Analysis of Autophosphorylating Kinase Activities of Arabidopsis and Human Cryptochromes†

Sezgin Özgür and Aziz Sancar*

Department of Biochemistry and Biophysics, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27599

Received August 1, 2006; Revised Manuscript Received September 5, 2006

ABSTRACT: Cryptochromes are FAD-based blue-light photoreceptors that regulate growth and development in plants and the circadian clock in animals. *Arabidopsis thaliana* and humans possess two cryptochromes. Recently, it was found that *Arabidopsis* cryptochrome 1 (AtCry1) binds ATP and exhibits autokinase activity that is simulated by blue light. Similarly, it was reported that human cryptochrome 1 (HsCry1) exhibited autophosphorylation activity under blue light. To test the generality of light stimulated kinase function of cryptochromes, we purified AtCry1, AtCry2, HsCry1, and HsCry2 and probed them for kinase activity under a variety of conditions. We find that AtCry1, which contains near stoichiometric amounts of FAD and human HsCry1 and HsCry2 (which contain only trace amounts of FAD), has autokinase activity, but AtCry2, which also contains stoichiometric amounts of FAD, does not. Finally, we find that the kinase activity of AtCry1 is not significantly affected by light or the redox status of the flavin cofactor.

Cryptochrome/photolyase family enzymes are flavoproteins that contain flavin adenine dinucleotide (FAD[1]) as the catalytic cofactor and are known or presumed to have a folate cofactor in the form of methenyltetrahydrofolate as the nonessential photoantenna chromophore (*1*−*3*). In rare cases, the second chromophore might be 8-hydroxy-5-deazariboflavin or FMN. The reaction mechanism of the photolyase members of the family is reasonably well understood: These enzymes repair UV-induced DNA damage by photoinduced electron transfer (*3*). In contrast, the reaction mechanism of cryptochromes either in plants or in animals is not known. Cryptochromes have been most extensively studied in *Arabidopsis thaliana*, *Drosophila melanogaster*, and mammals. *Arabidopsis* contains two cryptochromes, AtCry1 and AtCry2 (*2*). Another member of the family initially thought to be a cryptochrome and named AtCry3 (*4*, *5*) is now known to be a single strand specific DNA photolyase (*6*). Humans also have two cryptochromes named HsCry1 and HsCry2 (*7*). It should be noted, however, that the designation of cryptochrome 1 and 2 reflect the order of discovery in both *Arabidopsis* and humans, and consequently, HsCry1 and HsCry2 are not the mammalian counterparts of AtCry1 and AtCry2; they exhibit high sequence similarity to one another and are diverged equidistantly from the plant cryptochromes (*8*). The photochemical basis of cryptochrome photoreception is not known. In *Arabidopsis*, it has been found that AtCry1 and AtCry2 interact with a number of proteins including the E3 ubiquitin ligase COP1 (*9*, *10*) and that through these interactions they mediate blue-light induced gene activation (*9*, *10*). Light effects on *Arabidopsis* cryptochromes include conformational change of AtCry1 in vitro (*11*), nucleocytoplasmic shuttling of AtCry1 in vivo (*12*), proteolytic degradation of AtCry2 (*13*, *14*), and phosphorylation of both AtCry1 (*15*) and AtCry2 (*16*) in vivo. In contrast to the wealth of information available on the photobiological properties of *Arabidopsis* cryptochromes, there is at present only indirect evidence for a photoreceptive function of mammalian cryptochromes, and a widely held opinion is that the mammalian cryptochromes function solely as light-independent regulators of the circadian clock (*9*).

Against this background, recently, some potentially significant discoveries were made that may have bearing on the understanding of the cryptochrome photocycle. First, the crystal structure of the photolyase homology region (PHR) of AtCry1 was solved (*17*). The structure is very similar to that of *E.coli* photolyase with the notable absence of the positively charged DNA binding groove in AtCry1. Second, it was found that AtCry1 binds ATP stoichiometrically (*17*, *18*) and that the ATP is located in the active site cavity (*17*), which in the photolyase−DNA complex is occupied by the cyclobutane pyrimidine dimer. Third, and perhaps of greater functional significance, it was found that purified AtCry1 exhibited autokinase activity that was greatly stimulated by blue light (*15*, *18*). Moreover, it was reported that the kinase activity was dependent upon the presence of FAD in AtCry1 and that HsCry1 also exhibited similar light-activated autokinase activity (*18*). Finally, it was reported that the photoreduction of FAD by intraprotein electron transfer from an adjacent Trp residue was essential for light stimulation of AtCry1 autokinase (*19*). In light of these findings, we purified AtCry1, AtCry2, HsCry1, and HsCry2 and inves-

† This work was supported by NIH Grant GM31082.

