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ORIGINAL ARTICLE

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Profiles of pyrimidine biosynthesis, salvage and degradation in disks of potato (*Solanum tuberosum* L.) tubers

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Abstract In order to obtain general metabolic profiles of pyrimidine ribo- and deoxyribonucleotides in potato (*Solanum tuberosum* L.) plants, the in situ metabolic fate of various ^{14}C-labelled precursors in disks from growing potato tubers was investigated. The activities of key enzymes in potato tuber extracts were also studied. The following results were obtained. Of the intermediates in de novo pyrimidine biosynthesis, [^{14}C]carbamoylaspartate was converted to orotic acid and [2-^{14}C]orotic acid was metabolized to nucleotides and RNA. UMP synthase, a bifunctional enzyme with activities of orotate phosphoribosyltransferase (EC 2.4.2.10) and orotidine 5′-monophosphate decarboxylase (EC 4.1.1.23), exhibited high activity. The rates of uptake of pyrimidine ribo- and deoxyribonucleosides by the disks were high, in the range 2.0–2.8 nmol (g FW)$^{-1}$ h^{-1}. The pyrimidine ribonucleosides, uridine and cytidine, were salvaged exclusively to nucleotides, by uridine/cytidine kinase (EC 2.7.1.48) and non-specific nucleoside phosphotransferase (EC 2.7.1.77). Cytidine was also salvaged after conversion to uridine by cytidine deaminase (EC 3.5.4.5) and the presence of this enzyme was demonstrated in cell-free tuber extracts. Deoxycytidine, a deoxyribonucleoside, was efficiently salvaged. Since deoxycytidine kinase (EC 2.7.1.74) activity was extremely low, non-specific nucleoside phosphotransferase (EC 2.7.1.77) probably participates in deoxycytidine salvage. Thymidine, which is another pyrimidine deoxyribonucleoside, was degraded and was not a good precursor for nucleotide synthesis. Virtually all the thymidine 5′-monophosphate synthesis from thymidine appeared to be catalyzed by phosphotransferase activity, since little thymidine kinase (EC 2.7.1.21) activity was detected. Of the pyrimidine bases, uracil, but not cytosine, was salvaged for nucleotide synthesis. Since uridine phosphorylase (EC 2.4.2.3) activity was not detected, uracil phosphoribosyltransferase (EC 2.4.2.9) seems to play the major role in uracil salvage. Uracil was degraded by the reductive pathway via β-ureidopropionate, but cytosine was not degraded. The activities of the cytosine-metabolizing enzymes observed in other organisms, pyrimidine nucleoside phosphorylase (EC 2.4.2.2) and cytosine deaminase (EC 3.5.4.1), were not detected in potato tuber extracts. Operation of the de novo synthesis of deoxyribonucleotides via ribonucleotide reductase and of the salvage pathway of deoxycytidine was demonstrated via the incorporation of radioactivity from both [2-^{14}C]cytidine and [2-^{14}C]deoxycytidine into DNA. A novel pathway converting deoxycytidine to uracil nucleotides was found and deoxycytidine deaminase (EC 3.5.4.14), an enzyme that may participate in this pathway, was detected in the tuber extracts.

Keywords Deoxyribonucleotide synthesis · Nucleotide metabolism · Pyrimidine degradation · Pyrimidine salvage · *Solanum* (pyrimidine synthesis) · Uridine/cytidine kinase

Abbreviations CDP: cytidine 5′-diphosphat · PRPP: 5-phosphoribosyl-1-pyrophosphate · UDPGlc: UDP-glucose

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Introduction

Pyrimidine nucleotides are building blocks for RNA and DNA, and are also cofactors in the biosynthesis of phospholipids, glycolipids, sugars, and polysaccharides. The de novo synthesis of pyrimidine nucleotides begins with a series of six reactions to give orotate, the first pyrimidine intermediate. Orotate is then

converted to UMP, which is modified to form UTP and CTP. The de novo pathway is very highly conserved in both prokaryotes and eukaryotes (Santoso and Thornburg 1992). Deoxyribonucleotides are derived from ribonucleotides. In addition to the de novo pathways, there are several salvage pathways. The free ribonucleosides, deoxyribonucleosides and nucleobases formed during the breakdown of nucleotides and nucleic acids can be converted back to nucleotide by these salvage pathways. The salvage pathways of nucleotide biosynthesis are more diverse and are less understood (Sugiura and Takeda 2000). Furthermore, little is known about the catabolism of pyrimidine nucleotides in higher plants.

In the present study, potato tubers were used to investigate the relationship of pyrimidine metabolism and growth because changes in pyrimidine metabolism of potato must be closely related to other important aspects of metabolism that occur during tuber development. Within actively growing potato tubers, pyrimidine nucleotides, such as UTP and CTP, appear to be used mainly for nucleic acid synthesis while pyrimidine nucleotide sugars may be utilized for the synthesis of starch and phospholipids. The importance of pyrimidine nucleotides in starch synthesis has also been proposed. Loef et al. (1999) assert that a high concentration of uridine nucleotides increases the rate of starch synthesis from sucrose in growing potato tubers. In sprouting tubers, by contrast, stored starch is degraded and the resulting sucrose is transported to growing parts of the potato plant where UDPglucose (UDPGlc) also plays a key role in starch–sucrose conversion (Sowokinos et al. 1993). Pyrimidine nucleotides are therefore important at various stages of growth in potato tubers.

