Comparison of Purine Metabolism in Suspension Cultured Cells of Different Growth Phases and Stem Tissue of *Catharanthus roseus* *

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Catharanthus roseus (= *Vinca rosea*), Suspension Cultured Cells, Stem Tissue, Purine Metabolism

The overall metabolism of [8-^{14}C]adenine, [8-^{14}C]adenosine, [8-^{14}C]guanine, [8-^{14}C]hypoxanthine and [8-^{14}C]inosine in suspension cultured cells of the lag, cell division, cell expansion and stationary phases and stem tissue of *Catharanthus roseus* was studied. On a fresh weight basis, absorption of all purine bases and nucleotides by the cultured cells was 10–400 times higher than that by the stem tissue. The conventional pathways of purine salvage and degradation were operative both in the cultured cells and stem tissue. [8-^{14}C]Adenine and [8-^{14}C]adenosine were extensively salvaged to nucleotides and nucleic acids in both the cultured cells and the stem tissue. [8-^{14}C]-Guanine, [8-^{14}C]hypoxanthine and [8-^{14}C]inosine were incorporated into either salvage products (nucleotides and nucleic acids) or degradation products (CO_2, allantoin and allantoic acid) of cultured cells and stem tissue. The highest incorporation rate expressed as a percentage of absorbed ^{14}C into the degradation products was observed in the lag phase (12 h-old) cultured cells. Incorporation of all precursors into nucleotides and bases was much higher in the stem tissue than in the culltured cells.

Introduction

Since purine metabolism has a close relationship to energy metabolism and nucleic acid synthesis, biosynthesis and degradation of purine compounds is a key factor in the regulation of plant growth and development. However, little is known of purine metabolism in higher plants [1–3]. In addition, very few comprehensive studies have been executed on the overall metabolism of purine bases and nucleosides [4, 5].

Cultured plant cells are well suited to metabolic studies, because they grow in a defined medium under sterile conditions in a characteristic fashion. In addition, absorption of radioactive tracers by cultured cells is usually much higher than that of plant tissues and organs, which allows more detailed isotope studies. One reservation concerning the use of cultured plant cells is that they do not always exactly mimic the metabolism of the parent cells from which they were initially isolated [6, 7]. In consequence, caution must be exercised when extrapolating from one to the other.

To gain further information on this we determined the pattern of purine metabolism in cultured cells and stem tissue from which the culture originated. Metabolic investigations were carried out with radioactive adenine, adenosine, guanine, hypoxanthine and inosine supplied to the stem tissue and batch suspension cultured cells of *Catharanthus roseus*. Patterns of purine metabolism for these two systems are compared.

Materials and Methods

Plant materials

Stock cultures of *Catharanthus roseus* (= *Vinca rosea*) cells were isolated from stem sections of *Catharanthus roseus* plants in 1969. The stock culture (strain F1) was maintained in 50 ml of Murashige-Skoog basal medium [8] containing 2.2×10^{-6} M 2,4-dichlorophenoxyacetic acid in 300 ml Erlenmeyer flasks. Experimental cultures were initiated from 10 day-old stock cultures. The inoculum density of cell suspension was adjusted to give 2.4×10^5 cells · ml^{-1}. The cultures grown in 20 ml of medium in 100 ml flasks, were incubated at 27 °C on a horizontal rotary shaker operating at 90 strokes · min^{-1}, 8 cm amplitude, in the dark. The pattern of growth of *Catharanthus* cells was essentially the same as described previously [9]. Four

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growth phases (i) the lag phase (days 0–1), (ii) the cell division phase (days 1–4), (iii) the cell expansion phase (days 4–7) and (iv) the stationary phase (days 7–10), were recognized. Cells aged 12, 72, 144 and 216 h were used in this investigation.

Labelled compounds and chemicals

Labelled compounds were purchased from the radiochemical Centre, Amersham, UK. Purine bases, nucleosides and nucleotides were obtained from Kyowa Hakko Kogyo Co., Tokyo, Japan. Thin layer plates of Avicel SF microcrystalline cellulose and PEI-cellulose F were products of Asahi Kasei Kogyo Co., Tokyo, Japan and E. Merck, Darmstadt, respectively.

