Short Communication

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This communication presents some evidence to indicate that NAD kinase activity is controlled by phytochrome action. Seeds of *Pharbitis nil*, strain Violet (a short-day plant), were germinated after the description of Yoshida *et al.*¹⁾ After grown for 4 days under continuous illumination, seedlings were placed in the dark. Red light break is known to reduce most effectively the floral induction when it is given 8 hrs. after the start of darkness.²⁾ Twenty seedlings which received 8 hrs. of darkness were exposed to combinations of 5 min. each of red and far-red lights. Red (R) and far-red (FR) lights used in the present study were obtained in the same way as in Pjon and Furuya³⁾ and 800 and 12,000 ergs cm⁻² sec.⁻¹, respectively. After further exposure to darkness for 15 min, one of a pair of cotyledons of each seedling was used for NADP extraction and the other for NADPH extraction⁴⁾.

As shown in Fig. 1, R raised NADP level. The so-called R-FR reversibility was observed. These results suggest a close relation of phytochrome action to NADP synthesizing and/or decomposing systems. In the present work, however, NAD kinase which catalyzes the formation of NADP from NAD in the presence of ATP and Mg⁺⁺⁵⁾ was studied. *Pharbitis* cotyledons (4.0 g in fresh weight) exposed to the dark for 8 hrs. as above described were blended at 0° with 20 ml of 0.05 M Tris-HCl pH 7.6 in the presence of Polyclar AT (1 g) under the safelight. The homogenate was squeezed through 4 layers of gauze and centrifuged at 15,000×*g* for 30 min. in the dark. NAD kinase activity in the supernatant was greater in R than in darkness (Table 1).

If NAD kinase is promoted through phytochrome action, an association will be expected between phytochrome and NAD kinase. Seeds of *Pisum sativum* var. Alaska were germinated in the dark for 6 days, and the uppermost internodes including hook were separated. Phytochrome was extracted and purified by Manabe up to and including brushite chromatography step, by a modification of the method described by Siegelman and Firer⁶⁾. Hereafter, unless otherwise stated, this phytochrome preparation was used. Active phytochrome eluate from brushite column contained some NAD kinase activity which was elevated markedly by R (Table 2). As phytochrome purification was proceeded up to Sephadex G-200 step, NAD kinase was further concentrated (Table 2).

The components necessary for the NAD kinase reaction under R are summarized in Table 3. On omission of NAD, ATP, or Mg⁺⁺, there was practically no formation of NADP. Boiled phytochrome preparation was inactive.

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Fig. 1. Effects of red and far-red lights on nicotinamide coenzyme levels in cotyledons of *Parbitis nil* Violet.

Table 1. Effect of red light on NAD kinase in supernatant from *Pharbitis* cotyledons.

	NAD kinase activity*
Dark	0.304
Red light	0.467

* μ moles of NADP produced/mg protein per hr.

Table 2. Effect of red light on NAD kinase in a partially purified phytochrome preparation from pea stems.

	NAD kinase activity*	
	Dark	Red light
Brushite step	22.8	50.2
Sephadex G-200 step	45.3	83.9

* μ moles of NADP produced/mg protein per hr.

Table 3. Components required in NAD kinase system.

	NAD kinase activity*
Complete system (NAD+ATP+Mg ⁺⁺)	104.80
Without NAD	0.00
Without ATP	1.63
Without Mg ⁺⁺	4.59
With heated enzyme	0.00

* μ moles of NADP produced under red light/mg protein per hr.

Fig. 2. Effects of red and far-red lights on NAD kinase activity in a partially purified phytochrome preparation.

Fig. 3. K_m of NAD kinase for NAD Effect of red light.

In the experiments shown in Fig. 2, phytochrome fraction added to the assay system of NAD kinase was irradiated with several combinations of 5 min. each of R and FR at 0° before it was incubated for 30 min. at 30° in the dark for NADP formation. The promotion of NAD kinase activity by R seems to be reversibly removed by FR (Fig. 2). The exposure to R for 5 min. was nearly as effective as the exposure for 30 min.

K_m values for NAD of NAD kinase reaction under R or darkness were measured (Fig. 3). The K_m values calculated were $1.84 \times 10^{-3} \text{M}$ in darkness and $0.90 \times 10^{-3} \text{M}$ under R.

Each of the experiments reported in this communication was repeated at least twice with similar results.

At least two possibilities should be indicated. A) Phytochrome itself may have NAD kinase activity, or B) Phytochrome may only be closely associated with NAD kinase. The P_{fr} type of phytochrome may contribute to the decrease of the K_m value of NAD kinase reaction by introducing a conformational alteration of NAD kinase. Experimental studies are now in progress on these possibilities.

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