We demonstrate here the biosynthetic and catabolic pathways of pyrimidine nucleotides, including deoxyribonucleotides, in potato tubers, using in situ ^{14}C-tracer experiments and in vitro measurements of activities of related enzymes. To our knowledge this is the first comprehensive study of de novo and salvage biosynthetic pathways and interconversion and degradation of pyrimidine ribo- and deoxyribonucleotides in the same higher-plant material.

Materials and methods

Chemicals

All radiochemicals were MOR pure grade from Moravek Biochemicals (Brea, Calif., USA) and the purity was confirmed by TLC before use. Biochemicals and ACS-II scintillation fluid were purchased from Sigma Chemical Co. (St. Louis, Mo., USA) and from Amersham International (Amersham, UK), respectively.

Plant material

Seed potatoes (*Solanum tuberosum* L., cv. Danshaku) were obtained from the Hokuren Federation of Agricultural Cooperatives (Sapporo, Japan), and planted in March 2000 in a field of a farm at Machida, Tokyo. Growing tubers (ca. 30 g FW), obtained from 10- to 12-week-old plants, were used in all experiments. Cylinders, 6 mm in diameter, were cut parallel to the main axis of the tubers, and disks 1 mm thick (ca. 28 mg FW) were prepared aseptically, washed three times with sterilized 20 mM potassium phosphate buffer (pH 5.7) and used immediately as experimental material.

Metabolism of labelled pyrimidine precursors

Administration of ^{14}C-precursors and analysis of labelled metabolites were performed according to the protocols of Ashihara et al. (2000). All tracer experiments were performed aseptically and 37 kBq of each ^{14}C-labelled compound was used as precursor. The specific activities of these labelled compounds were: [2-^{14}C]orotic acid, 2.04 GBq mmol^{-1}; [carbamoyl-^{14}C]carbamoylaspartate, 2.04 GBq mmol^{-1}; [2-^{14}C]uracil, 1.96 GBq mmol^{-1}; [2-^{14}C]cytosine, 1.96 GBq mmol^{-1}; [2-^{14}C]uridine, 2.00 GBq mmol^{-1}; [2-^{14}C]cytidine, 2.04 GBq mmol^{-1}; [2-^{14}C]thymidine, 1.96 GBq mmol^{-1}; and [2-^{14}C]deoxycytidine, 2.07 GBq mmol^{-1}.

Six tuber disks and 1.8 ml of sterilized 20 mM potassium phosphate buffer (pH 5.7) were placed in the main compartment of a 30-ml Erlenmeyer flask fitted with a glass tube that contained a piece of filter paper impregnated with 0.1 ml of 20% KOH in a center well. Each reaction was started by the addition of 10 µl (37 kBq) of labelled compound to the main compartment of the flask. The flasks were incubated in an oscillating water bath at 22 °C. After 4 h, the glass tube was removed from the center well and placed in a 50-ml Erlenmeyer flask that contained 10 ml of distilled water. Simultaneously, the disks were collected, washed with distilled water, frozen with liquid nitrogen and then stored at –80 °C until extraction. Potassium bicarbonate that had been absorbed by the filter paper was allowed to diffuse into distilled water overnight, and aliquots of the resultant solution were used for the determination of radioactivity. The samples were extracted successively with cold 6% perchloric acid and a mixture of ethanol and ether (1:1, v/v) at 50 °C for 15 min. The ethanol and ether mixture insoluble fraction was hydrolyzed with 0.3 M KOH at 37 °C for 18 h, and adjusted to pH 2 with 6 N HCl and 60% perchloric acid. After centrifuging the mixture at 10,000 *g* for 5 min we removed the supernatant (RNA hydrolysates), and the DNA in the precipitate was hydrolyzed with 6% perchloric acid at 100 °C for 20 min. In some experiments, both RNA and DNA were simultaneously hydrolyzed with 6% perchloric acid at 100 °C for 20 min using the method of Schneider (1945). The perchloric acid-soluble metabolites and hydrolyzates of nucleic acids were neutralized with KOH, and radioactive compounds were separated by TLC using microcrystalline cellulose plates (200 mm×200 mm; Spotfilm; Tokyo Kasei Kogyo, Tokyo, Japan) and either *n*-butanol–acetic acid–water (4:1:2, v/v; system 1) or distilled water (system 2) as solvents. Some experiments employed ion-exchange PEI-cellulose plates (180 mm×200 mm). The solvent for these plates (system 3) was 0.2 M LiCl (2 min), 1.0 M LiCl (6 min) and 1.6 M LiCl (up to 13 cm above the origin; Randerath and Randerath 1967).

Radioactivity was determined using a multi-purpose scintillation counter (Type LS 6500; Beckman, Fullerton, Calif., USA) with scintillation fluid ACS-II. The distribution of ^{14}C radioactivity on the TLC plates was analyzed using a bio-imaging analyzer (Type FLA-2000; Fuji Photo Film Co., Tokyo, Japan).

Enzyme preparation

Potato tuber disks (ca. 2 g FW) were homogenized in a chilled extraction medium containing 50 mM Hepes–NaOH buffer (pH 7.6), 2 mM Na-EDTA, 2 mM DTT, 0.5% sodium ascorbate and 0.2% polyvinylpolypyrrolidone, using a pestle and mortar on ice. The homogenate was centrifuged at 20,000 *g* for 20 min at 4 °C. Aliquots of the supernatant (2.5 ml) were desalted on a prepacked column of Sephadex G-25 (PD-10) (Amersham Pharmacia Biotech, Uppsala, Sweden), and used for enzyme preparation.