Metabolism of labelled compounds

Aliquots (2.0 ml) of cell suspension containing approx. 200 mg fresh weight *Catharanthus* cells or segments of stems and 2 ml of potassium phosphate buffer (30 mM, pH 5.6) containing 40 mM sucrose were placed in the main compartment of 30 ml Erlenmeyer flasks fitted with a glass tube containing a piece of filter paper and 0.2 ml of 20% KOH.

Incubation was started by the addition of 0.2 ml (1.0 μCi) solution of [8-^{14}C]adenine (sp. act. 54 mCi/mmol), [8-^{14}C]adenosine (sp. act. 46 mCi/mmol), [8-^{14}C]guanine (sp. act. 51 mCi/mmol), [8-^{14}C]hypoxanthine (sp. act. 55 mCi/mmol) or [8-^{14}C]inosine (sp. act. 48 mCi/mmol) to the main compartment of the flask.

The flasks were incubated in an oscillating water bath operating at 120 strokes · min^{-1}, 5 cm amplitude at 27 °C for 1 h. After the incubation periods, the medium was removed and the cells or segments were washed and then frozen in liquid nitrogen as described previously [5, 10].

Cellular metabolites and nucleic acids were extracted as described in a previous paper [11]. Analysis of labelled metabolites and CO_2 was carried out according to Ashihara and Nobusawa [2].

Results

Absorption of labelled compounds

Absorption rate of labelled compounds are shown in Table I where the rates are expressed both on *g* fresh weight and 10^7 cells bases. Because of the difficulty of counting cell numbers in the stem tissue, the rates of absorption by the tissue are expressed on a fresh weight basis only.

The absorption of all ^{14}C-labelled precursors by the cultured cells was much higher than by the stem tissue. Absorption rate of each purine bases and nucleotides were similar in the stem tissue but quite different in the cultured cells. In every phase of cultured cells, absorption of purine nucleosides was higher than that of purine bases.

On a cell number basis, the highest absorption of adenine, adenosine, guanine and hypoxanthine occurred in the lag phase (12 h old) cells. In contrast, absorption of inosine by the lag phase cells was almost the same as that by the stationary phase cells.

Metabolism of [8-^{14}C]adenine

Metabolism of [8-^{14}C]adenine in the lag (12 h), cell division (72 h), cell expansion (144 h) and stationary (216 h) phases of cultured *Catharanthus* cells and stems are shown in Fig. 1 a.

The pattern of adenine metabolism of every phase of growth of cultured cells was similar except that incorporation of the label into the nucleoside plus base fraction was higher in the stationary phase

Table I. Absorption of labelled precursors by cultured cells and stem tissues of *Catharanthus roseus*. Values are shown as nmol · *g* fresh weight^{-1} and nmol · 10^7 cells^{-1} (in parentheses).

^{14}C-Precursor	Cultured cells				Stem tissues
	12 h	72 h	144 h	216 h	
[8-^{14}C]Adenine	715 (387)	433 (142)	313 (109)	425 (257)	4.97
[8-^{14}C]Adenosine	1332 (721)	854 (280)	1120 (390)	450 (272)	3.27
[8-^{14}C]Guanine	447 (242)	280 (92)	98 (34)	40 (24)	4.69
[8-^{14}C]Hypoxanthine	848 (459)	308 (101)	101 (35)	101 (61)	3.84
[8-^{14}C]Inosine	1000 (541)	573 (188)	934 (325)	985 (569)	4.32

