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Short communication

Nicotinate riboside salvage in plants: Presence of nicotinate riboside kinase in mungbean seedlings

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Abstract

Salvage of nicotinate riboside for NAD synthesis was investigated in mungbean seedlings. Nicotinate riboside kinase activity was detected in extracts from cotyledons. Exogenously supplied [carboxyl-^{14}C]nicotinate riboside was readily converted into pyridine nucleotides in cotyledons of mungbean seedlings. This conversion was also found in embryonic axes, but the rate was lower than in cotyledons. These results suggest that, in addition to the seven-component pyridine nucleotide cycle (PNC VII), an eight-component cycle (PNC VIII) involving nicotinate riboside kinase operates in plants.

Keywords: NAD synthesis; Nicotinate riboside; Nucleoside salvage; Pyridine nucleotide cycle

1. Introduction

Nicotinamide adenine dinucleotides (NAD and NADP) are important metabolites, and act as coenzymes for oxidoreductases. A further role of these nucleotides in signalling and gene regulation has recently been postulated [13,19], though no decisive evidence has yet been found in plants [10]. There are two different de novo biosynthetic pathways, derived from aspartate and kynurenine [6,11]. In *Arabidopsis thaliana*, quinolinic acid, an intermediate of the de novo pathway, is synthesised from aspartate and glyceraldehyde-3-phosphate [12]. Considerable diversity of the salvage pathways of pyridine nucleotides in different species has been found [15,18]. In animals and some bacteria and fungi, nicotinamide is salvaged by nicotinamide phosphoribosyltransferase (EC 2.4.2.12) [14]. Recently, salvage of nicotinamide riboside (NR) by NR kinases (EC 2.7.1.22) was discovered in yeast and humans [7], so that both nicotinamide and NR are salvaged in these organisms. In plants, nicotinamide is not salvaged directly, because nicotinamide phosphoribosyltransferase is not present [6]. As shown in Fig. 1, nicotinamide is converted into nicotinate by nicotinamidase (EC 3.5.1.19), and nicotinate is salvaged to nicotinate mononucleotide (NaMN) by nicotinate phosphoribosyltransferase (EC 2.4.2.11) [6,16]. NR seems to be produced in the course of catabolism of NAD, but NR kinase has not yet been found in plants.

In our previous studies of the pyridine nucleotide cycle in *Coffea arabica*, we found that nicotinate riboside (NaR) was labelled transiently when [carbonyl-^{14}C]nicotinamide was administered into leaf segments [18]. This fact suggests that the salvage route, nicotinamide → nicotinate → NaR → NaMN, is in operation. In the present study, we examined whether NaR kinase is involved in the plant pyridine salvage pathway, by in vitro determination of enzyme activity and in situ ^{14}C-tracer experiments using [carboxyl-^{14}C]NaR in mungbean seedlings. The results indicate that NaR salvage by NaR kinase occurs in plants, as well as the conventional nicotinate salvage by nicotinate phosphoribosyltransferase.

Abbreviations: NaAD, nicotinate adenine dinucleotide; NaMN, nicotinate mononucleotide; NaR, nicotinate riboside; NMN, nicotinamide mononucleotide; NR, nicotinamide riboside.

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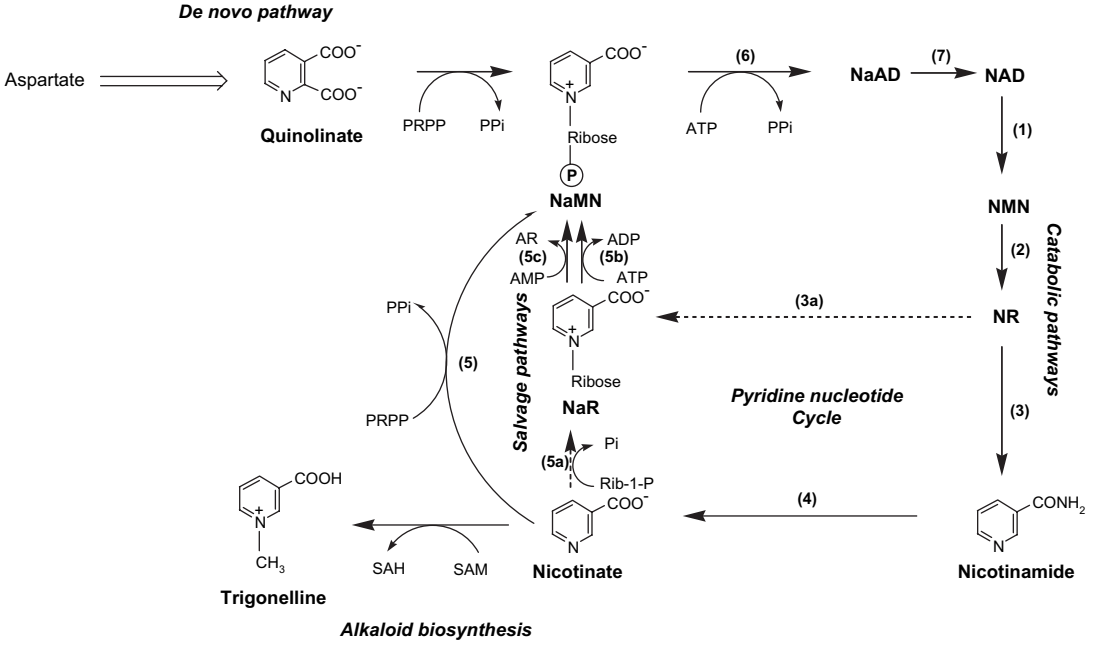