Determination of enzyme activity

Activity of all enzymes in this study was determined using ^{14}C-labelled substrates according to the method of Ashihara et al. (2000). To obtain proper assay conditions, various preliminary experiments were carried out before determining the activity of each enzyme. The proportionality of the reaction velocity to the amount of enzyme was confirmed by plotting initial velocities against at least three different amounts of the enzyme preparation.

The total volume of the reaction mixture was 100 µl, and incubation was performed at 30 °C. The enzyme reactions were terminated by adding 10 µl of 60% perchloric acid. The reaction mixture was neutralized with KOH as described above. After removal of the precipitate, the neutralized samples were evaporated to dryness and the pellets were dissolved in 50% (v/v) ethanol and loaded onto the cellulose TLC plates. The labelled substrate and product were separated as described above, with solvent system 1. The specific activities of [2-^{14}C]orotic acid, [2-^{14}C]uracil, [2-^{14}C]cytosine, [2-^{14}C]uridine, [2-^{14}C]cytidine, [2-^{14}C]thymidine and [2-^{14}C]deoxycytidine used for the enzyme assays were all 340 kBq µmol^{-1}, except for [2-^{14}C]uracil for uridine phosphorylase at 185 kBq µmol^{-1}. The compositions of the reaction mixtures for the enzyme assays were as follows:

- Phosphoribosyltransferases (uracil-, cytosine- and orotate phosphoribosyltransferase): 30 mM Hepes–NaOH buffer (pH 7.6), 10 mM $MgCl_2$, 1 mM DTT, 0.6 mM 5-phosphoribosyl-1-pyrophosphate (PRPP) and 55 µM labelled substrate ([2-^{14}C]uracil, [2-^{14}C]cytosine or [2-^{14}C]orotic acid).
- Nucleoside kinases (uridine-, cytidine-, deoxycytidine- and thymidine kinase): 30 mM Hepes–NaOH buffer (pH 7.6), 10 mM $MgCl_2$, 1 mM DTT, 3.75 mM ATP and 55 µM labelled substrate ([2-^{14}C]uridine, [2-^{14}C]cytidine, [2-^{14}C]deoxycytidine, or [2-^{14}C]thymidine). To prevent decomposition of ATP in the enzyme preparation, 1 mM phosphoenolpyruvate with 16.7 nkat rabbit muscle pyruvate kinase (Sigma No. P9136) and 10 mM NaF were added to the reaction mixture as an ATP-generating system and a phosphatase inhibitor.
- Nucleoside phosphotransferase: as for nucleoside kinases, except that ATP was replaced by 3.75 mM AMP and the ATP re-generating system and NaF were left out.
- Nucleoside deaminases (cytidine- and deoxycytidine deaminase): as for nucleoside phosphotransferase, except that AMP was left out.
- Cytosine deaminases: 30 mM Hepes–NaOH buffer (pH 7.6), 10 mM $MgCl_2$, 1 mM DTT and 55 µM [2-^{14}C]cytosine.
- Nucleoside phosphorylase (cytidine- and uridine): 30 mM Hepes–NaOH buffer (pH 7.6), 10 mM $MgCl_2$, 1 mM DTT, 0.17 mM ribose-1-phosphate and 100 µM labelled substrate ([2-^{14}C]cytosine and [2-^{14}C]uracil).

Results

Metabolism of intermediates of de novo UMP synthesis

To estimate the contribution of the de novo pathway to nucleotide biosynthesis, the metabolic fate of [carbamoyl-^{14}C]carbamoylaspartate and [2-^{14}C]orotate in disks taken from growing potato tubers was investigated. Incorporation of the radioactivity from [carbamoyl-^{14}C]carbamoylaspartate and [2-^{14}C]orotate into metabolites was investigated. The rate of uptake of [carbamoyl-^{14}C]carbamoylaspartate by the disks was very low [0.42 ± 0.07 nmol (g FW)$^{-1}$ (4 h)$^{-1}$]. More than 40% of the radioactivity from [carbamoyl-^{14}C]carbamoylaspartate metabolised by the disks was distributed in orotic acid. Low levels of radioactivity were also found in two unidentified compounds, but the low level made further analysis difficult. Substantial radioactivity from [2-^{14}C]orotate was distributed in the uracil ribonucleotides (UMP, UDP and UTP), UDPGlc and RNA (Fig. 1). It follows that pyrimidine nucleotide biosynthesis de novo is a physiologically significant process in potato tubers. A small amount of radioactivity from [2-^{14}C]orotate was found in uridine and CO_2; these compounds may be degradation products of uridine nucleotides.

Metabolism of pyrimidine ribonucleosides

[2-^{14}C]Uridine and [2-^{14}C]cytidine were readily taken up by the potato disks (see legend for Fig. 2). More than 90% of the radioactivity taken up by the disks was incorporated into salvage products, nucleotides and nucleic acids (Fig. 2). Approximately 40% of the radioactivity from [2-^{14}C]uridine was recovered as uracil nucleotides, a further 40% showed as UDPGlc, and small amounts of radioactivity were found as cytosine nucleotides (0.5%) and cytidine 5′-diphosphate (CDP) lipids (4%). A substantial amount of radioactivity (14.2%) was incorporated into RNA. Almost 75% of radioactivity was distributed in uracil residues of RNA, and the rest (ca. 25%) was in cytosine residues. Release of $^{14}CO_2$ from [2-^{14}C]uridine was low (less than 1% of total radioactivity).

