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# EFFECT OF HORMONES ON THE INCREASE AND THE SUBSEQUENT DECREASE IN NAD KINASE IN POTATO TUBER SLICES

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## Introduction

The tissue of resting plant storage organs such as carrots, red beets, sugar beets or potato tubers causes the formation of various enzymes by cutting into thin slices and incubation of the fragments in a moist atmosphere<sup>12</sup>. In potato tuber tissue for example, phenylalanineammonialyase (PAL), cinnamic acid 4-hydroxylase (CA4H), RNase, glucose-6-phosphate dehydrogenase and other enzymes were reported to be induced in response to cutting<sup>11,13,16,21,24</sup>. Furthermore, there are several informations on the synthesis or activation of plant hormones of different kinds after wounding of plant storage organ<sup>6,10,18,22</sup>. These findings gave a possibility that plant growth regulators might play a role of the metabolic change in aging slices. However, little was known the effect of plant hormones on the enzyme induction in potato slices<sup>19</sup>.

On the other hand, the activity of oxidative pentose phosphate pathway rises considerably and reaches a maximum one day later in potato slices<sup>1</sup>. This phenomenon is considered to be due to the increase of NADP content<sup>2</sup>, because the level of NADP is related to the participation of this pathway<sup>20</sup>. Thus, NAD kinase (EC. 2, 7, 1, 23), which produces NADP from NAD and ATP, is of particular interest in connection with activity of the oxidative pentose phosphate pathway in potato slices. This paper deals with the effect of several plant hormones on the change in NAD kinase activity in potato slices during aging.

## Materials and Methods

### Plant materials

Unless otherwise stated, the tubers of potato (*Solanum tuberosum* L. var. Irish Cobbler) stored for 6 months at 4°C in darkness were used as materials. The disc-shaped slices (1 mm in thickness and 7 mm in diameter) were prepared from the parenchymatous tissue of these tubers.

### Incubation of slices

Ten slices were aseptically explanted onto 30 ml of modified White's inorganic medium<sup>20</sup> with 0.7% agar in a 100 ml-Erlenmeyer's flask autoclaved at 1.0 kg/cm<sup>2</sup> pressure for 10 min. Plant hormone and cycloheximide were added to the agar medium at an appropriate concentration. The slices were incubated in a dark room at 25°C. For ethylene application, slices were placed on agar medium in an air-tight chamber (inside volume, 350 ml) and incubated at 25°C in the presence of ethylene.

### NAD kinase extraction

Thirty slices were taken at appropriate intervals and ground in 20 ml of 50 mM tris-HCl, pH 7.6, (Buffer A) containing 0.5 M sucrose, 0.5% (w/v) cysteine and 4 mM EDTA with mortar and glass homogenizer. The homogenate was centrifuged at 15,000 × g for 10 min. The supernatant was brought to 50% saturation with ammonium sulfate, and then precipitate was collected by centrifugation at 12,000 × g for 10 min and suspended in a small volume of Buffer A. The solution was then applied to a column (25 × 90 mm) of Sephadex G-25 and eluted with Buffer A. Thus the obtained protein fraction were used as the enzyme solution.

### Assay of NAD kinase activity

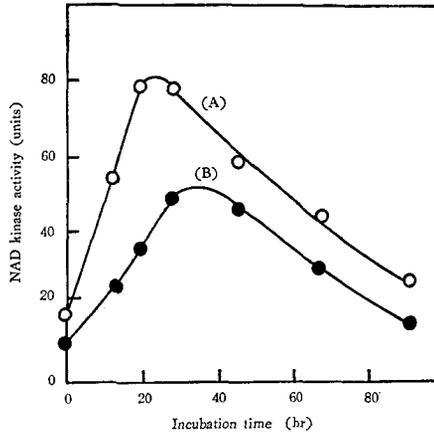
The standard assay system contained 0.6 μmol NAD, 0.8 μmol ATP, 7.3 μmol MgCl<sub>2</sub>, 25 μmol tris-HCl, pH 7.6, and a suitable amount of enzyme solution in a total volume of 0.5 ml. After incubation for 30 min at 30°C, the NADP formed was determined as described in the previous paper<sup>9</sup>. One unit of activity is defined as an amount of enzyme which produces one n mole of NADP per hr per g fresh weight.

