosyltransferase

Extracts from all parts of the tomato plant catalysed conversion of [U-^{14}C]adenine into [U-^{14}C]AMP.

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When subjected to ion-exchange chromatography on DEAE-cellulose, adenine phosphoribosyltransferase activity from either roots or leaves eluted as a single component (Figs 1a and 1b). The enzymes from root or leaf tissues, however, did differ from one another. Whilst both enzymes showed quite alkaline pH optima, that of the leaf enzyme occurred at a considerably higher pH (Fig. 2). The pH optimum of around 8.0 for the root enzymes is near the upper end of the range reported for plant adenine phosphoribosyltransferases [7, 8, 13, 17] whilst the value of approximately 9.0 for the leaf enzyme falls outside this range.

The M_r estimated for root adenine phosphoribosyltransferase (*ca* 27 000) was comparable with that reported for the enzyme from other plants [7] and from rat liver [20] whilst the leaf enzyme (M_r 47 000) was somewhat larger. The K_m values for adenine were 4.0 and 2.0 μM for root and leaf enzyme, respectively, and those for 5-phosphoribosyl 1-pyrophosphate 2.0 and 1.0 μM for the enzyme from the same sources.

The enzymes from both roots and leaves catalysed conversion of [8-^{14}C]benzyladenine into its ribotide. The K_m value for benzyladenine for the leaf enzyme was approximately 125 μM and that for the root enzyme 50 μM. These values are comparable with the value of 154 μM for the K_m of the wheat germ enzyme [7].

Conversion of adenine into AMP was inhibited by the presence of cytokinin bases (Table 1). The leaf enzyme was inhibited more by zeatin than by benzyladenine; for the root enzyme the reverse was true. The enzyme with the lowest K_m for benzyladenine (root) showed the greatest inhibition by benzyladenine.

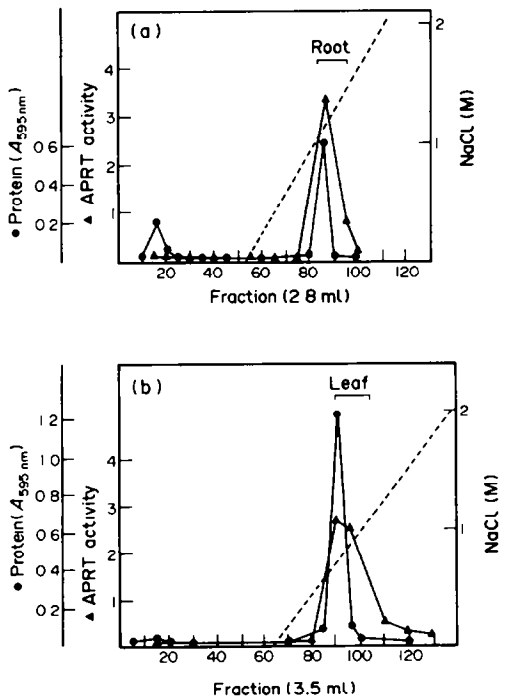

Fig. 1. Elution of tomato adenine phosphoribosyltransferase (APRT) from DEAE-cellulose as described in Experimental. (a) Adenine phosphoribosyltransferase from roots; (b) adenine phosphoribosyltransferase from leaves.

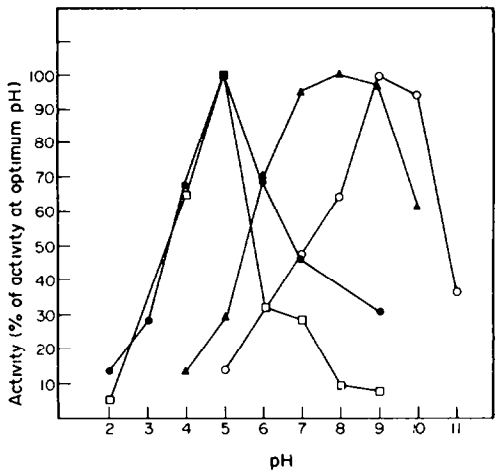

Fig. 2. pH–activity profiles for adenine phosphoribosyltransferase (APRT) and 5'-nucleotidase (5'-ND) from tomato. ●, Root 5'-ND; □, leaf 5'-ND; ▲, root APRT; ○, leaf APRT.