More than half of the radioactivity (50.8%) from [2-^{14}C]cytidine was incorporated into RNA, but only 0.7% into DNA. Nearly 20% was recovered as cytosine nucleotides. Some radioactivity was also distributed in

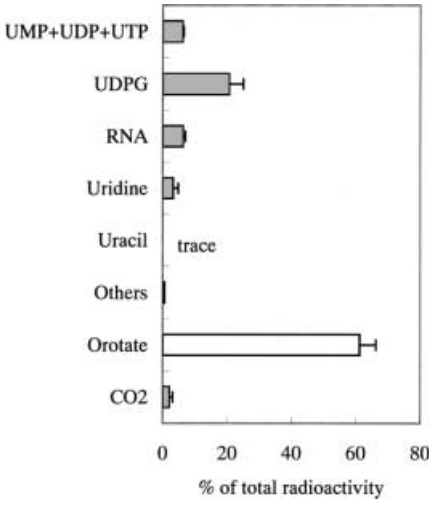

Fig. 1. Metabolism of [2-^{14}C]orotate in growing tubers of potato (*Solanum tuberosum*). Slices (ca. 200 mg FW) were incubated for 4 h with 9.1 µM [2-^{14}C]orotic acid in 1.8 ml of 20 mM potassium phosphate buffer (pH 5.7) at 22 °C. Rates of incorporation are expressed as a percentage of total uptake. The average value ± SD of the total radioactivity taken up by the slices is 2.1 ± 0.4 nmol (g FW)$^{-1}$

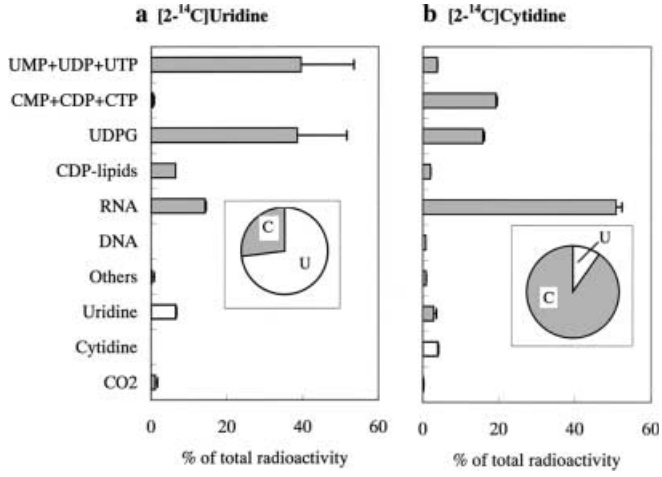

Fig. 2. Metabolism of [2-^{14}C]uridine (**a**) and [2-^{14}C]cytidine (**b**) in growing potato tubers. Slices (ca. 200 mg FW) were incubated for 4 h with 9.3 μM [2-^{14}C]uridine and 9.1 μM [2-^{14}C]cytidine in 1.8 ml of 20 mM potassium phosphate buffer (pH 5.7) at 22 °C. Rates of incorporation are expressed as a percentage of total uptake. Average values ± SD of the total radioactivity taken up by the slices are 11.3 ± 0.9 nmol (g FW)$^{-1}$ (**a**) and 9.3 ± 0.2 nmol (g FW)$^{-1}$ (**b**). The *inset* shows the proportional distribution of radioactivity in uracil (*U*) and cytosine (*C*) bases of RNA

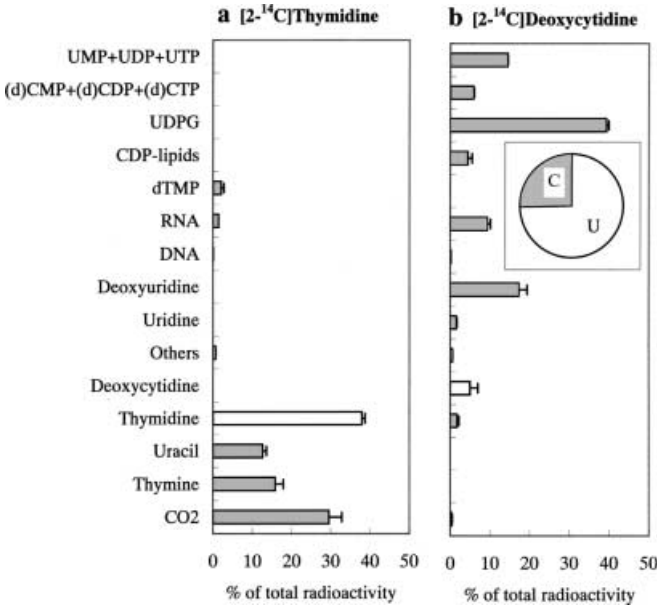

Fig. 3. Metabolism of [2-^{14}C]thymidine (**a**) and [2-^{14}C]deoxycytidine (**b**) in growing potato tubers. Slices (ca. 200 mg FW) were incubated for 4 h with 9.4 μM [2-^{14}C]thymidine and 9.1 μM [2-^{14}C]deoxycytidine in 1.8 ml of 20 mM potassium phosphate buffer (pH 5.7) at 22 °C. Rates of incorporation are expressed as a percentage of total uptake. Average values ± SD of the total radioactivity taken up by the slices are 7.9 ± 0.2 nmol (g FW)$^{-1}$ (**a**) and 9.8 ± 0.9 nmol (g FW)$^{-1}$ (**b**). The proportional distribution of radioactivity in uracil (*U*) and cytosine (*C*) bases of RNA from [2-^{14}C]deoxycytidine is also shown in the figure. *(d)CMP + (d)CDP + (d)CTP* Cytosine deoxyribonucleotides plus cytosine ribonucleotides, *dTMP* thymidine 5′-monophosphate

uracil nucleotides (ca. 4%), UDPGlc (ca. 16%) and CDP lipids (ca. 2%). More than 90% of the radioactivity in the RNA fraction was distributed in cytosine and less than 10% in uracil residues. A small amount of radioactivity (ca. 3%) was recovered as uridine.