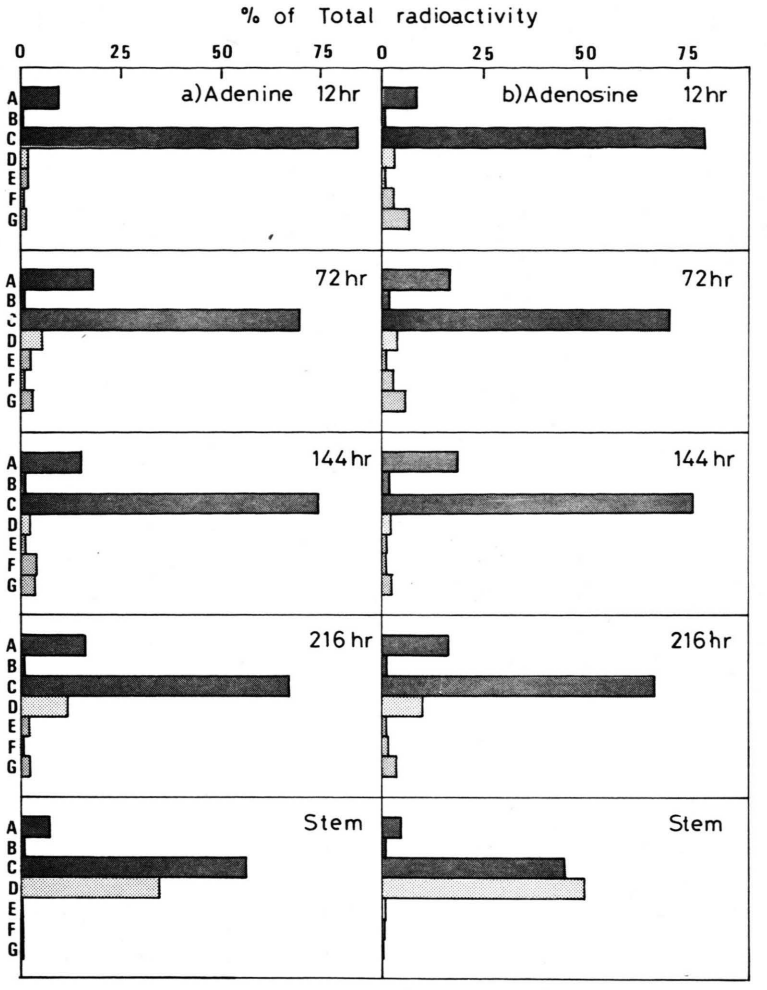

Fig. 1. Incorporation of radioactivity from [8-^{14}C]adenine(a) and [8-^{14}C]adenosine(b) into the RNA(A), DNA(B), nucleotide(C), nucleoside plus base(D), allantoin plus allantoic acid(E), CO_2(F) and other degradation product(G) fractions in the lag (12 h old), the cell division (72 h old), the cell expansion (144 h old) and the stationary (216 h old) phase cultured cells and stem tissue of *Catharanthus roseus*. The rate of incorporation of radioactivity is expressed as % of total radioactivity.

cells. Almost all (more than 90%) labelled adenine was converted to adenine nucleotides and RNA-adenine in every phase of cultured cells and incorporation of the label into the degradation products was extremely low (Fig. 1).

When the incorporation rate was expressed as a percentage of the total absorbed [8-^{14}C]adenine, the lag phase (12 h) cells had the highest incorporation rate into nucleotides and the lowest rate into RNA among the four growth stages studied.

In the stems, a considerable amount (65%) of adenine was also salvaged for nucleotide and nucleic acid synthesis. However, approx. 35% of absorbed [8-^{14}C]adenine was recovered in the nucleoside plus base fraction. Degradation of adenine in the stem was also of limited significance (less than 0.2% of total radioactivity).

Metabolism of [8-^{14}C]adenosine

The pattern of adenosine metabolism in cultured cells and stems (Fig. 1b) resembled that of adenine metabolism shown in Fig. 1a. Most absorbed adenosine (50–95%) was salvaged and only a little (less than 10%) was degraded in both cultured cells and stems.

Compared with [8-^{14}C]adenine, a large amount (approx. 50%) of the absorbed adenosine was recovered as nucleosides and bases in the stem.