## Results

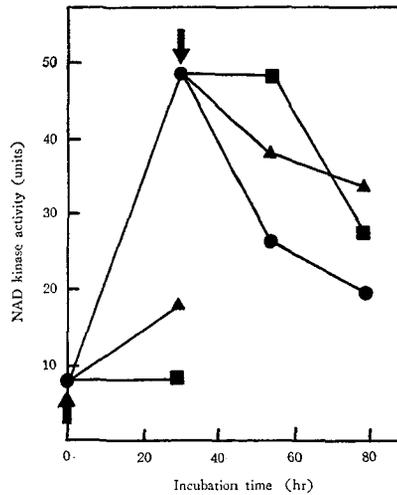
### The increase and subsequent decrease in NAD kinase activity during incubation

Figure 1 illustrates an arithmetic plot of the time course of the wound-induced NAD kinase in potato tuber slices. The activity of NAD kinase

was spontaneously increased immediately after preparation of slices and reached a peak at about 18 hr or 30 hr after the initiation of the incubation (defined as phase I), and the activity then gradually declined thereafter (phase II). It became apparent from a series of experiments that the peak in the enzyme activity was varied depending upon batches of potato tubers. Espe-



**Fig. 1.** Time course of NAD kinase activity in potato tuber slices at 25°C. (A) Two months after harvest of tuber. (B) 15 months after harvest.



**Fig. 2.** Effect of cycloheximide (CHI) on the decrease in NAD kinase activity. Slices were transferred to the agar medium containing cycloheximide at the time shown by the arrow. ●: control, ■: 3 µg/ml CHI, ▲: 1 µg/ml CHI.

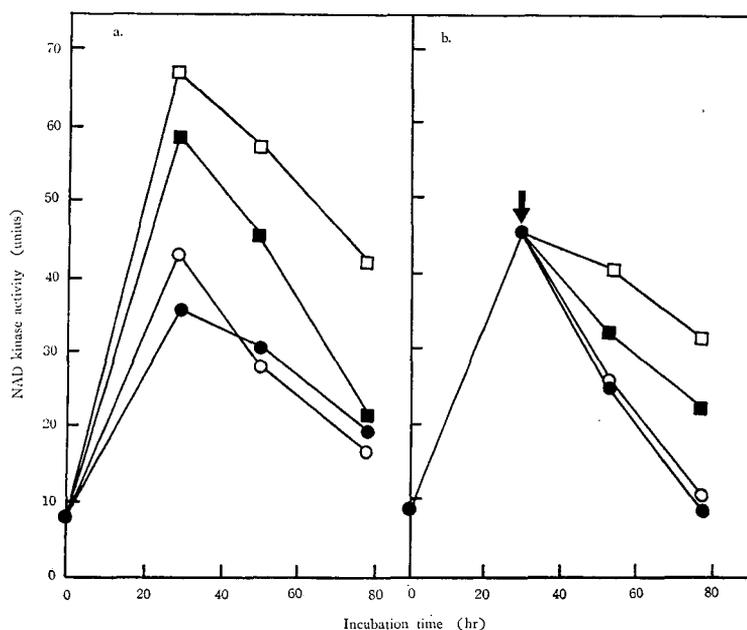
cially, the peak height was decreased with increasing storage time of potato tubers. The potato tubers used in this paper almost belong to (B) type in Fig. 1.

### Effect of cycloheximide

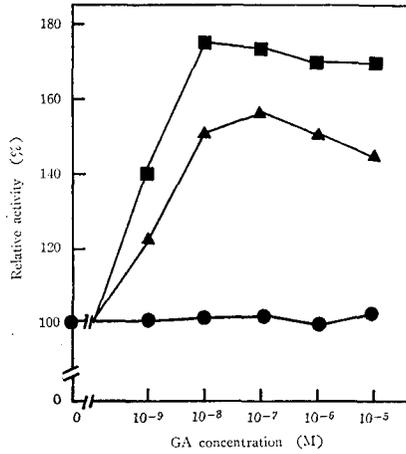
The increase in NAD kinase activity was completely inhibited by the addition of cycloheximide. However, when the inhibitor ( $3 \mu\text{g/ml}$ ) was added to aging slices at 30 hr after preparation of slices, the decrease in the activity (i. e. phase II) was considerably suppressed and followed by marked declining (Fig. 2). The low concentration of cycloheximide ( $1 \mu\text{g/ml}$ ) was good enough to suppress ca. 50% of the decrease in enzyme activity to the control throughout in phase II. These results suggest that the protein synthesis is a prerequisite for NAD kinase degradation on potato slices.