Metabolism of pyrimidine deoxyribonucleosides

Uptake of [2-^{14}C]thymidine and [2-^{14}C]deoxycytidine by potato disks was rapid (see legend for Fig. 3). Less than 5% of [2-^{14}C]thymidine taken up by the disks was salvaged to nucleotides, and almost all of the thymidine metabolized was recovered as thymine, uracil and CO_2. In contrast, [2-^{14}C]deoxycytidine was easily salvaged. However, substantial amounts of radioactivity from [2-^{14}C]deoxycytidine were distributed in uracil ribonucleotides (14.5%), UDPGlc (39.3%) and RNA (9.4%). Approximately 75% of the radioactivity in RNA was recovered as uracil residues, and the rest (ca. 25%) as cytosine residues. Less than 6% of the radioactivity was incorporated into deoxyribonucleotides and DNA. Approximately 17% of the radioactivity was recovered as deoxyuridine. In contrast to thymidine metabolism, the catabolic pathway of deoxycytidine seems not to be functional, since release of $^{14}CO_2$ from [2-^{14}C]deoxycytidine was extremely low (less than 0.5% of total radioactivity).

Metabolism of pyrimidine bases

We studied the metabolic fate of the two pyrimidine bases, [2-^{14}C]uracil and [2-^{14}C]cytosine in the potato disks (Fig. 4). Nearly 40% of the total radioactivity [6.7 ± 0.1 nmol (g FW)$^{-1}$ (4 h)$^{-1}$] from [2-^{14}C]uracil was incorporated into the salvage products, uracil nucleotides (21.4%), UDPGlc (11.5%) and RNA (4.4%). Approximately 80% of the radioactivity in RNA was recovered as uracil residues, and 20% as cytosine residues. Approximately half of the total radioactivity from [2-^{14}C]uracil was recovered as degradation metabolites, β-ureidopropionate (< 0.1%) and CO_2 (47.4%). [2-^{14}C] Cytosine taken up by the disks [2.67 ± 0.25 nmol (g FW)$^{-1}$ (4 h)$^{-1}$] remained unmetabolised; as a result, data are not shown in Fig. 4.

Enzyme activity

Table 1 shows profiles of the activity of various key enzymes in pyrimidine nucleotide metabolism, measured in a desalted enzyme preparation from growing potato tubers. Orotate phosphoribosyltransferase, which is a key enzyme in de novo pyrimidine nucleotide biosynthesis, had the highest activity [ca. 28 pkat (mg protein)$^{-1}$] of all the enzymes measured here. Kinase activities were also high for uridine [ca. 10 pkat (mg protein)$^{-1}$] and cytidine [ca. 6 pkat (mg protein)$^{-1}$]. Since a single uridine/cytidine kinase protein has been found in most organisms including plants, these two activities are most

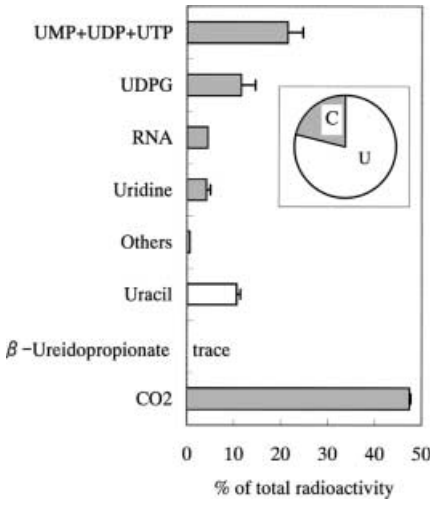

Fig. 4. Metabolism of [2-^{14}C]uracil in growing potato tubers. Slices (ca. 200 mg FW) were incubated for 4 h with 10.0 μM [2-^{14}C]uracil in 1.8 ml of 20 mM potassium phosphate buffer (pH 5.7) at 22 °C. Rates of incorporation are expressed as a percentage of total uptake. Average values ± SD of total radioactivity taken up by the slices are 6.7 ± 0.1 nmol (g FW)$^{-1}$. The proportional distribution of radioactivity in uracil (*U*) and cytosine (*C*) bases of RNA from [2-^{14}C]uracil is also shown in the figure

likely to arise from this single kinase. We also detected uridine and cytidine phosphotransferase activity measured with AMP [ca. 4 and 3 pkat (mg protein)$^{-1}$, respectively], but these activities were roughly half of the kinase activity with ATP. Substantial activities of deoxyribonucleoside kinases such as thymidine kinase and deoxycytidine kinase have been measured in other organisms, but their activities were extremely low in growing potato tubers. However, the activities of nucleoside phosphotransferase(s) for thymidine and deoxycytidine using AMP as a phosphate donor were extremely high [ca. 18 and 9 pkat (mg protein)$^{-1}$, respectively]. Low uracil phosphoribosyltransferase activity [ca. 1 pkat (mg protein)$^{-1}$] was observed, but no cytosine phosphoribosyltransferase activity was found. The activities of uridine phosphorylase and cytidine phosphorylase were not detected. Cytidine deaminase [ca. 3 pkat (mg protein)$^{-1}$] and deoxycytidine deaminase [ca. 5 pkat (mg protein)$^{-1}$] activities were detected, but not that of cytosine deaminase.