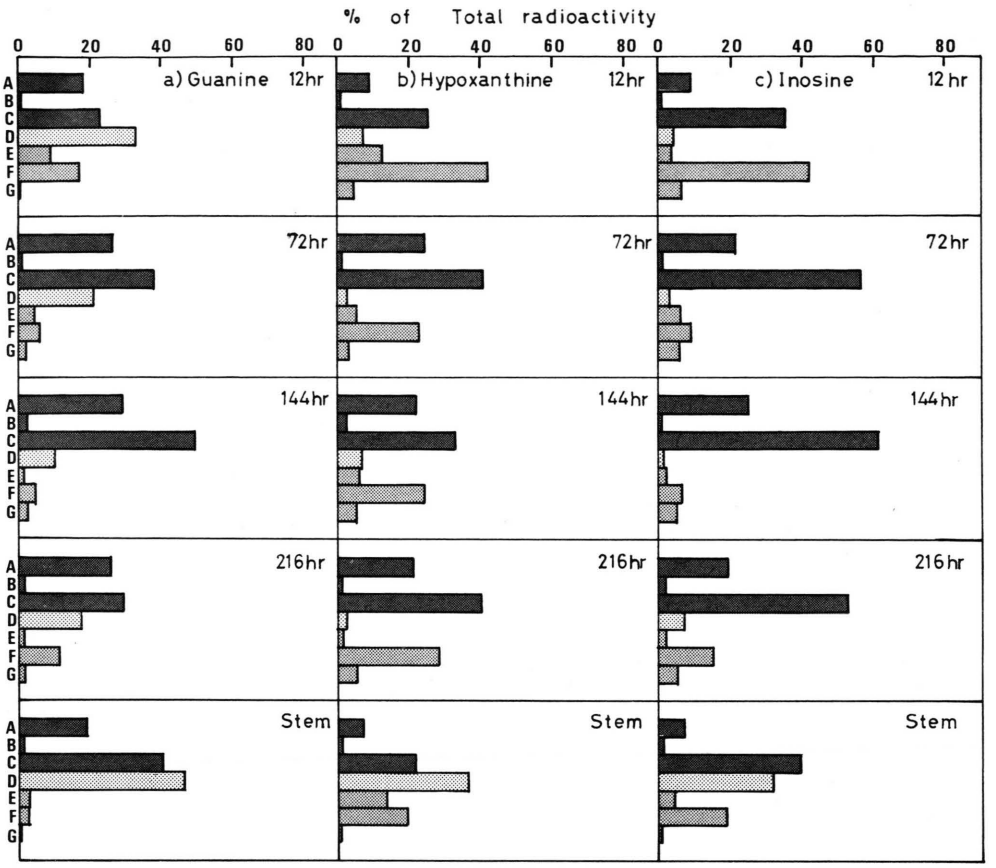

Fig. 2. Incorporation of radioactivity from [8-^{14}C]guanine (a), [8-^{14}C]hypoxanthine (b) and [8-^{14}C]inosine (c) into RNA (A), DNA (B), nucleotide (C), nucleoside plus base (D), allantoin plus allantoic acid (E), CO_2 (F) and other degradation product (G) fractions in the lag (12 h old), the cell division (72 h old), the cell expansion (144 h old) and the stationary (216 h old) phase cultured cells and stem tissue of *Catharanthus roseus*. The rate of incorporation of radioactivity is expressed as % of total radioactivity.

Metabolism of [8-^{14}C]guanine

In the lag phase of *Catharanthus* cells, incorporation of [8-^{14}C]guanine into the nucleoside plus base fraction was higher than that into the RNA and nucleotide fractions and a significant amount (approx. 25% of total radioactivity) of [8-^{14}C]guanine was degraded into ureide and CO_2 (Fig. 2a).

On the other hand, most of absorbed guanine (approx. 65–80%) was recovered as nucleotides and RNA in the other three stages of cultured cells. The degradation of [8-^{14}C]guanine was less than that in the lag phase cells.

The metabolic pattern of [8-^{14}C]guanine observed in the stem tissue was similar to that of its adenosine metabolism: most of the radioactivity was recovered in the nucleoside plus base, nucleotide and RNA fractions.

Metabolism of [8-^{14}C]hypoxanthine

Metabolic patterns of [8-^{14}C]hypoxanthine in *Catharanthus* cells and stem tissue (Fig. 2b) were similar to that of [8-^{14}C]guanine. However, incorporation of [8-^{14}C]hypoxanthine into degradation products, allantoin, allantoic acid and CO_2, was much higher than that of [8-^{14}C]guanine. Approx. 55% of [8-^{14}C]hypoxanthine was degraded in the lag phase of the cells.