### Effects of NAA and kinetin

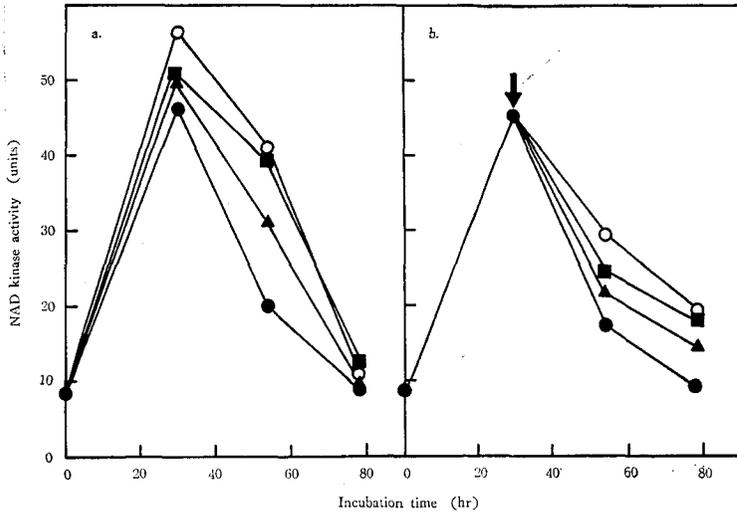
When NAA or kinetin was applied to potato tuber slices immediately after slicing, NAA caused the enhancement of the increase in NAD kinase



**Fig. 3.** Effect of NAA ( $160 \mu\text{M}$ ) and kinetin ( $14 \mu\text{M}$ ) on the change in NAD kinase activity. a. Slices were incubated on the agar medium containing each hormones. b. Slices were transferred to the agar medium containing each hormones at the time shown by the arrow. ●: control, ■: NAA, ○: kinetin, □: NAA+kinetin.



**Fig. 4.** Effect of GA concentration on the increase in NAD kinase activity. Activities are expressed as a percentage of the control after 20 hr. ■: 44 days after harvest of tuber, ▲: 55 days after harvest, ●: 85 days after harvest.



**Fig. 5.** Effect of GA on the change in NAD kinase activity. a. Potato slices were incubated on the agar medium containing various concentration of GA. b. Slices were transferred to the agar medium containing various concentration of GA at the time shown by the arrow. ●: control, ○: 10<sup>-7</sup> M GA, ■: 10<sup>-8</sup> M GA, ▲: 10<sup>-9</sup> M GA.

activity during phase I. On the other hand, kinetin did not show any effect on the enzyme activity during phase I. When kinetin was added to the incubation medium with NAA, it intensified the effect of NAA (Fig. 3a). In order to investigate the effect of NAA and kinetin on the decrease in enzyme activity during phase II, the slices which had been pre-incubated for 30 hr in hormone-free medium were transferred to agar medium containing NAA and kinetin (Fig. 3b). NAA prevented the decrease of the activity of NAD kinase, and this effect was also accentuated by the concomitant presence of kinetin which did not show any significant stimulation during phase II.

#### **Effect of GA**

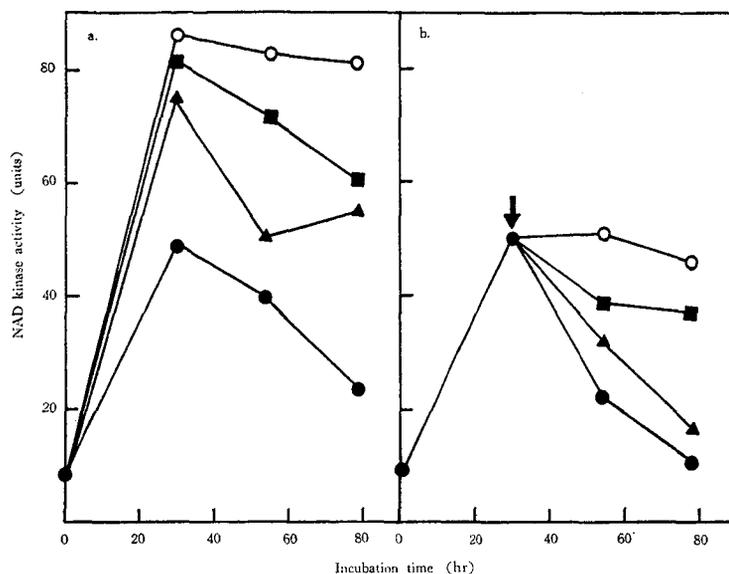
As shown in Fig. 4, GA enhanced the increase in NAD kinase activity during phase I and  $10^{-8}$  M of this hormone brought about the maximum activity to enzyme development. However, the more aged potato tuber was used, the lesser stimulative effect of GA was obtained. From these results, it is appeared to change in the susceptibility of potato tuber to this hormone during storage. By using of older tuber, GA was slightly inhibited the decrease in the activity during phase II (Fig. 5).

