Phytochemistry, Vol. 22, No. 2, pp. 343–346, 1983.
Printed in Great Britain.

0031–9422/83/020343–04$03.00/0

NICOTINIC ACID AND NICOTINAMIDE METABOLISM AND PROMOTION OF CELL ARREST IN G2 IN *PISUM SATIVUM*

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(*Received* 8 *March* 1982)

Key Word Index—*Pisum sativum*; Leguminosae; pea; trigonelline; nicotinic acid; nicotinamide; pyridine nucleotide metabolic pathway; cell arrest in G2.

Abstract—Nicotinic acid and nicotinamide are immediate precursors of trigonelline, a hormone present in cotyledons of *Pisum sativum* L. which promotes cell arrest in G2 during cell maturation in roots and shoots. All three compounds are members of the pyridine nucleotide pathway for the synthesis of NAD and NADP. Concentrations of nicotinic acid and nicotinamide in excised roots grown for 3 days in White's medium with sucrose were determined by HPLC. Results suggest that nicotinamide is rapidly converted first to nicotinic acid and then trigonelline. High nicotinic acid concentrations may occur in excised roots. Conversion of trigonelline to nicotinic acid in excised roots did not occur in these experiments. The concentrations of either nicotinamide or nicotinic acid in roots are not related to the proportions of cells arrested in G2. Trigonelline promotes cell arrest in G2, and nicotinic acid and nicotinamide are active only because they are converted to trigonelline.

INTRODUCTION

Recent results demonstrate that trigonelline is a plant hormone [1–3]. Excised roots exposed to concentrations of at least 10^{-7} M trigonelline exhibited increased cell arrest in G2 [4]. Concentrations of 10^{-6} M of either nicotinic acid, nicotinamide or nicotinamide adenine dinucleotide are ineffective in promoting predominant cell arrest in G2, but at 10^{-4} M, all three test compounds produced predominant cell arrest in G2 [1]. When excised roots were cultured with other intermediates of the pyridine nucleotide pathway, the concentration of trigonelline within the roots increased [5], and this increased concentration was correlated with an increased cell arrest in G2.

Joshi and Handler [6] have shown with radioactive tracer experiments that yeast (*Torula cremoris*) can degrade trigonelline for use in the pyridine nucleotide pathway. *Pisum sativum* seedlings were incapable of the same transformation [6]. The identification of a physiological role for trigonelline [2, 3] prompted a reinvestigation of trigonelline metabolism in *Pisum* relative to the concentrations necessary for G2 cell arrest in root meristems. Radioactive tracer experiments [5] confirmed the experimental results of Joshi and Handler by showing that there is only a one-way flux of pyridine nucleotide intermediates towards trigonelline.

Nicotinic acid and nicotinamide are immediate precursors of trigonelline in the pyridine nucleotide metabolic pathway [7]. These precursors are readily converted to trigonelline in excised roots, resulting in an increased percentage of cells in G2 [5]. The present experiments were performed (a) to determine concentrations of nicotinic acid and nicotinamide in cultured roots when concentrations of various components of the pyridine nucleotide pathway were added to the media, and (b) to relate these concentrations with the ability of the pathway components to promote cell arrest in G2. In order to determine tissue concentrations of several pyridine nucleotide pathway intermediates, as well as their levels in tissue culture systems containing high levels of trigonelline precursors, a rapid quantitative detection system for nicotinic acid (NA) and nicotinamide (NAM) has been developed. Direct monitoring of NA and NAM concentrations in culture is now possible and their role in trigonelline metabolism and cellular arrest in G2 has been investigated.

RESULTS

Figure 1 shows tissue concentrations of nicotinamide in cultured roots of *Pisum sativum* as a function of added pyridine nucleotide pathway intermediates to culture media. Trigonelline, nicotinic acid, NAD^+, and nicotinamide are each plotted on separate graphs. Regardless of the concentrations of component added, tissue concentrations of nicotinamide remained constant between 0.12 and 0.73 μg/g tissue. As the concentration surpassed the threshold (10^{-4} M for NAD^+ and nicotinamide; 10^{-5} M for nicotinic acid; and 10^{-6} M for trigonelline), there was a marked increase in the number of cells arrested in G2.

Figure 2 shows tissue concentrations of nicotinic acid as a function of the concentration of pyridine nucleotide pathway intermediates in aseptic culture. The concentration of nicotinic acid increased markedly as the concentrations of other intermediates in the media were increased, except trigonelline. Addition of 10^{-4} M NAD^+ to the culture medium gave a nicotinic acid concentration in tissues of over 1 mg/g. Addition of either nicotinic acid or nicotinamide at 10^{-4} M increased tissue concentrations of nicotinic acid to over 400 μg/g tissue.