Metabolism of [8-^{14}C]inosine

The pattern of [8-^{14}C]inosine metabolism was intermediate between that of adenine and hypoxanthine (Fig. 2c). Compared with adenine and adenosine, the degradation rate of inosine, especially in the lag phase of the cells, was much higher and, as a result, inosine salvage was less stronger.

Discussion

This work indicated that the same routes of purine metabolism exist both in the intact stem tissue and in the cultured cells of *Catharanthus roseus*; they are the salvage pathways of nucleotide biosynthesis and the degradation pathways (Fig. 3). However, there are two major differences in the tracer experiments between the stem tissue and cultured cells. One of them appeared in the absorption of radioactive purine bases and nucleosides. On the fresh weight basis, absorption of these compounds by the cultured cells was much higher than that by the segments of the stems.

Another difference is found in the incorporation of labelled compounds into the nucleoside plus base fraction. The incorporation into the fraction in the stem tissue was always much higher than that in the cultured cells. Most of the radioactivity of the fraction seems to be unmetabolized precursors or degradation products of the preformed nucleotides. It has been suggested that nucleotides are readily hydrolysed in the damaged tissues by phosphatases [12]. Since we used segments of the stem tissue, the hydrolysation of preformed nucleotides by phosphatases may be higher than that in the intact cells.

Apart from these two major differences, the pattern of the purine metabolism in the stem tissue is essentially the same as observed in the cultured cells. Thus, the cultured cells of *Catharanthus roseus* are suitable experimental materials for metabolic research including tracer experiments.

Adenine and adenosine were extensively salvaged in both the cultured cells and the stem tissues. The salvage enzymes, adenine phosphoribosyltransferase and adenosine kinase, which catalyse the conversion of adenine and adenosine into the nucleotide have been found in several plant species [4, 13–17] including cultured cells of *Catharanthus roseus* [18]. The possibility of adenosine phosphotransferase and adenosine nucleosidase to adenine and adenosine salvage cannot be excluded, but the former enzyme was present in only a limited number of plant species and its activity was lower than adenine phosphoribosyltransferase [3, 19]. The latter enzyme was also detected in *Catharanthus* cells but its

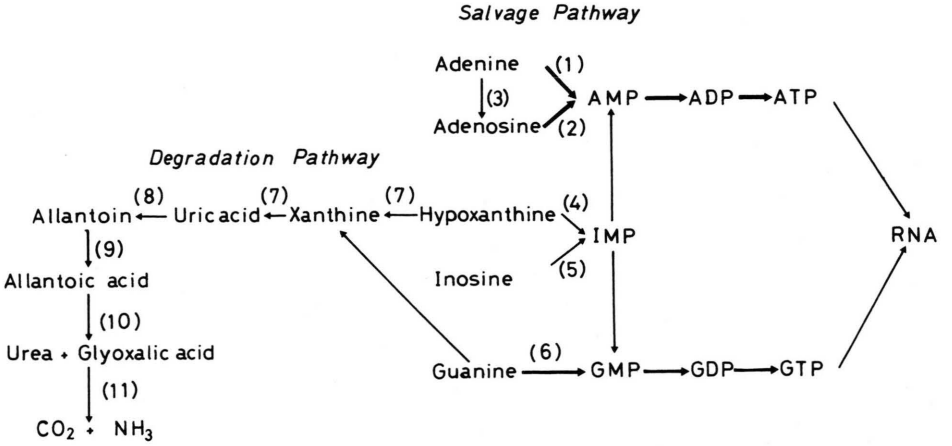

Fig. 3. Pathways of purine salvage and degradation in the cultured cells and the stem tissue of *Catharanthus roseus*. Numbers represent enzymes as follows. (1) adenine phosphoribosyltransferase (EC 2.4.2.7), (2) adenosine kinase (EC 2.7.1.20), (3) purine nucleosidase (EC 3.2.2.1), (4) hypoxanthine phosphoribosyltransferase (EC 2.4.2.8), (5) nucleoside phosphotransferase (EC 2.7.1.77), (6) guanine phosphoribosyltransferase (EC 2.4.2.8), (7) xanthine dehydrogenase (EC 1.2.1.37), (8) urate oxidase (EC 1.7.3.3), (9) allantoinase (EC 3.5.2.5), (10) allantoicase (EC 3.5.3.4), (11) urease (EC 3.5.1.5).

activity was much lower than adenine phosphoribosyltransferase (Hirose, unpublished data).