5'-Nucleotidase

Extracts made from all parts of the tomato plant catalysed conversion of [8-^{14}C]AMP to [8-^{14}C]adenosine. The enzyme catalysing this reaction was purified by ion-exchange chromatography on DEAE-cellulose. The enzyme from leaves eluted as a single component (Fig. 3b) whilst that from roots was resolved into two fractions, root 1 and root 2 (Fig. 3a). Further purification and complete removal of acid phosphatase activity were achieved by chromatography on Blue Sepharose CL-6B (Fig. 4).

Root 2 and leaf, which were eluted from DEAE-cellulose by approximately 0.7 M NaCl, had a M_r close to 68 000, considerably greater than that of root 1 (M_r 18 000). The value obtained for root 1 is very approximate due to the very low enzyme activity. The two components of 5'-nucleotidase isolated from wheat germ have been reported to have M_rs of 57 000 (FI) and 110 000 (FII) [6].

The K_m values for AMP for the enzyme from tomato roots and leaves, determined as described in Experimental, were quite similar and were as follows: root

Table 1. Percentage inhibition of adenine phosphoribosyltransferase by purine bases*

Compound	Source of enzyme	
	Root	Leaf
Hypoxanthine	0	0
Zeatin	14.4	64.1
Isopentenyladenine	35.8	37.0
Benzyladenine	24.4	11.5

*The partially purified enzyme was incubated at 30° for 5 min with 1.99 μM [U^{14}-C]adenine, 25.0 mM glycine–NaOH (pH 9.0), 0.38 mM phosphoribosylpyrophosphate and 0.63 mM inhibitor in a total volume of 80 μl. [U-^{14}C]AMP was estimated as described in Experimental.

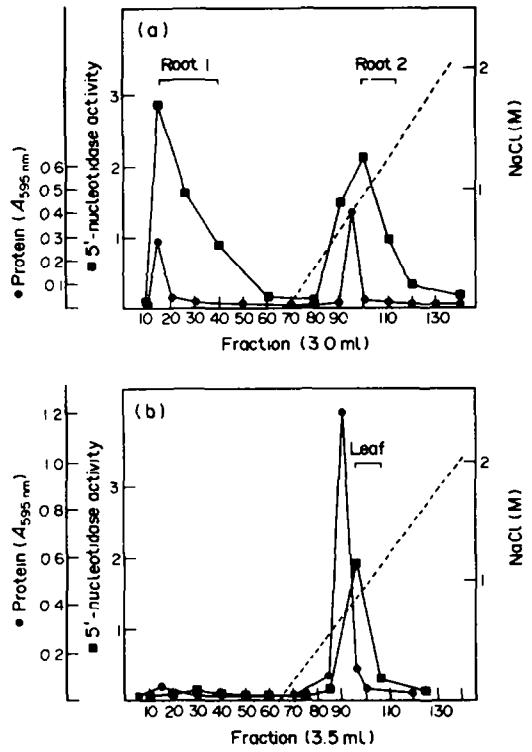

Fig. 3. Elution of tomato 5'-nucleotidase from DEAE-cellulose as described in Experimental. (a) 5'-Nucleotidase from roots; (b) 5'-nucleotidase from leaves.

1, 13 μM; root 2, 23 μM; leaf, 17 μM. All three showed a pH optimum of *ca* 5.0 (Fig. 2), considerably lower than the values reported for 5'-nucleotidase from other sources [6, 21–23].

The ability of root 2 5'-nucleotidase to hydrolyse cytokinin ribotides was examined using HPLC to follow conversion of isopentenylAMP into isopentenyladenosine. At a concentration of 2 mM, isopentenylAMP was hydrolysed at 25% of the rate of hydrolysis of AMP.