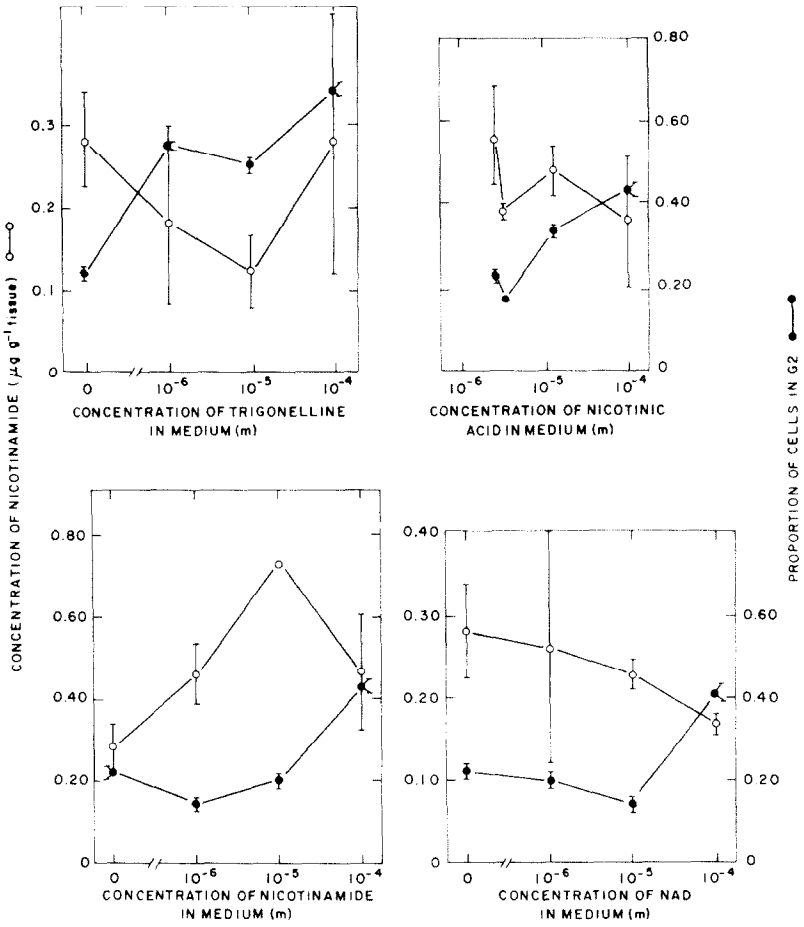

Fig. 1. Concentrations (μg/g tissue) of nicotinamide and proportions of cells arrested in G2 in stationary phase meristems of excised roots of *Pisum sativum* exposed to various substances in aseptic culture. Excised roots from 3-day-old seedlings were exposed to various concentrations of trigonelline (upper left), nicotinic acid (upper right), nicotinamide (lower left) and nicotinamide adenine dinucleotide (NAD) (lower right) while in medium with sucrose. After 3 days in medium with sucrose some roots were harvested to determine the concentrations of nicotinamide, while the remainder was transferred to medium without sucrose to establish a stationary phase.

DISCUSSION

Using a combination of prep. TLC and HPLC, a system has been developed to monitor levels of NA and NAM in cultured root meristems. This method enables the monitoring of tissue concentrations of these two members of the pyridine nucleotide pathway when cultured in the presence of varying levels of other members of the metabolic pathway. Results indicate that increasing media concentrations of NA, NAM, NAD^+, or trigonelline, reach a threshold above which preferential cell arrest in G2 occurs. Recent evidence [5] suggests that these intermediates are metabolically converted to trigonelline, and that G2 arrest is directly correlated with trigonelline concentration. The trigonelline concentration of cultured *Pisum* roots has been shown to increase to over 200 μg/g tissue [5].

Although all three intermediates tested are converted into trigonelline, the level of nicotinamide never exceeds 0.7 μg/g tissue, and its concentration is unaffected by the addition of all pathway intermediates tested. In contrast, nicotinic acid concentrations rise to over 400 μg/g tissue with additions of 10^{-4} M NA, NAM and NAD^+. Concentrations of 10^{-4} M trigonelline did not result in elevated NA levels [5]. The low tissue NAM concentration implies more efficient metabolic conversions or certainly better metabolic regulation of this intermediate, which may be understandable in the light of the plant-growth regulator effects of NAM [8, 9]. *Pisum* is quite tolerant of high NA and trigonelline concentrations.