* To whom correspondence should be addressed. Tel: 919-962-0115. Fax: 919-843-8627. E-mail: Aziz_Sancar@med.unc.edu.

[1] Abbreviations: FAD, flavin adenine dinucleotide; AtCry, Arabidopsis cryptochrome; HsCry, human cryptochrome; FMN, flavinmononucleotide; ATP, adenosine triphosphate; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; Tris-HCl, tris-(hydroxymethyl) aminoethane; BME, β-mercaptoethanol; ITC, isothermal titration calorimetry; DTT, dithiotreitol; Hepes, *N*-(2-hydroxyethyl) piperazine-*N*′-2-propanesulfonic acid; μCi, microcurie.

10.1021/bi061556n CCC: $33.50
Published on Web 10/17/2006

tigated their autokinase activities. We find that AtCry1, HsCry1, and HsCry2 have kinase activities, but AtCry2 does not. We also find that FAD is not required for kinase activity, that under our experimental conditions light does not stimulate cryptochrome kinase, and that the kinase activities of AtCry1 containing oxidized or reduced FAD are indistinguishable.

MATERIALS AND METHODS

Construction of Baculovirus Cryptochrome Vectors. The cDNA clones of AtCry1, HsCry1, and HsCry2 have been described before (*20*, *21*). All *Arabidopsis* and human cryptochrome genes were cloned into the baculovirus pFastBacHTa vector. The cDNA of the AtCry2 gene (*13*) was obtained from Dr. Chentao Lin (University of California, Los Angeles). All four constructs have a 6X His tag at their *N*-termini. The corresponding viruses were produced in Sf21 insect cells according to the manufacturer's procedures (Invitrogen). The integrity of the genes was confirmed by DNA sequencing. The baculoviruses were amplified in Sf21 insect cells and had approximate titers of 1×10^9 pfu/mL.

Purification of the Recombinant Cryptochromes. To purify cryptochromes, 250−300 mL of Sf21 cells (1×10^6 cells/mL) were inoculated with the appropriate baculovirus at MOI = 10 and incubated at 27 °C for 48 h. Then, the cells were spun down at 2000 rpm in a Sorvall SS34 rotor for 10 min and washed once with cold phosphate buffered saline (PBS) solution. The cell pellet was frozen and kept at −80 °C until further use. The proteins were purified using affinity chromatography according to the manufacturer's protocol (Qiagen). Briefly, the cells were lysed in a lysis buffer (10 mM Tris-HCl at pH 7.4, 130 mM NaCl, 10 mM β-mercaptoethanol (BME), and 0.5% NP-40) for 30 min on ice. The cell lysate was spun down at 15000 rpm in a Sorvall SS34 rotor at 4 °C for 30 min. The supernatant (adjusted with lysis buffer to 10−15 mg/mL protein) was incubated with 0.5 mL of NTA resin (Qiagen) equilibrated with lysis buffer at 4 °C for at least 2 h. The resin was washed five times with 13 mL of wash buffer (10 mM Tris-HCl at pH 7.4, 300 mM NaCl, 20 mM imidazole at pH 8.0, 10 mM BME, and 10% Glycerol). The cryptochromes were eluted in 3 mL of elution buffer (10 mM Tris-HCl at pH 7.4, 130 mM NaCl, and 250 mM imidazole at pH 8.0). The purity of the proteins was checked by SDS−PAGE and Coomassie blue staining. The proteins were dialyzed against a storage buffer (20 mM Tris-HCl at pH 7.4, 130 mM NaCl, and 50% Glycerol) and stored at −20 °C until further use. The protein concentrations and flavin content were determined by absorption spectroscopy using the following molar extinction coefficient: AtCry1 = 154000, AtCry2 = 147000, HsCry1 = 123200, HsCry2 = 121800 M^{-1} cm^{-1} at 280 nm and FAD = 11400 M^{-1} cm^{-1} at 440 nm. The proteins were denatured in 6 M guanidine-HCl for absorbance measurements to avoid errors introduced by aromatic interactions within the native proteins. In addition, in calculating the concentrations of *Arabidopsis* cryptochromes from absorbance at 280 nm, the significant contribution of the flavin cofactor to absorbance at this wavelength (27346 M^{-1} cm^{-1}) was taken into account. The human cryptochromes contained no or a negligible amount of FAD, and hence, no such correction was needed.