The conversion of AMP to adenosine was inhibited by cytokinin and other riboside monophosphates (Table 2),

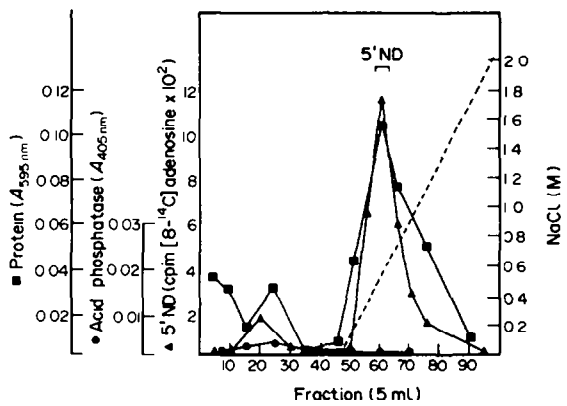

Fig. 4. Elution of tomato root 5'-nucleotidase (5'-ND) from Blue Sepharose CL-6B. Loading and elution of the column were carried out as described in Experimental.

Table 2. Percentage inhibition of tomato 5'-nucleotidase by phosphate esters*

Compound	Enzyme		
	Root 1	Root 2	Leaf
β-Glycerophosphate	0	0	0
IsopentenylAMP	49.4	55.1	70.3
IMP	85.1	80.5	83.9
GMP	83.5	87.1	85.8
UMP	47.0	66.8	80.8
CMP	42.5	64.6	43.1
ATP	41.7	—	34.9
ITP	24.1	—	18.0

*Purified tomato 5'-nucleotidase was incubated at 30° for 10 min with 5.75 μM [8-^{14}C]AMP, 50 mM Tris–HCl (pH 7.0), 5 mM 2-mercaptoethanol, 6 mM $MgCl_2$ and 0.67 mM various phosphate esters in a total volume of 60 μl. [8-^{14}C]Adenosine was estimated as described in Experimental.

as might be expected from the broad substrate specificity of this enzyme reported by others. The enzyme was also inhibited by a number of other nucleotides (ATP, NAD and ITP) and by Blue Dextran (results not shown).

DISCUSSION

Adenine phosphoribosyltransferase appears to exist in single but distinct forms in roots and leaves of the tomato plant. 5'-Nucleotidase from the roots, however, was fractionated into two forms by ion exchange chromatography. When root 1, which passed straight through the DEAE-cellulose column, was rechromatographed on the same column it again passed straight through so that resolution of the root enzyme into two components was unlikely to be the result of overloading the column. In addition, Chen and Kristopeit [6] reported that 5'-nucleotidase from wheat germ was resolved into two components during ion-exchange chromatography. These components also differed in M_r. It is not known whether the two tomato root enzymes show tissue or organelle specific distribution, or whether changes in their relative importance accompany large changes in 5'-nucleotidase activity occurring during development of the plants (L. R. Burch and T. Stuchbury, unpublished work).

Inhibition, by cytokinin derivatives, of the action of adenine phosphoribosyltransferase and 5'-nucleotidase on adenine and AMP respectively provides evidence that metabolism of N^6-substituted and unsubstituted purines is catalysed by single enzymes rather than by unresolved enzymes of different specificities. The greater inhibition of leaf adenine phosphoribosyltransferase by zeatin than by benzyladenine and the reverse for the root enzyme suggests that the leaf enzyme may bind more polar cytokinins than the root enzyme.

ATP was an inhibitor of root 2 5'-nucleotidase. 5'-Nucleotidase from animal tissues is also inhibited by ATP and this may be a means of controlling nucleotide breakdown [21, 22].

In animal tissues, 5'-nucleotidase has been proposed to control the release from cells of adenosine, produced during hydrolysis of AMP catalysed by 5'-nucleotidase

[24]. In some cells this enzyme may be membrane-bound but in cultured tomato cells it is cytosolic [25].

The uptake of both purine and cytokinin bases is accompanied by their rapid conversion into nucleotides catalysed by adenine phosphoribosyltransferase [1, 5, 26, 27]. In bacterial cells, uptake of bases and their phosphoribosylation may be linked processes [28] and this may also be the case in higher plants [29].