Discussion

Successful cloning has recently taken place of nearly all the genes that encode enzymes for de novo pyrimidine biosynthesis and some enzymes for salvage pathways, and the molecular properties of the enzymes have been determined (Moffatt and Ashihara 2002). However, the function and regulation of pyrimidine metabolism in vivo cannot be inferred from this information alone. We therefore examined the metabolic fate of various ^{14}C-substrates in situ and the activities of several related enzymes in growing potato tubers.

^{14}C-Labeled orotic acid, uridine and uracil have been often used to evaluate the activity of de novo, salvage and degradation pathways of pyrimidine nucleotides (e.g. Ashihara et al. 2000), but there have been very few studies of other pyrimidines in plant tissues (Ross 1965; Ross and Cole 1968). To obtain comprehensive profiles of pyrimidine metabolism in potato plants, we also studied the metabolism of labelled carbamoylaspartate, cytidine, cytosine, thymidine and deoxycytidine. The results allow us to propose major and minor pathways for pyrimidine biosynthesis de novo, and salvage and degradation of ribonucleotides (Fig. 5) and deoxyribonucleotides (Fig. 6) in growing potato tubers.

Table 1. Maximum activities of enzymes involved in pyrimidine metabolism in growing tubers of potato (*Solanum tuberosum*). The activities are expressed as pkat (mg protein)$^{-1}$. *dTMP* Thymidine 5′-monophosphate, *dCMP* deoxycytidine 5′-monophosphate, *ND* no activity detected

	Reaction Substrate→Product	Enzyme (EC)	Enzyme activity
	(Step number in Figs. 5, 6)		
De novo pathway	Orotate→UMP (3 and 4 in Fig. 5)	UMP synthase (2.4.2.10 + 4.1.1.23)	27.94 ± 0.17
Ribonucleoside salvage	Uridine→UMP (5 in Fig. 5)	Uridine/cytidine kinase (2.7.1.48)	10.30 ± 0.27
	Uridine→UMP (6 in Fig. 5)	Nucleoside phosphotransferase (2.7.1.77)	4.45 ± 0.45
	Cytidine→CMP (5 in Fig. 5)	Uridine/cytidine kinase (2.7.1.48)	5.88 ± 0.11
	Cytidine→CMP (6 in Fig. 5)	Nucleoside phosphotransferase (2.7.1.77)	2.82 ± 0.12
Deoxyribonucleoside salvage	Thymidine→dTMP(1 in Fig. 6)	Thymidine kinase (2.7.1.21)	Trace
	Thymidine→dTMP(2 in Fig. 6)	Nucleoside phosphotransferase (2.7.1.77)	17.48 ± 0.28
	Deoxycytidine→dCMP (6 in Fig. 6)	Deoxycytidine kinase (2.7.1.74)	Trace
	Deoxycytidine→dCMP (2 in Fig. 6)	Nucleoside phosphotransferase (2.7.1.77)	9.05 ± 0.81
Nucleobase salvage	Uracil→Uridine (10 in Fig. 5)	Uridine phosphorylase (2.4.2.3)	ND
	Uracil→UMP (8 in Fig. 5)	Uracil phosphoribosyltransferase (2.4.2.9)	1.27 ± 0.29
	Cytosine→Cytidine (21 in Fig. 5)	Pyrimidine nucleoside phosphorylase (2.4.2.2)	ND
	Cytosine→CMP (22 in Fig. 5)	Cytosine phosphoribosyltransferase	ND
Deamination	Cytidine→Uridine (19 in Fig. 5)	Cytidine deaminase (3.5.4.5)	2.89 ± 0.06
	Deoxycytidine→Deoxyuridine (8 in Fig. 6)	Deoxycytidine deaminase (3.5.4.14)	5.16 ± 0.37
	Cytosine→Uracil (20 in Fig. 5)	Cytosine deaminase (3.5.4.1)	ND