Spectroscopy. The absorption spectra were recorded with a Shimadzu UV-1601 spectrophotometer. The amount of flavin in human cryptochromes was estimated by fluorescence spectroscopy. The cryptochromes were denatured by boiling for 5 min in 0.8% sodium dodecyl sulfate in 0.1 N HCl. Then, emission spectra were obtained for excitation at 440 nm using a Shimadzu RF5000U spectrofluorometer. The concentration of the released FAD was estimated from a standard curve generated using FAD solutions of known concentrations.

Micro Isothermal Titration Calorimetry. The equilibrium binding constants of AtCry1 and AtCry2 with ATP were determined by isothermal titration calorimetry (ITC). A solution containing 6−22 μM AtCry was dialyzed overnight against an ITC buffer (50 mM Tris-HCl at pH 7.4, 100 mM NaCl, 1 mM $MgCl_2$, and 0.5 mM DTT). ATP was dissolved in the same buffer. The concentrations of cryptochromes and ATP were determined from the absorption spectra using a molar extinction coefficient of 181346 M^{-1} cm^{-1} for AtCry1, 174350 M^{-1} cm^{-1} for AtCry2 at 280 nm, and 14300 M^{-1} cm^{-1} at 260 nm for ATP. The experiments consisted of a series of injections of 5 μL of ATP (180−380 μM) into a cell containing 2.5 mL of protein at 26° C and measuring heat changes in the chamber as a result of the AtCry1−ATP interaction using a MicroCalVP-ITC. The association constant derived from these data were calculated using SigmaPlot.

Autophosphorylation. Cryptochrome phosphorylation was done as described previously (*18*). Unless stated otherwise, the reaction mixture (20 μL) contained 50 mM Tris-HCl at pH 7.4, 5 mM $MgCl_2$, 100 μM ATP (plus 1 μCi of γ-^{32}P-ATP (3000 Ci/mmol)), 10 mM BME, and 3 μg of cryptochrome. Unless otherwise stated, the reaction was carried out at 23° C for 1 h under yellow light from general electric "Gold" fluorescent light bulbs to prevent an uncontrolled blue-light effect. To determine the potential effect of light on Cry kinase activity, the experiment was done as follows. A reaction master mix was prepared and divided in two sets with a final concentration as described above. One set was wrapped in aluminum foil, and the other remained uncovered with the lids open. The tubes were then exposed to 366 nm light from a Sylvania F15T8/BLB battery at a fluence rate of 31 μmol cm^{-2} s^{-1} for the indicated times. The light passes through a heat-absorbing filter as well as two glass plates to prevent heating and to cut out shorter wavelengths and, thus, eliminate artifacts that may arise from uncontrolled heating or exposure to UV light. The reaction was stopped by the addition of 2X SDS−PAGE loading dye and boiling for 5 min. The protein was separated on 10% SDS−PAGE and analyzed by Coomassie staining and phosphoimaging. Quantitative analysis of phosphorylation was carried out by densitometry using ImageQuant 5.0 software (Molecular Dynamics).

Kinase Assay with Chemically Reduced Cryptochrome. To 100 μL of AtCry1 at 20 μM in 50 mM Hepes at pH 7.5, 5 mM $MgCl_2$, and 10 mM BME in an anaerobic cuvette, sodium dithionite was added to 20 μM. The cuvette was placed on ice, and ATP was added to 10 μM plus 2 μCi of γ-^{32}P-ATP (3000 Ci/mmol). The cuvette was sealed with a rubber cap and flushed with Argon gas for 5 min. Then, the cuvette was placed sideways and incubated in the dark or exposed to 366 nm light for 15 min at 31 μmol cm^{-2} s^{-1}. Then, the reaction product was processed as in the standard autophosphorylation assay. The reaction with preilluminated