Since both adenine phosphoribosyltransferase and 5'-nucleotidase act on cytokinins they may be important in the control of cytokinin uptake and release by plant cells. In particular, the proportion of cytokinins present as nucleotides may be influenced by the relative activities of adenine phosphoribosyltransferase and 5'-nucleotidase. In view of the very different pH optima of these enzymes the nucleotide levels may be extremely pH-dependent within the normal range of pH found in plant cells (Fig. 2). Since cell membranes are thought to be impermeable to cytokinin nucleotides but not to bases or ribosides [1] hydrolytic removal of the 5'-phosphate group may be crucial in controlling the release of cytokinins from cells.

Within the tomato plant the distribution of these enzymes differs and very substantial changes in the activity of the enzymes occur during development (L. R. Burch and T. Stuchbury, unpublished work). Such changes may determine the competence of plant cells, tissues or organs to release or take-up cytokinins or other purine derivatives at specific times during their development.

EXPERIMENTAL

Chemicals. [8-^{14}C]AMP (2.07 GBq/mmol), [U-^{14}C]adenine (10.58 GBq/mmol) and [8-^{14}C]benzyladenine (2.11 GBq/mmol) were obtained from Amersham International. Polyethylene glycol (PEG) (M_r 8000), tetrabutyl ammonium hydrogen sulphate, isopentenyladenosine and Blue Sepharose CL-6B were from Sigma. IsopentenylAMP was supplied by P-L Biochemicals and Bio-gel P200 (50–150 mesh) by Bio-Rad Laboratories.

Analytical techniques. Protein concn was determined according to the method of Bradford [30].

Plants. Tomato plants (*Lycopersicon esculentum* Mill. cv. Bellina; Asmer Seeds, Leicester, U.K.) were grown in a greenhouse under natural lighting conditions, watered as required and fed a commercial liquid fertilizer (Fison's Liquinure) as directed by the manufacturer. After 8 weeks' growth, the roots and leaves were frozen in liquid N_2 and stored at $-80°$.

Extraction of adenine phosphoribosyltransferase. Frozen tomato roots (103 g) or leaves (107 g) were homogenized at 4° in 500 ml 50 mM Tris–HCl (pH 7.5) containing 5 mM $MgCl_2$ and 10 mM 2-mercaptoethanol. The homogenate was filtered through double layers of muslin and centrifuged for 10 min at 15 000 *g*. The supernatant was centrifuged again for 30 min at 20 000 *g*. The supernatant from this spin was purified by a modification of the method of Chen *et al.* [7].

Heat treatment. AMP and $(NH_4)_2SO_4$ were added to the crude extract, which was treated as described in ref. [7].

$(NH_4)_2SO_4$ *fractionation.* Solid $(NH_4)_2SO_4$ was added to the supernatant to 30% saturation. After 1 hr the sample was centrifuged at 20 000 *g* for 20 min. The 30% supernatant was brought to 80% saturation by addition of solid $(NH_4)_2SO_4$ and the ppt. was collected by centrifugation at 20 000 *g* for 20 min. The pellet was resuspended in homogenizing buffer and dialysed for 18 hr against 2 × 1 l. of the same buffer. The dialysate was reduced to 8 ml by dialysis against 25% PEG.

DEAE-cellulose chromatography. The conc root or leaf protein soln was applied to a DEAE-cellulose column (Whatman DE 32; 2.5 × 28 cm) equilibrated with homogenizing buffer. The column was eluted with 200 ml of this buffer followed by a linear gradient of NaCl (0–2.0 M; total vol. 200 ml). The adenine phosphoribosyltransferase fractions (shown as root or leaf in Figs 1a and 1b) were pooled and dialysed against 2 l. of homogenizing buffer for 18 hr and the vol. was reduced to 8 ml by dialysis against 25% PEG.