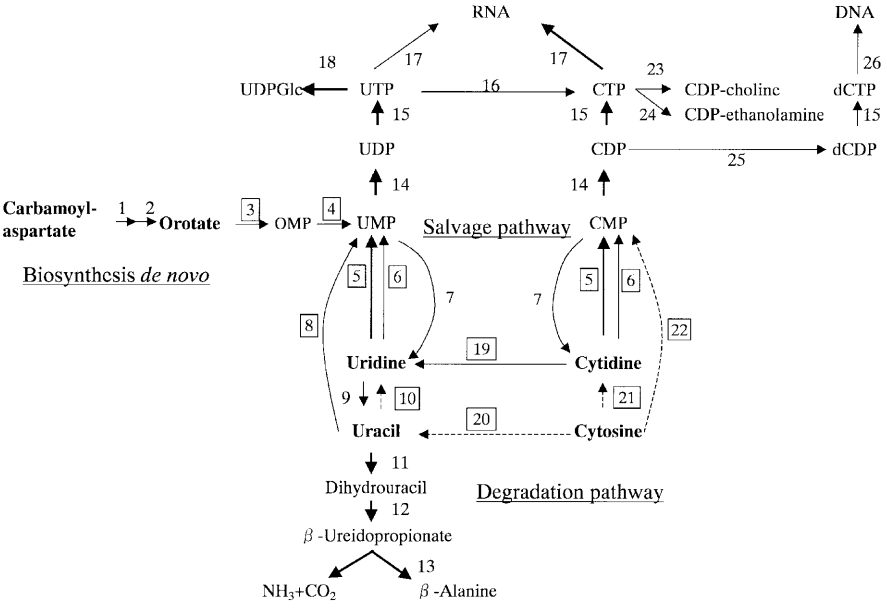

Fig. 5. Possible metabolic fate of exogenously supplied [carbamoyl^{14}C]carbamoylaspartate, [2-^{14}C]orotic acid, [2-^{14}C]uridine, [2-^{14}C]cytidine, [2-^{14}C]uracil and [2-^{14}C]cytosine in growing potato tubers. Enzymes: (*1*) dihydroorotase; (*2*) dihydroorotate dehydrogenase; (*3*) orotate phosphoribosyltransferase; (*4*) orotidine 5′-monophosphate decarboxylase; (*5*) uridine/cytidine kinase; (*6*) nucleoside phosphotransferase; (*7*) 5′-nucleotidase and/or acid phosphatase; (*8*) uracil phosphoribosyltransferase; (*9*) uridine nucleosidase; (*10*) uridine phosphorylase; (*11*) uracil reductase and/or dihydrouracil dehydrogenase; (*12*) dihydropyriminase; (*13*) β-ureidopropionase; (*14*) nucleoside monophosphate kinase; (*15*) nucleoside diphosphate kinase; (*16*) CTP synthetase; (*17*) RNA polymerase; (*18*) UDPGlc pyrophosphorylase; (*19*) cytidine deaminase; (*20*) cytosine deaminase; (*21*) pyrimidine nucleoside phosphorylase; (*22*) cytosine phosphoribosyltransferase; (*23*) choline-phosphate cytidyltransferase; (*24*) ethanolamine-phosphate cytidyltransferase; (*25*) ribonucleotide reductase; (*26*) DNA polymerase. The *boxed numbers* are the enzymes measured in this study

Uptake of pyrimidine nucleosides and bases was much faster than for intermediates of the de novo pyrimidine pathways. This observation strongly suggests the presence of transporters of those nucleosides and bases. Such transporters have already been discovered in bacteria (Nygaard and Saxild 2000), yeast (Jund et al. 1988) and mammalian cells (Cass et al. 1998).

The high activity of orotate phosphoribosyltransferase (step 3, Fig. 5) detected in potato tuber extracts (Table 1) supports the operation of the de novo pathway of pyrimidine nucleotide biosynthesis. Loef et al. (1999) showed that supplying exogenous orotate to the disks from growing potato tubers increased the level of uridine nucleotides. The report also supports the observation that orotate phosphoribosyltransferase activity is very high in potato tubers (Table 1). Furthermore, the data also suggest that the supply of PRPP in the tubers is sufficient for de novo pyrimidine biosynthesis in vivo. In general, the availability of PRPP seems to exceed the requirement for the de novo and salvage pathways of purine and pyrimidine nucleotide biosynthesis, because nucleotide synthesis is always stimulated by exogenously administered purine and pyrimidine bases (Hirose and Ashihara 1983, 1984a).

In plants as well as animals, UMP is synthesized from orotate by two successive reactions, catalyzed by orotate phosphoribosyltransferase (step 3, Fig. 5) and orotidine 5′-monophosphate decarboxylase (step 4, Fig. 5). Since these two activities reside on a single peptide in the organisms studied, this enzyme is now called UMP synthase (see Santoso and Thornburg 1992; Moffatt and Ashihara 2002). In potato tubers, conversion of UMP to UTP by nucleoside monophosphate kinase and nucleoside diphosphate kinase seems to be very fast, as has been demonstrated in other plant cells (Hirose and Ashihara 1984b). Likewise, the synthesis of UDP-sugars from UTP by UDPGlc pyrophosphorylase is also rapid (Sowokinos et al. 1993). In many plant materials, including potato tubers, UDPGlc is the most abundant pyrimidine. For example, Loef et al. (1999) reported that the concentration of UDPGlc in slices of growing potato tubers obtained from ca. 10-week-old potato plants was ca. 140 nmol (g FW)$^{-1}$, and that it comprised more than 70% of total uridine nucleotides. In growing potato tubers, UTP is always present in higher amounts than UMP and UDP (Loef et al. 1999; Farré et al. 2001; own data, not shown).

We observed salvage of various pyrimidine nucleosides in potato tubers. Uridine and cytidine were exclusively salvaged to UMP and CMP respectively, either by uridine/cytidine kinase (step 5, Fig. 5) or non-specific nucleoside phosphotransferase (step 6, Fig. 5) or both. Small amounts of cytidine were deaminated to uridine by cytidine deaminase (step 19, Fig. 5) and then converted to UMP (step 5/6, Fig. 5). Deoxycytidine and thymidine salvage for deoxy CMP and thymidine 5′-monophosphate was very limited, however (steps 1, 2 and 6, Fig. 6). Both conversions appeared to be catalyzed by non-specific nucleoside phosphotransferase, (step 2, Fig. 6) because the activities of deoxycytidine