Only limited amounts of adenine and adenosine were degraded in both the cultured cells and the stem tissue of *Catharanthus roseus*. This suggests that deamination activity of adenine and adenosine was extremely low in the plant.

The catabolic activity of adenine and adenosine varied between plant species. For example, adenine was extensively deaminated to hypoxanthine in tobacco protoplasts [4] which in turn was degraded into allantoin and allantoic acid in black gram seedlings [5].

Guanine was also utilised for nucleotide and nucleic acid syntheses in the stem tissue as well as cultured cells. Guanine phosphoribosyltransferase activity was detected in *Catharanthus* cells (Hirose and Ashihara, unpublished results). A part of absorbed [8-^{14}C]guanine was catabolized and the radioactivity was incorporated into allantoin, allantoic acid and CO_2. This suggests that guanine is deaminated to xanthine and then catabolized *via* the conventional purine degradation pathway (Fig. 3). Guanine deaminase activity has been reported in tobacco leaf protoplasts [4].

The salvage activity of hypoxanthine in *Catharanthus* tissue and cells was lower than that of adenine, adenosine and guanine. However, it should be emphasized that hypoxanthine salvage observed in the cell division, the cell expansion and the stationary phase cells (approx. 60% of absorbed hypoxanthine) was much higher than that of other plant materials [2]. Active hypoxanthine phosphoribosyltransferase presented in *Catharanthus roseus* (Hirose and Ashihara, unpublished results) indicated that direct phosphoribosylation of hypoxanthine was the major route of hypoxanthine salvage.

Hypoxanthine was degraded by the conventional purine catabolic pathway. In contrast with leguminous plants [5], a large quantity of $^{14}CO_2$ released from [8-^{14}C]hypoxanthine was observed in the tissue and the cultured cells of *Catharanthus roseus*. This suggests hypoxanthine was converted to allantoin and allantoic acid and then further catabolysed to CO_2, NH_3 and glyoxalic acid.

Inosine was converted to nucleotides more effectively than hypoxanthine. Since inosine kinase was not detected in *Catharanthus* cells, inosine salvage seemed to be catalysed by non-specific nucleoside phosphotransferase which was found in *Catharanthus* cells (Hirose and Ashihara, unpublished results). Significant radioactivity of [8-^{14}C]inosine was observed in the CO_2 fraction. This indicates that inosine was hydrolysed to hypoxanthine and then degraded.

When the incorporation rate was expressed on the percentage of absorbed precursors, the lowest incorporation of all precursors into RNA was found in the lag phase (12 h-old cells) among the four stages of growth of *Catharanthus* cells. In contrast, incorporation of adenine and adenosine into the nucleotide fraction was highest in the lag phase cells. This suggests that rapid adenine and adenosine salvage observed in the lag phase cells provides ATP for several biosyntheses including RNA synthesis in the subsequent phases. A ten-fold increase in ATP content observed between 24 h of the culture of *Catharanthus* cells [20] is consistent with this assumption.

Degradation of guanine, hypoxanthine and inosine was extremely high in the lag phase cells. Although the metabolic fate of degradation products could not be detected in this study because of using [8-^{14}C]precursors, the products, glyoxalic acid and ammonia seem to be reutilised for the synthesis of nitrogen and carbon compounds in the lag phase cells. In this stage, cells may provide the energy and some essential building blocks prepared for subsequent cell division.

However, it should be remembered that the data in this paper indicate the metabolic pattern of an individual [8-^{14}C]purine compounds rather than the metabolic activity *in vivo*. When the incorporation rate was expressed as moles per cell, the highest incorporation of labelled purine compounds into nucleotides and nucleic acids was observed in the lag phase cells. The absolute metabolic potential of purine salvage seems to be highest in the lag phase of *Catharanthus* cells where the highest activity of enzymes of purine salvage was observed (Hirose and Ashihara, unpublished results).

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