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De novo and salvage biosynthetic pathways of pyridine nucleotides and nicotinic acid conjugates in cultured plant cells

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Abstract

To estimate the operation of the de novo and salvage pathways of pyridine nucleotide synthesis, [^{3}H]quinolinic acid, an intermediate of the de novo synthesis, and [^{14}C]nicotinamide and [^{14}C]nicotinic acid, substrates of the salvage pathways, were administered to cultured white spruce (*Picea glauca*) and Madagascar periwinkle (*Catharanthus roseus*) cells and overall metabolism of these radioactive compounds was examined over the culture period. In *P. glauca* cells, all three precursors were able to generate pyridine nucleotides (mainly NAD and NADP) and trigonelline. Supplied [^{3}H]quinolinic acid was efficiently converted to pyridine nucleotides and trigonelline in both at the logarithmic and the stationary stages of cell growth, although uptake of [^{3}H]quinolinic acid by *P. glauca* cells was very low. [^{14}C]Nicotinic acid and [^{14}C]nicotinamide were taken up by the cells in a relatively facile manner, and 15–20 and 32–58% of total radioactivity from these compounds was found in pyridine nucleotides (mainly NAD and NADP) and trigonelline, respectively, after 18 h incubation. In *C. roseus* cells, these three precursors were utilised for pyridine nucleotides, but in contrast to *P. glauca*, nicotinic acid glucoside, but not trigonelline, was heavily labeled. Nicotinic acid and nicotinamide were better precursors for pyridine nucleotide synthesis than quinolinic acid. In Pi-starved cells, the uptake of quinolinic acid, nicotinic acid and nicotinamide was markedly decreased. Pyridine nucleotide synthesis de novo was greatly reduced in Pi-starved *C. roseus* cells, while little effect was found in the salvage pathway of nicotinic acid. Pi-deficiency slightly increased the rate of nicotinic acid-glucoside synthesis from nicotinic acid and nicotinamide. From the in vitro determination of enzyme activity, it is concluded that quinolinic acid and nicotinic acid are converted to nucleotides by quinolinate phosphoribosyltransferase (2.4.2.19) and nicotinate phosphoribosyltransferase (2.4.1.11), respectively. High activity of nicotinamidase (3.5.1.19) but no detectable activity of nicotinamide phosphoribosyltransferase (2.4.2.12) suggests that nicotinamide is converted to nicotinic acid, and then salvaged by nicotinate phosphoribosyltransferase.

Keywords: Nicotinamide; Nicotinic acid; Nicotinic acid glucoside; Quinolinic acid; Trigonelline

1. Introduction

During our research on nucleotide metabolism during growth and development of plant cells, we have examined purine and pyrimidine nucleotide metabolism in cultured cells of *Catharanthus roseus* and *Picea glauca* (for review [1,2]). Little is known about the biosynthesis and metabolism of pyridine nucleotides (nicotinamide adenine nucleotides) in plant cells. NAD and NADP are coenzymes that participate in many redox reactions in cells and the NADH/NAD$^+$ and NADPH/NADP$^+$ ratios in cells regulate the activities of many oxidoreductase reactions. Recently, it has been shown that pyridine nucleotides also regulate gene expression by modulating activities of some transcription

Abbreviations: SAM, *S*-adenosyl-L-methionine

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factors in animals [3]. Similar to purine and pyrimidine biosynthesis, pyridine nucleotides are synthesized by the de novo and salvage pathways. Two different de novo pathways have been proposed for pyridine nucleotide synthesis. In bacteria and plants, quinolinic acid is synthesized from aspartic acid and glyceraldehyde-3-phosphate by the so-called ''aspartate pathway'', but in animals, pyridine nucleotides are synthesized from tryptophan by the ''kynurenine pathway''. Recent bioinformatic search of genome databases suggests that the ''aspartate pathway'' is operative in *Arabidopsis thaliana*, while *Oryza sativa* may utilise both aspartic acid and tryptophan for the de novo synthesis of pyridine nucleotides [3]. Regardless of whether an aspartate or kynurenine pathway is operative, quinolinic acid is formed and converted to nicotinic acid mononucleotide by quinolinate phosphoribosyltransferase. Subsequently, nicotinic acid adenine dinucleotide is formed by the reaction of nicotinic acid mononucleotide-adenylyltransferase, and finally NAD is produced. Part of NAD is further converted to NADP by NAD kinase. In many organisms, nicotinamide and nicotinic acid, degradation products of NAD and NADP, are re-utilised for the synthesis of pyridine nucleotides by salvage pathways. The route, which consists of several reactions involved in the degradation and re-synthesis of pyridine nucleotides, has been referred to as the pyridine nucleotide cycle [4,5]. As the number of enzymes involved in pyridine nucleotide cycle are different between species, several distinct cycles are present in different organisms [6,7].