#### **Effect of ABA**

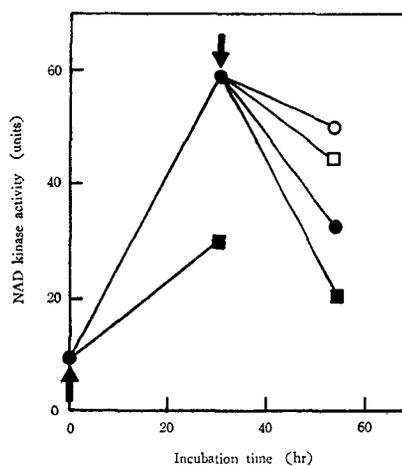
Figure 6 shows the effect of ABA on the changes in NAD kinase activity during the incubation period. When ABA was applied to slices immediately after slicing, it enhanced the increase in NAD kinase activity during phase I (Fig. 6 a). The activity enhanced by ABA ( $10^{-5}$  M), which was distinct from the effect of GA, was not decreased during phase II. Similar results were observed by the addition of ABA at the end of phase I to the slices (Fig. 6 b).

#### **Effect of ethylene**

The increase in NAD kinase activity in slices incubated in the presence of ethylene during phase I was suppressed by ca. 50% of control (Fig. 7). Treatment of ethylene at 30 hr after slicing caused the stimulation of the decrease in enzyme activity during phase II. However, this stimulation of the decrease was partially counteracted by the presence of NAA and kinetin. Since carbon dioxide is known to be an antagonist of ethylene<sup>9</sup>, the effect of removing carbon dioxide was investigated. As shown in Table 1, although the carbon dioxide removal slightly inhibited the increase in the enzyme activity, the most strong inhibition of the development of NAD kinase during phase I was found in slices incubated by the combination of ethylene applica-



**Fig. 6.** Effect of ABA on the change in NAD kinase activity. a. Potato slices were incubated on the agar medium containing various concentration of ABA. b. Slices were transferred to the agar medium containing various concentration of ABA at the time shown by the arrow. ●: control, ○:  $10^{-5}$  M ABA, ■:  $10^{-6}$  M ABA, ▲:  $10^{-7}$  M ABA.



**Fig. 7.** Effect of ethylene (5 ppm) on the change in NAD kinase activity. Slices were transferred to the agar medium containing various hormones (NAA  $160 \mu\text{M}$ , Kinetin  $14 \mu\text{M}$ ) or treated with ethylene in an air-tight chamber at the time shown by the arrow. ●: control, ■: ethylene, ○: NAA+Kinetin, □: NAA+Kinetin+ethylene.

TABLE 1. Relation of an ethylene addition and carbon dioxide removal to the development of NAD kinase activity

Conditions	Activity (Units*)
Control	47.5
-CO <sub>2</sub>	44.3
+5 ppm C <sub>2</sub> H <sub>4</sub>	29.6
+5 ppm C <sub>2</sub> H <sub>4</sub> -CO <sub>2</sub>	25.3

\* *n* moles NADP formed hr<sup>-1</sup> gFW<sup>-1</sup>

Slices were incubated for 30 hr in an air-tight chamber. Carbon dioxide released during incubation was removed by 10 ml of 20% potassium hydroxide.

tion and carbon dioxide removal. These results suggest that carbon dioxide antagonizes ethylene during the aging of potato slices.

### Discussion

The spontaneous increase in NAD kinase activity during aging of potato tuber tissue after preparation of slices has been found to be de novo synthesis, because cycloheximide completely inhibited the development of NAD kinase (Fig. 1). However, the subsequent decrease in the activity during phase II was partially prevented by the presence of cycloheximide. KAHL and STEGEMANN<sup>14</sup> reported that the decrease in phosphoglucomutase activity which enhanced by slicing of resting potato tuber tissue was prevented by inhibitors of translation. ZUCKER<sup>30</sup> also reported that the decrease in PAL activity at the later stage in aging of potato slices was prevented by cycloheximide. On the other hand, the wound-induced RNase in bean pod tissue and acid invertase in sweet potato were stimulated the decrease in their activities by cycloheximide<sup>17,23</sup>. Discrepancy between these effects of cycloheximide on the enzyme activities are considered to be due to the difference between the instability of enzymes and their inactivating system<sup>30</sup>. Thus, it is suggests that the formation of inactivating system of NAD kinase is inhibited by cycloheximide, and that the inactivating system is more labile than the protein molecules of NAD kinase.