Fig. 1. Presumed pyridine nucleotide cycle and related reactions in plants. The seven-member PNC VII (steps 1–7) cycle, eight-member PNC VIII (steps 1–4, 5a, 5b, 6–7) cycle and six-member PNC VI (steps 1–2, 3a, 5b, 6–7) cycle are shown. The de novo pathway to NaMN synthesis and trigonelline synthesis is also shown for reference. Reaction of NaR kinase is 5b.

2. Results and discussion

2.1. Activities of NaR kinase

Fig. 2 shows the time-course of NaR kinase activity in the desalted protein fraction from 3-day-old cotyledons of mungbean seedlings. The reaction was linear at least for 2 h and was absolutely dependent on the presence of ATP. Table 1 compares the activities of enzymes involved in NaR and nicotinate metabolisms. NaR kinase activity was 0.15 pkat/mg protein in cotyledons, but its activity was very low in the embryonic axes. Activity of phosphotransferase, which catalyses NaMN synthesis from NaR and AMP, was lower than NaR kinase activity. The activity of nicotinate phosphoribosyltransferase, which is a conventional pyridine salvage enzyme, is higher than NaR kinase activity, especially in the embryonic axes. In the embryonic axes, therefore, NaR kinase does not make a significant contribution to salvage for pyridine nucleotide synthesis.

2.2. In situ determination of NaR salvage

The observed in vitro enzyme activity suggests the conversion of NaR into NaMN by NaR kinase in cotyledons of mungbean seedlings. To determine whether the salvage pathway of NaR is actually operative in cotyledons, in situ metabolism of [carboxyl-^{14}C]NaR was investigated. Fig. 3A shows the time-course of the conversion of [carboxyl-^{14}C]NaR to pyridine nucleotides, NaMN, NMN, NAD and NADP. Radioactivity from [carboxyl-^{14}C]NaR was incorporated into the pyridine nucleotides gradually, but labelling in nicotinate was low and almost constant during the time of incubation up to 4 h.

2.3. Comparison of the metabolic fate of [carboxyl-^{14}C]NaR in cotyledons and in embryonic axes

The metabolic fate of [carboxyl-^{14}C]NaR 1 h after administration was compared in cotyledons and in embryonic axes

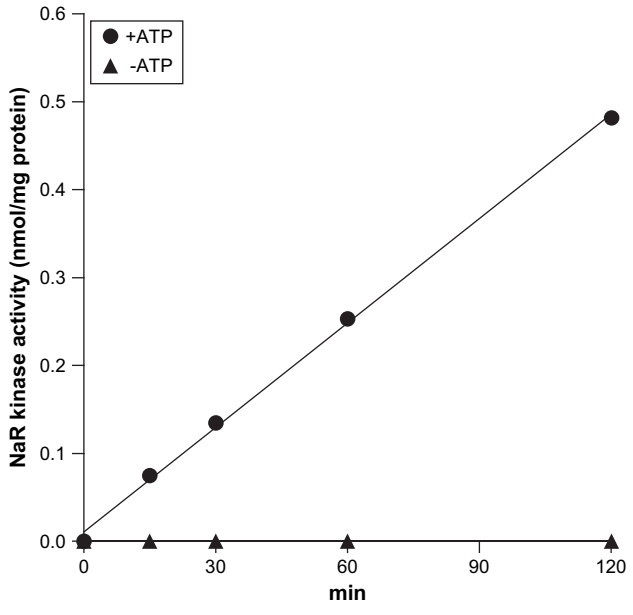

Fig. 2. Time-course of NaR kinase activity in the enzyme preparation from cotyledons taken from 3-day-old mungbean seedlings. In the presence of ATP, NaR was converted into NaMN, and part of NaMN was further converted into NaAD by NaMN adenylyltransferase in the extracts. Consequently, NaR kinase activity is estimated from the conversion of NaR into these pyridine nucleotides. Typical data expressed as nanomoles of NaMN plus NaAD formed per milligram protein are shown.