In higher plants, nicotinic acid is generally converted to nicotinic acid conjugates, trigonelline (*N*-methylnicotinic acid) and nicotinic acid *N*-glucoside. In many cases, formation of these two compounds is species specific and conversion of each compound does not occur [8,9]. In a limited number of plant species, nicotinic acid is also utilised as a building block of specific secondary metabolites, such as nicotine, anabasin and ricinine [5,10].

In the present study, which is our first investigation on pyridine nucleotide metabolism in cultured plant tissues, we examined aspects of pyridine metabolism in suspension-cultured cells of a gymnosperm *P. glauca* and an angiosperm *C. roseus*. This was done by determining the metabolic fate of radio-labeled quinolinic acid, nicotinic acid and nicotinamide, and the activity of some key enzymes. The effect of Pi, one of the most important effectors of cellular purine and pyrimidine nucleotide biosynthesis [11], on pyridine metabolism is also examined in the present study.

2. Materials and methods

2.1. Plant materials

White spruce (*P. glauca* [Moench] Voss) embryogenic tissues were generated from mature and immature embryos as shown in previous paper [12]. Embryogenic tissues were transferred to the liquid maintenance medium (von Arnold and Erikson medium containing 10 μM 2,4-D, 2 μM BA, and 3% sucrose) and were subcultured every 7 days. Suspension cultured cells of Madagascar periwinkle (*C. roseus* (L.) G. Don) maintained and sub-cultured in MS medium containing 2.2 μM 2,4-D and 3% sucrose were also used. Cells of both species were sampled at the logarithmic stage of growth (day 4) and those at the stationary phase (day 7). The effect of Pi on pyridine metabolism was examined using a *C. roseus* suspension culture, since this culture system has been previously used as a model for studies on the relations between Pi and metabolism [13–18]. Phosphate starvation cell cultures were obtained as described by Nagano et al. [13,14].

2.2. Tracer experiments

[^{3}H]quinolinic acid (specific activity 200 GBq mmol^{-1}), [carbonyl-^{14}C]nicotinamide (specific activity 1.96 GBq mmol^{-1}) and [carboxyl-^{14}C]nicotinic acid (specific activity 1.92 GBq mmol^{-1}) were obtained from Moravek Biochemicals, Inc., Brea, CA, USA.

Administration of pyridine compounds into the cultured plant cells were carried out according to the procedure of Ashihara et al. [12]. Suspension-cultured cells (ca. 100 mg fresh weight) and 1.9 ml of the culture medium in which the cells had been grown 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 centre well. Each reaction was started by the addition of 10 μl of a solution of labeled compounds to the main compartment of the flask. The flasks were incubated in an oscillating water bath at 27 °C. After incubation, the glass tube was removed from the centre well and placed in a 50 ml Erlenmeyer flask that contained 10 ml of distilled water. Simultaneously, the cells were harvested by filtration over Miracloth, washed with distilled water, and frozen with liquid nitrogen and 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 (usually 0.5 ml) were used for the determination of radioactivity. The radioactivity was measured with a liquid scintillation analyzer.

The frozen cells were extracted with cold 6% perchloric acid (PCA). After extraction, the mixture was centrifuged at 12,000 × *g* for 7 min, the resultant supernatant was collected and the precipitate was re-suspended in the same extraction reagent and washed using centrifugation. The PCA-soluble fraction was neutralized with KOH. After removal of the precipitated potassium perchlorate by brief centrifugation, the supernatant was evaporated in vacuo until dryness at 37 °C. The cold PCA-soluble metabolites were then fractionated by TLC using microcrystalline cellulose TLC plates (Merck, Darmstadt, Germany). Solvent systems used were *n*-butanol–acetic acid–water (4:1:2, v/v/v) [19] and isobutyric acid–ammonia–water (660:17:330, v/v/v) [20]. The R_f values of pyridine and related compounds were shown in the previous paper [21].