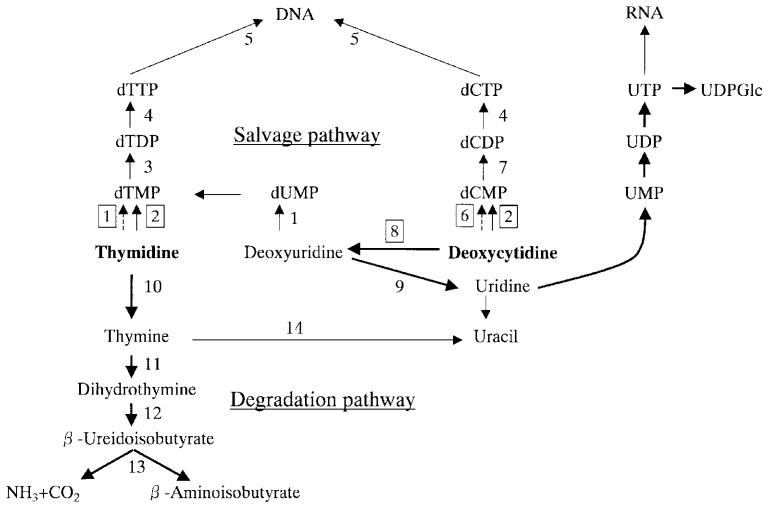

Fig. 6. Possible metabolic fate of exogenously supplied [2-^{14}C]thymidine and [2-^{14}C]deoxycytidine in growing potato tubers. Enzymes: (*1*) thymidine kinase; (*2*) nucleoside phosphotransferase; (*3*) thymidine monophosphate kinase; (*4*) nucleoside diphosphate kinase; (*5*) DNA polymerase; (*6*) deoxycytidine kinase; (*7*) nucleoside monophosphate kinase; (*8*) deoxycytidine deaminase; (*9*) pyrimidine deoxyribonucleoside 2′-hydroxylase; (*10*) thymidine phosphorylase and/or thymidine nucleosidase; (*11*) uracil reductase and/or dihydrouracil dehydrogenase; (*12*) dihydropyriminase; (*13*) β-ureidopropionase; (*14*) demethylation enzyme(s) of thymine. The *boxed numbers* are the enzymes measured in this study

kinase (step 6, Fig. 6) and thymidine kinase (step 1, Fig. 6) were extremely low in tuber extracts (Table 1). Of the pyrimidine bases, only uracil is salvaged by uracil phosphoribosyltransferase (step 8, Fig. 5). It is not salvaged by uridine phosphorylase (step 10, Fig. 5) because this enzyme was not detected in tuber extracts (Table 1). In contrast to uridine salvage, uracil salvage was low, although uracil-salvage activity seems to be higher in potato tubers than in other plants (Kanamori-Fukuda et al. 1981; Ashihara et al. 2000; Stasolla et al. 2001). Another pyrimidine base, cytosine, was not metabolized in potato tubers. Cytosine-metabolizing enzymes including cytosine deaminase (step 20, Fig. 5), found in yeast (Ipata et al. 1971) and in *Escherichia coli* (Katsuragi et al. 1986) and other gram-negative bacteria (Sakai et al. 1976), could not be found in potato tuber extracts. No cytosine phosphoribosyltransferase activity has been found in any organisms, including potato tubers (step 22, Fig. 5).

We found an active reductive uracil- and thymine-degradation pathway (Figs. 5, 6) in growing potato tubers. Extensive degradation of exogenously supplied uracil has been reported in cultured *Catharanthus roseus* cells (Kanamori-Fukuda et al. 1981), white spruce cells (Ashihara et al. 2000) and white spruce somatic embryos during partial drying treatment (Stasolla et al. 2001), and similar degradation of [2-^{14}C]thymidine has been observed in cotyledons of *Phaseolus mungo* seedlings (Kameyama et al. 1985).

The present results show that biosynthesis and metabolism of deoxyribonucleotides are similar in higher plants and animal cells, though details are different. We first demonstrated using in situ tracer experiments that the de novo synthesis of deoxyribonucleotides by ribonucleotide reductase (step 25, Fig. 5) and salvage pathways utilizing deoxycytidine (steps 2, 7 and 4, Fig. 6) were both operating in plants. Our present study found that substantial amounts of deoxycytidine were converted to UDPGlc. This conversion has not yet been observed elsewhere. The labelling pattern from the tracer experiments with [2-^{14}C]deoxycytidine suggests that UDPGlc synthesis from deoxycytidine proceeds by the pathway deoxycytidine → deoxyuridine → uridine → UMP → UDP → UTP → UDPGlc. Incorporation of [2-^{14}C]deoxycytidine into uracil residues of RNA, which we observed in growing potato tubers, also supports the operation of this pathway. To our knowledge this is the first report showing this pathway for production of uracil ribonucleotides from deoxycytidine. Conversion of deoxycytidine to uridine could be catalyzed by two enzymes, namely deoxycytidine deaminase and pyrimidine deoxyribonucleoside 2′-hydroxylase (steps 8 and 9, Fig. 6). We detected appreciable activity of the former enzyme in our potato tuber extracts. This enzyme has been also reported in maize leaves (le Floc'h and Guillot 1974) and seedlings (Wanka and Bauer 1967) and rye grass seedlings (Frisch and Charles 1965). The second enzyme, pyrimidine deoxyribonucleoside 2′-hydroxylase (2-deoxyuridine, 2-oxoglutarate: oxygen oxidoreductase, EC 1.14.11.3), has not yet been found in any plant materials, but it exists in some microorganisms, such as *Nuerospora crassa* (Bankel et al. 1972) and *Rhodotorula glutinis* (Warn-Cramer et al. 1983; Stubbe 1985).

Further studies of the function of various pathways of pyrimidine metabolism during development, dormancy and sprouting of potato plants are under way.

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