Studies on Photoperiodic Responses of Salvinia natans (VII)

On Ultraviolet Light-Absorbing Components, NAD and NADP, Extracted from Photoinduced Plants

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(Received Nov. 30, 1965)

Photosensitive plants have been considered to produce floral hormone in their leaves under a suitable day-length for floral formation, and some evidences [2,3,4] for such facts have already been found. If the floral hormone is synthesized, or is activated from any material in the leaves, a metabolic system should be formed newly to produce the hormone.

Recently, the author [7] reported that a water soluble fraction of *Pharbitis* leaves enhanced an ultraviolet (UV) light-absorbing ability with increasing number of photoperiodic induction, and suggested that the UV light-absorbing component may be some enzyme like substances.

The present paper deals with a UV light-absorbing component which seems to be a coenzyme in a metabolic system contributing to the formation of photoperiodic stimulus.

Materials and Methods

Concerning to the culture conditions and experimental materials applied in this study, the previous reports [6,8] are to be referred to.

Inductive photoperiod, consisting of 8 hour light and 16 hour dark periods, was given with the required cycles by each experiment. As it was supposed, by the previous observations[7], that the UV light-absorbing component may be a coenzyme, the method for the extraction of the component consists in a heating process followed by the previous procedures.

The extraction of a UV light-absorbing component: The leaves were collected 1 or 14 hours after the last photoinductive dark period, weighed after the removal of moisture on the leaf surface by a blotting paper, immersed in hot water for 15 minutes at 90±3°C, and ground in the water after cooling to a room temperature. Cold aceton of 6-7 times as much volume was poured into the blended solution. After the solution was kept in a refrigerator over-night,

it was centrifuged. The sediment obtained was dissolved in distiled water, and the solution of crude protein was obtained by centrifugation. To simplify a quantitative comparison among the results obtained from different experiments, the water used in the last procedure was proportioned in volume to the weight of the leaves collected at the first time.

The separation of nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) from the protein solution: To separate NAD and NADP from the protein solution, a paper chromatographic technique was used. Filter paper for chromatograph (TOYO filter paper, No. 50) was banded with 0.2 ml of the protein solution obtained by above procedures, and was developed by pyridine–water (2:1)[4] for 15 hours at a room temperature. Then, the part corresponding to the Rf value of NAD or NADP was cut off, and it was extracted into 4 ml of water. In this case, this volume of water used for the extraction of coenzymes always held the component contained in 0.113g of the fresh leaves. In order to obtain the relative quantities of coenzyms, the absorbances of the extracts were observed at 260mμ by using a photoelectric spectrophotometer of Bechman type. Therefore, the absorbances measured express a relative level of NAD or NADP contained in the same weight of the leaves.

The chromatographic identification tests for NAD and NADP were made by using chemicals manufactured by Sigma Chemical Co. Ltd., U. S. A., under the same condition as the above procedures.

Results

1. *General characters of the crude protein solution.*

The protein solution obtained from the leaves revealed a strong absorption in UV light, and a sharp decrease of the absorption was found with a transition to visible ray. A difference in the absorbance between two groups of plant, photoinduced and non–photoinduced, was found at the wave length ranging 260 to 270mμ. That is, in a comparison with the absorbance in the non–photoinduced plants, a little increase was found in the plants receiving 1 photoinduction, and a much increase was given by 3 photoinductions.

In the paper chromatograph of the leaf extract, the UV light-absorbing component was detected in two Rf values, which were in agreement with those of NAD(0.63) and NADP(0.51) in an identification test. Therefore, the following experiments were undertaken under a consideration of the fact that the UV light–absorbing component in the leaf extract consists of two factors, NAD and NADP.

2. *The influences of a lapse of time after photoinduction on NAD and NADP levels.*

In order to know the influences of a lapse of time after photoinduction on

Table 1. The influence of time length from photoinduction to extraction on coenzyme levels.

the number of photoinductions	coenzyme	absorbance	
		1 hr.	14 hrs.
1	NAD	0.094	0.089
	NADP	0.107	0.087
3	NAD	0.121	0.123
	NADP	0.118	0.105

NAD and NADP levels, the coenzymes were extracted 1 and 14 hours after the last photoinductive dark pariod.

The results were shown in table 1. The UV light-absorbing ability was generally greater in the extract after 1 hour than after 14 hours, but its difference was neglezible for the result that the absorbance of NAD or NADP made little difference between both times. Subsequently, the extraction procedure was made 1 hour after the dark period as follows.

