Phototransformation of the Phytochrome-Cyclic AMP and Phytochrome-Estriol Complexes

Hak Yong Kim and Quae Chae*

Department of Chemistry, Chungbuk National University, Cheongju, *Received March 19, 1987*

Phytochrome which is a chromoprotein consisting of the tetrapyrrole pigment linked covalently to the apoprotein triggers various photomorphogenic responses in plant kingdom. It exists as the two different forms depending upon the wavelength of light irradiated to the plant tissues. One is a red light absorbing form (Pr; λ_{max}=666 nm) and the other is a far-red light absorbing form (Pfr; λ_{max}=730 nm). Photoreversible reaction is taking place between these two forms; $Pr \underset{730\ nm}{\overset{660\ nm}{\rightleftharpoons}} Pfr$. The latter (Pfr) produced from the former by red light is believed to be the physiologically active form[1,2]. The most interesting aim for the scientists working in this area has been focused to understand how the light signal perceived by phytochrome is transduced to the metabolic processes. One aspect of the many advances for the elucidation of the primary physiological reactions is that binding of Pfr to the membranes[3,4] can change Ca^{++} ion influx into the cytosol[5,6]. The hypothetical mode of action of phytochrome was proposed as following[6,7];

$$Pr \underset{730\ nm}{\overset{660\ nm}{\rightleftharpoons}} Pfr \xrightarrow{\text{receptor X}} Pfr\text{-}X \longrightarrow \text{Physiological responses}$$

where the presumed receptors X were considered to be a protein, cyclic AMP, hormones, membrane, and genetic molecules, respectively. Considering X as a membrane, many studies have been carried out and the meaningful results have been reported[8~11].

In addition to membranes, the second messenger c-AMP and a kind of steroids estriol can be regarded as the primary receptor in the physiological reaction of phytochrome. Since the existence of c-AMP was discovered in higher plants[12], the exact role of this compound in plants has not been reported yet. Steroid compounds also play an important physiological role in plants[13]. One example is a dramatic induction of flowering upon injection of steroid estriol[14]. In an attempt to compare the binding properties of these two possible receptors to phytochrome, phototransformation experiments of the complexes were carried out. Phytochrome was isolated and purified by the methods described in[15]. Phytochrome-c-AMP and phytochrome-estriol complexes were prepared and assayed by following the procedures in the reference[15]. Phototransformation of phytochromes was checked by monitoring the absorbance changes at 666 nm and 730 nm with the continuous irradiation of samples with red or far-red light as described by Hahn[16]. Figure 1 shows the effects of c-AMP, estriol and 8-anilino-naphthalene-sulfonate (ANS) on the phototransformation of phytochrome. In all cases, the phototransformation is nearly completed within 2 min of red or far-red light irradiation and it was not much inhibited in the phytochrome-c-AMP complex compared with the one of ANS and estriol bound complexes. This result implies that the binding site of c-AMP in phytochrome must not be the hydrophobic surface area of the phytochrome, which is the vacant chromophore binding site upon photoconversion of $Pr \underset{}{\overset{660\ nm}{\longrightarrow}} Pfr$[16]. The working model for the phototransformation of phytochrome[17] is strongly supported by the binding experiment of hydrophobic fluorescence probe ANS to the phytochrome. ANS accelerates the Pr→Pfr photoconversion, and it inhibits Pfr→Pr photoconversion[16]. These effects are interpretable in terms of competitive binding of ANS to the chromophore binding site.

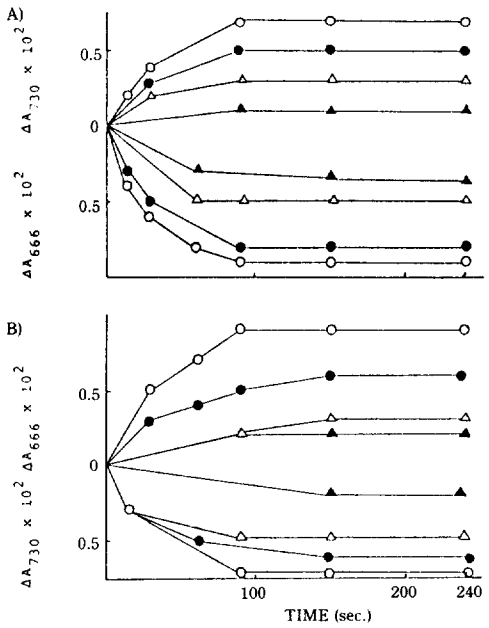

Figure 1. Phototransformation of phytochrome (20 mM KPB, pH 7.8, at 4°C; A_{666}/A_{280}=0.7, 0.07 mg) by measuring the absorbance changes at 666 nm and 730 nm upon irradiation of free phytochrome (○-○), phytochrome-cyclic AMP (20 μM) complex (●-●), phytochrome-estriol (20 μM) complex(△-△), and phytochrome-ANS (1 mM) complex(▲-▲), respectively. (A) a red light irradiation for Pr→Pfr phototransformation. (B) a far-red light irradiation for Pfr→Pr phototransformation.