Gel filtration. The partially purified enzyme solns were passed through a column of Bio-gel P200 (50–150 mesh; 2.5 × 45 cm) or a column of Sephadex G-75 (2.5 × 44 cm) (flow rate 0.3 ml/min, fractions 4.4 ml) to purify the enzymes further. An approximate M_r for root 2 and leaf 5'-nucleotidase was also determined by comparison of their elution from the same P200 or Sephadex G-75 columns with that of standard proteins of known M_r.

Extraction of 5'-nucleotidase. Tomato roots (100 g) or leaves (80 g) were homogenized separately at 4° in a Townson Mercer top drive macerator in 400 ml 50 mM Tris–HCl (pH 7.0) containing 10 mM 2-mercaptoethanol and 12 mM $MgCl_2$. The homogenate was filtered through double layers of muslin and the purification procedure of Chen and Kristopeit [6] was followed with slight modifications.

$(NH_4)_2SO_4$ *fractionation.* Solid $(NH_4)_2SO_4$ was added to the extracts to 20% saturation. After 1 hr, the ppt. was removed by centrifugation at 20 000 *g* for 20 min and the supernatant was brought to 60% saturation by adding more $(NH_4)_2SO_4$. After 30 min, the ppt. was collected by centrifugation at 25 000 *g* for 20 min and redissolved in 20 ml homogenizing buffer. The protein soln was dialysed against 2 l. homogenizing buffer for 18 hr and reduced to 10 ml by dialysis against 25% PEG.

DEAE-cellulose chromatography. The root and leaf extracts were applied separately to a column of DEAE-cellulose (Whatman DE-32, 2.5 × 24 cm) equilibrated with homogenizing buffer. The column was eluted with 200 ml buffer followed by a linear gradient of NaCl (0–2.0 M, total vol. 180 ml) in the same buffer. 5'-Nucleotidase fractions shown as root 1 and root 2 (Fig. 3a) and leaf (Fig. 3b) were pooled and concentrated using 25% PEG.

Blue Sepharose CL-6B affinity chromatography. The enzyme solns were passed through the affinity chromatography column to remove contaminating acid phosphatase. The column (1.0 × 9.0 cm) was washed with homogenizing buffer which eluted the acid phosphatase. A linear gradient of NaCl (0–1.5 M; total vol. 150 ml) was used to elute root or leaf 5'-nucleotidase (Fig. 4). 10 mM ATP could also be used to elute root 2 enzyme. Fractions containing 5'-nucleotidase activity were pooled and used without further purification.

Enzyme assays. 5'-Nucleotidase: 5'-Nucleotidase was assayed in the following incubation mixture (total vol. 100 μl): 10 mM Tris–HCl (pH 7.0), 2 mM 2-mercaptoethanol, 2.4 mM $MgCl_2$, 5.70 μM [8-^{14}C]AMP and the enzyme preparation (10–80 μl). After 10 min incubation at 30° the reaction was terminated by placing the incubation vials in boiling water for 1 min. 20 μl of the reaction mixture was immediately chromatographed on Whatman DE-81 ion exchange paper developed in H_2O in an ascending fashion for 8–9 cm. Radioactivity associated with AMP (R_f 0–0.06), adenine (R_f 0.17–0.19) or adenosine (R_f 0.39–0.44) was estimated by counting 0.2–0.5 cm pieces in vials containing 2 ml scintillation fluid (Supersolve; Koch-Light Ltd.) in a Beckman LS-150 scintillation counter. When comparing the rates of hydrolysis of isopentenylAMP and AMP, isopentenyladenosine and adenosine were separated from isopentenylAMP and AMP, respectively, by reversed-phase HPLC on an Altex system using a Whatman Partisil PXS 10/25 ODS-3 column (4.6 mM i.d. × 25 cm) and the following solvent systems: 0.1 M acetate buffer (pH 5.0), 0.5 mM tetrabutyl ammonium hydrogen sulphate: MeOH (4:1 for separation of

AMP and adenosine; 3:2 for separation of isopentenylAMP and isopentenyladenosine).