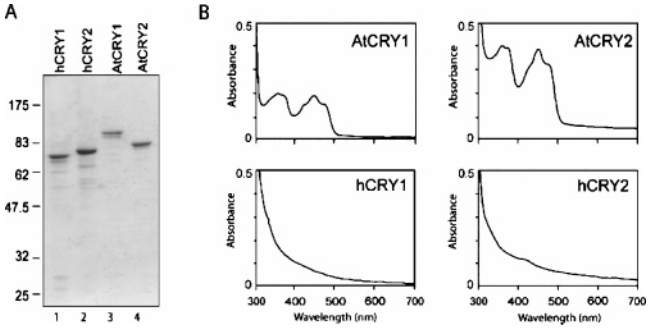

FIGURE 1: Purification and spectroscopic properties of human and *Arabidopsis* cryptochromes. All four cryptochromes were purified from Sf21 cells infected with appropriate baculoviruses and were purified as 6X His tagged proteins using nickel resin. (A) Analysis of purified proteins on 10% SDS−PAGE followed by Coomassie blue staining. Each lane contained about 3 μg of protein. The numbers on the left margin indicate the positions of the molecular weight standards. (B) Absorption spectra of cryptochromes in the near-UV/vis region. The concentrations of the proteins were in the range of 20−40 μM.

AtCry1 was conducted in a similar manner except that the reaction buffer contained 5 mM dithiothreitol instead of mercaptoethanol. Preillumination was carried out on ice for 5 min with 31 μmol cm^{-2} s^{-1} of 366 nm light. Then ATP was added, and the kinase reaction was carried out at 27 °C in the absence or presence of light.

RESULTS

Purification and Spectroscopic Properties of Arabidopsis AtCry1 and AtCry2 and Human HsCry1 and HsCry2. To investigate the kinase activity of *Arabidopsis* and human cryptochromes and the effects of flavin cofactor and light on this activity, we expressed all four proteins in a baculovirus/insect cell system and purified them by affinity chromatography. From 300 mL of insect cells, we obtained at least 1 mg of the appropriate proteins at >95% purity (Figure 1A). The *Arabidopsis* cryptochromes exhibited bright yellow color like most flavoproteins, whereas the human cryptochromes, at comparable or higher concentrations, were colorless. In agreement with the visual observation, AtCry1 and AtCry2 have absorption spectra in the near-UV/visible wavelength range typical of oxidized flavin with vibrational fine structure as seen in most flavoproteins (Figure 1B). In contrast, HsCry1, at the highest concentrations available to us, had no absorption band in the near-UV/visible wavelength, indicative of flavin, and HsCry2 exhibited a minor shoulder in the 410−420 nm region superimposed on the tail absorption of the apoprotein (Figure 1B). From the extinction coefficients of the apoproteins at 280 nm and FAD at 440 nm, we calculated that the stoichiometries of FAD-to-apoprotein in various preparations of AtCry1 and AtCry2 were in the range of 0.7 to 0.9. In contrast, the absorption of the HsCry1 and HsCry2 preparations in the near-UV/visible region was too low for an accurate determination of flavin content in these proteins. To obtain approximate estimates, we denatured the human cryptochromes and measured fluorescence at 520 nm with 440 nm excitation. From the fluorescence measurements, we estimated <0.1% FAD in HsCry1 and ~0.2% in HsCry2.

ATP Binding of Cryptochromes. It has been shown previously that AtCry1 binds ATP with 1-to-1 stoichiometry (*17*, *18*), and the crystallographic structure of AtCry1 immersed in an AMP-PNP solution revealed that the nucleotide analog was inserted into the cavity leading to the FAD, in a manner similar to the binding of the cyclobutane pyrimidine dimer to photolyase (*17*). We wished to know if ATP binding was a general property of the cryptochromes. We measured cryptochrome−ATP binding by isothermal titration calorimetry (ITC). The results obtained with AtCry1 and AtCry2 are shown in Figure 2. From the data, we calculated that AtCry1 binds ATP with a *K*d = 4.2 μM and a stoichiometry of 0.9 ATP to 1.0 AtCry1 enzyme, values that are in reasonable agreement with those published previously (*17*, *18*). For AtCry2, we obtained a *K*d = 0.9 μM and a stoichiometry of 0.6 ATP to 1.0 AtCry2. On the basis of the structural and evolutionary considerations of