None of the pyridine nucleotide pathway intermediates tested appear to have adverse affects on cultured roots. Previous work has shown that all intermediates are absorbed by roots and metabolized to some extent. Products of metabolism appear both in roots, as well as in culture medium, suggesting some leakage or excretion of metabolic products [5]. Although the metabolic controls of the pathway are not understood, it is clear that nicotinamide concentrations are highly regulated. Levels of NA are less highly regulated, and may in some way be compartmentalized for storage. This is important in relation to trigonelline metabolism and its physiological

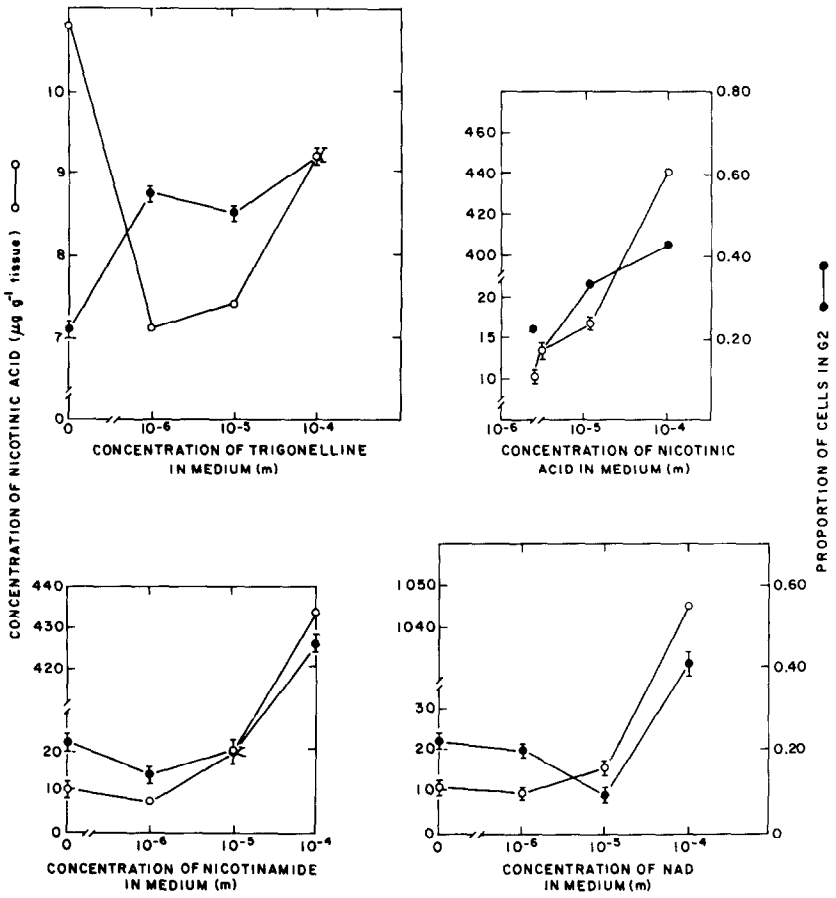

Fig. 2. Concentrations (μg/g tissue) of nicotinic acid and proportions of cells arrested in G2 in stationary phase meristems of excised roots of *Pisum sativum* exposed to various substances in aseptic culture. Excised roots from 3-day-old seedlings were exposed to various concentrations of trigonelline (upper left), nicotinic acid (upper right), nicotinamide (lower left) and nicotinamide adenine dinucleotide (NAD) (lower right) while in media with sucrose. After 3 days in media with sucrose some roots were harvested to determine the concentrations of nicotinic acid while the remainder was transferred to media without sucrose to establish a stationary phase.

role in G2 arrest. Trigonelline is accumulated in high concentrations in *Pisum* root tissue. Most interesting, G2 arrest occurs in response to the extra tissue concentration of trigonelline rather than its intra-tissue concentration [5]. This response may be explained by a compartmental storage of excess trigonelline and nicotinic acid.

EXPERIMENTAL

General culture conditions. Bioassay for promotion of cell arrest in G2, and DNA measurements were described in detail previously [5].

Nicotinic acid and nicotinamide concentrations. To determine the concn of nicotinic acid and nicotinamide in roots, 0–1 cm terminal root segments of 3-day-old seedling roots were excised and extracted in an EtOH series [10]. Each extract was concd, spotted on Analtech Si gel TLC UV plates, 250 μm thickness (Analtech, Newark, DE 19711), and developed in *iso*-PrOH–H_2O–HOAc (29:5:6). Plates were allowed to air dry and the area from nicotinic acid to nicotinamide was scraped and eluted with EtOH. This soln was evaporated to dryness and the residue resuspended in 0.5 ml H_2O. This soln was chromatographed a second time (HOAc–Me_2CO–MeOH–C_6H_6, 1:1:4:14). Nicotinamide and nicotinic acid were eluted individually. Quantities of nicotinic acid and nicotinamide were determined using a Whatman C-18 reverse phase column using KPi (pH 5.8), 0.001 M, at a flow rate of 2–3 ml/min on a Waters 300 HPLC [11]. Authentic samples were injected with each sample to confirm peak identity. Efficiency of recovery of each compound was determined by subjecting known amounts of NA and NAM to the entire isolation procedure.

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