The changes in quantity of hormones in tissue slices were reported to increase by slicing of potato tuber<sup>6,11,18,22</sup>. We thus made an attempt to investigate the effect of hormones on the changes in NAD kinase activity during aging in potato slices. NAA promoted the increase in activity during phase I (Fig. 3). This was stimulated by the presence of kinetin, while

kinetin showed any significant changes of NAD kinase activity when compared with slices incubated in medium lacking NAA and kinetin. On the other hand, it was reported that the addition of hormone such as auxin suppressed the development of RNase of leaf tissue<sup>27</sup>. From these different effects of NAA or kinetin on an enzyme development, it is suggested that these hormones may regulate the synthesis or stability of mRNA for a specific enzyme. NAA and kinetin also prevented the decrease in NAD kinase activity during phase II (Fig. 4). This effect is not briefly considered to be due to stimulation of protein synthesis, because cycloheximide also prevented the decrease in the activity. NAA and kinetin might suppress the synthesis of the degradation system of the enzyme activity.

When the freshly sliced Jerusalem artichoke tissue is supplied with GA, the synthesis of invertase is markedly increased<sup>5</sup>. In potato tuber slices, the activity of DNA-dependent RNA polymerase is greatly enhanced by the presence of GA<sup>15</sup>. NAD kinase activity in potato slices was also increased by the addition of this hormone (Fig. 7). This effect, however, was obtained by using only potato tubers stored for short period of time from their harvest season. It was reported to increase in the amount of GA before breaking of the dormancy in potato tuber<sup>26</sup>. The lacking of GA effect on the development of NAD kinase activity may be due to the change in the hormone content in potato tubers during the storage. ABA has been generally recognized to inhibit the protein and RNA synthesis in plant tissue<sup>9</sup>. However, some enzymes such as invertase in sugar cane<sup>8</sup> and acid phosphatase and RNase in bean endocarp tissue<sup>7</sup> were reported to increase by the application of ABA. The incubation of excised bean axes with ABA caused PAL activity to increase, and the continued incubation of the axes in ABA solution resulted in only a 20% to 30% drop in PAL activity, so that by 24 hr the activity was 2 to 4 times that observed in axes which had been incubated in buffer during the same period of time<sup>28</sup>. NAD kinase in potato slices was also influenced on both the development and retention by ABA. From the inhibitory action of ABA on protein and RNA synthesis, it is difficult to explain the enhancing mechanism of NAD kinase activity by ABA during phase I. This hormone, however, may be at least partially successful in reducing the effectiveness of the inactivating system.

The increases in PAL, CA4H and peroxidase activities are known to be stimulated by ethylene in wounded sweet potato tuber<sup>17,19</sup>. On the other hand, the increases in acid invertase and DNA synthesis were inhibited by this hormone<sup>17,25</sup>. Ethylene inhibited the development of NAD kinase during phase I and stimulated the decrease during phase II, respectively (Fig. 7).

These results indicate that the effect of ethylene on the change in NAD kinase activity completely differs from that of the developments of PAL, CA4H or peroxidase.

From these results, it may be appeared that plant hormones play some regulatory role on the development of NAD kinase in potato slices during aging.

### Summary

The effects of auxin (NAA), cytokinin (Kinetin), gibberellic acid (GA), abscisic acid (ABA) and ethylene on the changes of NAD kinase activity in potato tuber tissue induced by slicing were examined. The activity of NAD kinase greatly increased, and thereafter decreased during the process of aging in potato slices. The increase in enzyme activity was completely inhibited by cycloheximide, and the decrease in the activity was suppressed by this inhibitor. NAA promoted the increase in NAD kinase activity. Kinetin did not show any effect, but, it further stimulated the increase in enzyme activity upon the addition of NAA. The decrease in enzyme activity at the later stage of incubation was counteracted by the combination of NAA and kinetin. GA enhanced the increase in enzyme development in the slices of potato tuber which had stored for a short period after harvest. ABA enhanced the increase in enzyme activity and suppressed the subsequent decrease. On the other hand, ethylene suppressed the increase and stimulated the decrease in enzyme activity. These hormonal effects on the changes in NAD kinase activity were discussed with the results from the experiment using cycloheximide.

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