Phototransformation aspects of estriol bound phytochrome were essentially the same as the one of ANS bound phytochrome (unpublished results). This means that estriol binds to the chromophore binding site of phytochrome and relatively inhibits the photochemical reaction from Pfr to Pr. However, the phototransformation behavior of phytochrome-c-AMP complex was shown to be essentially the same with the one of free phytochromes (Figure 1 and absorption spectra which are not listed in this paper). This suggests that c-AMP binding site to the phytochrome must be different with the one of the chromophore. For this reason, c-AMP bound phytochrome (Pfr form) which still has the vacant chromophore binding site preferentially binds to the lipo-

some through the hydrophobic interactions[15]. The conclusive two remarks for this experiment are following;

1) the binding sites of c-AMP and estriol to phytochrome are different, (the fromer is a hydrophilic domain and the latter is the chromophore binding site-a hydrophobic domain)

2) both receptors can bind more preferentially to the Pfr form of phytochrome than to the Pr form.

When we combine this result with the one[15] suggesting us that receptor-phytochrome complexes incorporate better into the liposome than the one of free phytochrome, one of the roles of c-AMP and estriol in the photomorphogenic process would be speculated to be the enhancement of phytochrome binding to the membrane by the complexation.

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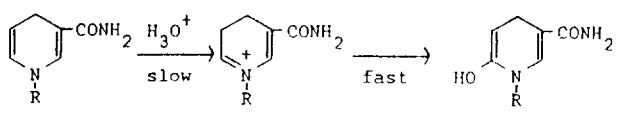
Substituent Effects on the Hydration Reactions of Dihydronicotinamides

Kwanghee Koh Park*, Jae Gug Yoo and Joon Woo Park+

Department of Chemistry, Chungnam National University, Daejeon 300-31; +Department of Chemistry, Ewha Womans University, Seoul 120. *Received March 30, 1987*

Reduced form of nicotinamide adenine dinucleotide (NADH) and its phosphate derivative (NADPH) are coenzymes for many dehydrogenases. In recent years various NAD(P)H model compounds, mainly 1,4-dihydronicotinamides (3-carbamoyl-1,4-dihydropyridines), were utilized in exploring the reactions and mechanisms involving the coenzymes and also in variety of synthetic reactions.[1] Dihydronicotinamides reduce various unsaturated funtionalities, transfer nucleophiles to substrates, and are used for recycling NAD(P)H in NAD(P)H dependent enzyme-catalyzed organic synthesis.[2] Meanwhile, dihydronicotinamides as well as NAD(P)H are known to be unstable in acidic medium and undergo acid catalyzed hydration reaction.[3-9]



1 **2** **3**

This deters the effectiveness of the compounds in various applications in organic reactions. In this communication we wish to report the results of kinetic studies on the hydration reactions of 1-aryl-1,4-dihydronicotinamides, which show great sensitivity of the reaction rate on the nature of 1-substituents.

1-aryl-1,4-dihydronicotinamides were prepared by reduction of the corresponding 1-aryl-3-carbamoylpyridinium salts, which were obtained by reaction between 1-(2,4-dinitrophenyl)-3-carbamoylpyridinium salts with the corresponding aniline derivatives as described elsewhere.[10] Kinetic studies were performed in 2% 2-propanol-water medium containing a desired HCl concentration (5×10^{-5}M ~0.1 M) depending on the substituents of dihydronicotinamides. The reactions were followed by decrease in the absorbance of the characteristic absorption of dihydronicotinamides at 345-365 nm. The reactions were first order with respect to both the substrate and H^+.[11] This is in good agreement with the results on other dihydronicotinamides.[7,8] The second order rate constants k_H are summarized in Table 1.

It is evident from Table 1 that k_H becomes greater as the substituent at 1-position of dihydronicotinamide has greater electron-donating power. This agrees well with the conclusion that protonation of 1,4-dihydronicotinamide is involved in the rate-determining step[6-8], since the formation of iminium salts **2** would be more easily formed as electron density on the ring nitrogen is greater.

To correlate the hydration rate constants with character of 1-substituents of the dihydronicotinamides, the Hammett plots were made in Figure 1. The plots of log k_H for the hy-