3. *The influences of the number of photoinductive cycles on NAD and NADP levels.*

The coenzyme levels measured when given with 1 or 3 cycles of the photoperiodic induction were shown in figure 1. The plants which was given with 1 photoinductive cycle revealed almost equal level of NAD and some more

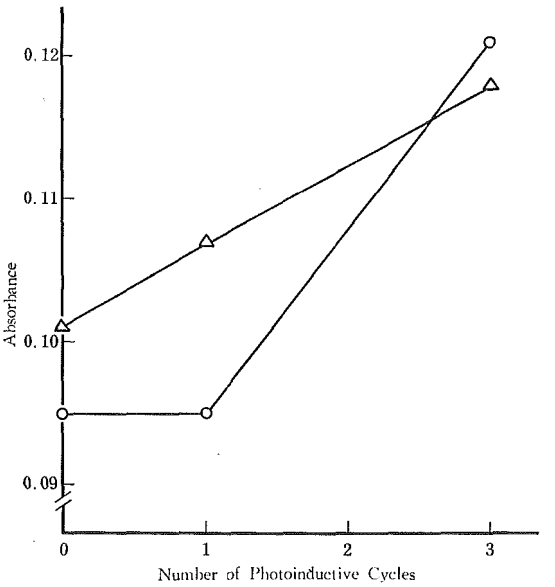

Figure 1. A relation between the number of photoinductive cycles and NAD or NADP level. Triangles and circles illustrate NADP and NAD, respectively.

level of NADP as compared to those of control plants. With 3 photoinductive cycles, however, NAD which made a considerable increase exceeded in the level of NADP which made a moderate increase continually from the first cycle.

4. *The influences of KCN or CO applied on NAD and NADP levels during the dark periods.*

In the previous report[8], the author reported that the inhibitors of heavy metal enzymes, such as CO, KCN or NaN_3, accelerated sporocarp initiation in *Salvinia natans*. In order to know the cause of such effect, it was investigated whether an application of CO or KCN affects to the level of NAD or NADP as mentioned above. During 3 dark periods, 10^{-4}M KCN or 95% CO was applied. After the plants treated with KCN were rinsed in running water for 15 minutes after its application, they were transferred to a medium without KCN in the light periods.

Table 2. The influences of certain enzymatic inhibitors on coenzyme levels and the number of sporocarps during the dark periods.

inhibitor	coenzyme	absorbance	NAD/NADP ratio	the number of sporocarps
non	NAD	0.121	1.02	7.5
	NADP	0.118		
KCN	NAD	0.150	1.17	14.7
	NADP	0.090		
CO	NAD	0.105	1.04	9.8
	NADP	0.101		

These results as well as the number of sporocarps were shown in table 2. The ratio of NAD to NADP levels was found to be higher with KCN than with CO, and was also the case of the number of sporocarps.

Discussion

As it was found that a UV light absorbing substance in *Pharbitis* plant may contribute to its photoperiodic response, experiments were undertaken to verify the hypothesis developed with *Salvinia natans* that such substance can be a coenzyme. Such expectation may be supported by the fact that the protein solution extracted was similar to the oxidized coenzymes in the point that UV light was absorbed in the maximum at about 260mμ, and additional evidence for the expectation was given from the result of the paper chromatographic experiment that the Rf values of the UV light-absorbing component in the solution were in agreement with those of NAD and NADP.

YAMAMOTO [11] has reported that, independent of the development of a reproductive phase in plants, coenzyme levels in the plants were influenced by

environmental factors such as the light or the air. In the results obtaind here, though the coenzyme levels in *Salvinia* plant changed with time when the plants were lightened after the last dark period, the light did not affected to the coenzyme levels accompanied by the photoperiodic induction. Therefore, the change in NAD or NADP level with the induction seems to relate with the development of the reproductive phase in the plants, and it is suggested that the photoperiodic treatment raises the coenzyme levels.

By the inhibition of heavy metal enzymes during the dark periods, both components of the coenzyme decreased more or less in a practical level than those in the control plants, but NAD increased in a relative value to NADP level. In this case, it is interesting to say that the relative value is higher by KCN inhibition which induced more sporocarps than by CO inhibition. From the fact that the change in NAD level followed by varying photoinductive cycles makes us recall the status in the number of sporocarps reported in the previous paper[6], it may be concluded that the treatment such as enables the plants to form more sporocarps increases a relative level of NAD to NADP. Practically, its increase may be resulted from the increase of NAD or from the decrease of NADP.

Concerning to the physiological function of NAD in photoperiodic responses, the fact that the coenzymes as well as uracil having a specific acceleratory effect on floral [5,10] or sporocarp formation [9] are pyridine derivertives leads a supposition that both substances have a similar effect on sporocarp formation. However, it is likely that NAD contrasts with NADP in an enzymatic function, because the level of NAD changes in contrast to that of NADP. Whether such a metabolic system is a known one, such as hexose-pentose pathway to Embden-Meyerhof or glyoxyl cycle to TCA, is remained to be known.

Summary

1. NAD and NADP were paper-chromatographically separated from the leaves of *Salvinia natans* receiving short day treatment, and they were compared with those in control plant.
2. In accordance with the increase in the number of short day treatment, NAD level increased remarkablly in contrast with less increment of NADP.
3. When the dark processes were inhibited by applying 95% CO or 10^{-4}M KCN, a NAD/NADP ratio was found to be higher with KCN than with CO, and was also the case of the number of sporocarps.
4. From these results, it is suggested that the NAD/NADP ratio is closely related with sporocarp initiation, and that the development of a reproductive phase may be caused by activation of a metabolic system concerning NAD

or by inacitvation of that concerning NADP.

The author wishes to express his hearty gratitude to Prof. K. NAKAYAMA for his kind help and constant encourgement in the course of this study.

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