Adenine phosphoribosyltransferase: This enzyme was assayed in the following incubation mixture (total vol. 60 μl): 0.33 mM phosphoribosylpyrophosphate, 2.7 μM [U^{14}-C]adenine, 16.7 mM glycine–NaOH (pH 9.0) and enzyme preparation (10 μl). After 5 min incubation at 30°, the reaction was terminated and the products were fractionated as described for the 5′-nucleotidase assay. Similar separations were obtained for benzyladenine and benzylAMP as for the corresponding adenine derivatives.

REFERENCES

1. Laloue, M., Pethe-Terrine, C. and Guern, J. (1981) in *Metabolism and Molecular Activities of Cytokinins* (Guern, J. and Peaud-Lenoel, C. eds) p. 80. Springer, Berlin.
2. Scott, I. M. and Horgan, R. (1984) *Planta* **161**, 345.
3. Bieleski, R. L. (1964) *Analyt. Biochem.* **9**, 431.
4. Chen, C.-M. and Eckert, R. L. (1977) *Plant Physiol.* **59**, 443.
5. Miernyk, J. A. (1979) *Physiol. Plant.* **45**, 63.
6. Chen, C.-M. and Kristopeit, S. M. (1981) *Plant Physiol.* **67**, 494.
7. Chen, C.-M., Melitz, D. K. and Clough, F. W. (1982) *Arch. Biochem. Biophys.* **214**, 634.
8. Nicholls, P. B. and Murray, A. W. (1986) *Plant Physiol.* **43**, 645.
9. Nobusawa, E. and Ashihara, H. (1983) *Int. J. Biochem.* **15**, 1059.
10. Price, C. E. and Murray, A. W. (1969) *Biochem. J.* **115**, 129.
11. Sadorge, P., Doree, M., Terrine, C. and Guern, J. (1970) *Physiol. Veg.* **8**, 499.
12. Doree, M. (1973) *Phytochemistry* **12**, 2101.
13. Le Floc'H, F. and Lefleuriel, J. (1978) *Phytochemistry* **17**, 643.
14. Guranowski, A. and Barankiewicz, J. (1979) *FEBS Letters* **104**, 95.
15. Barankiewicz, J. and Paszkowski, J. (1980) *Biochem. J.* **186**, 343.
16. Anderson, J. D. (1977) *Plant Physiol.* **60**, 689.
17. Hirose, F. and Ashihara, H. (1983) *Z. Pflanzenphysiol.* **110**, 135.
18. Doree, M. and Guern, J. (1973) *Biochim. Biophys. Acta* **304**, 611.
19. Fox, I. H. and Kelly, W. N. (1978) *Annu. Rev. Biochem.* **47**, 655.
20. Kenimer, J. G., Young, L. G. and Groth, D. P. (1975) *Biochim. Biophys. Acta* **384**, 87.
21. Ipata, P. L. (1968) *Biochemistry* **2**, 507.
22. Murray, A. W. and Friedrichs, B. (1969) *Biochem. J.* **111**, 83.
23. Neu, H. C. and Heppel, L. A. (1965) *J. Biol. Chem.* **240**, 3685.
24. Arch, J. R. S. and Newsholme, E. A. (1978) *Essays Biochem.* **14**, 82.
25. Wasternack, C., Guranowski, A., Glund, K., Tewes, A. and Walther, R. (1985) *J. Plant Physiol.* **120**, 19.
26. Stuchbury, T., Palni, L. M. S., Horgan, R. and Wareing, P. F. (1979) *Planta* **147**, 97.
27. Palni, L. M. S., Horgan, R., Darrall, N. M., Stuchbury, T. and Wareing, P. F. (1983) *Planta* **159**, 50.
28. Hochstadt-Ozer, J. and Stadtman, E. R. (1971) *J. Biol. Chem.* **246**, 5304.
29. Letham, D. S., Tao, G. Q. and Parker, C. W. (1982) in *Plant Growth Substances*, 1982 (Wareing, P. F., ed.) p. 143. Academic Press, London.
30. Bradford, M. M. (1976) *Analyt. Biochem.* **72**, 248.