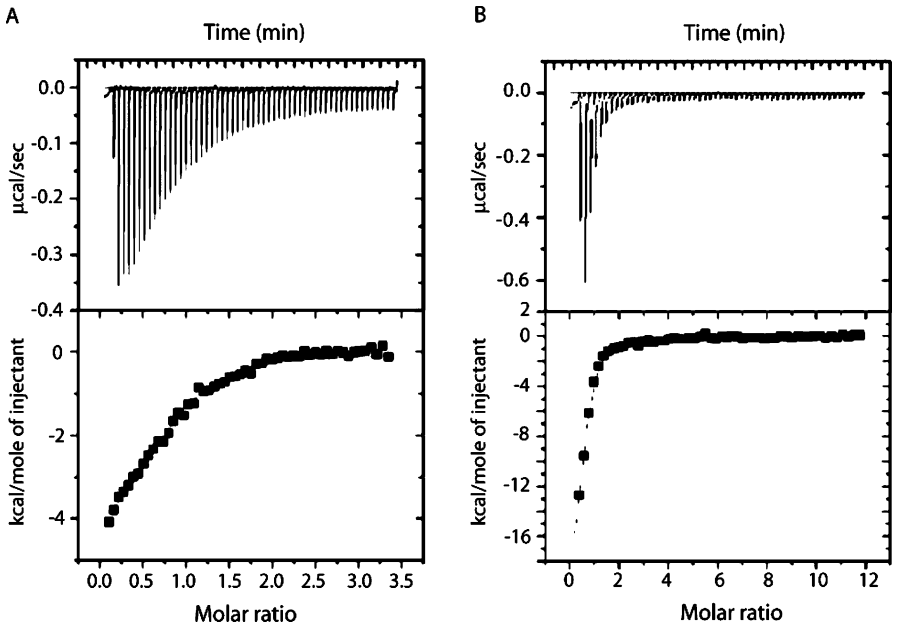

FIGURE 2: Binding of AtCry1 and AtCry2 to ATP analyzed by isothermal titration calorimetry. (A) ITC data for AtCry1. (B) ITC data for AtCry2. The top panels show time evolution, and the bottom panels show the heat released by each injection in terms of kcal/mol of ATP.

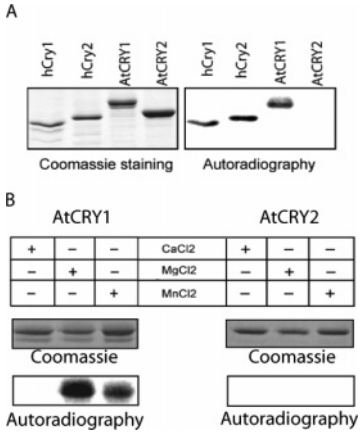

FIGURE 3: Autophosphorylation of *Arabidopsis* and human cryptochromes. (A) Kinase assays with cryptochromes. The reactions were performed in the dark at 23° C. Left panel: Coomassie blue stained SDS−PAGE of cryptochromes after kinase reactions. Right panel: Autoradiography of the gel shown on the left panel. (B) Effect of divalent cations on *Arabidopsis* cryptochrome kinase activity. The kinase assays were conducted in the presence of the indicated divalent cations, and the proteins were separated on SDS−PAGE and analyzed by Coomassie blue staining and autoradiography, as indicated.

AtCry1 and AtCry2, we suspect that both proteins bind ATP with one-to-one stoichiometry and that the range of values we obtained is a reflection of the resolution limit of ITC. We were unable to conduct ATP-binding experiments with human cryptochromes by ITC because at the high protein concentrations necessary to conduct the experiments, the addition of $MgCl_2$ to the protein sample caused extensive aggregation. Moreover, we encountered similar problems in our attempts to measure the binding of human cryptochromes to ATP by the method of Hummel and Dreyer (*17*). Therefore, at present, no statement can be made regarding the binding of human cryptochromes to ATP on the basis of equilibrium-binding experiments alone. However, the kinase assays detailed below show that human cryptochromes bind ATP.

Autokinase Activity of Cryptochromes. It has been reported that both AtCry1 (*15*, *18*) and HsCry1 (*18*) have autokinase activities. To find out whether kinase is a general property of cryptochromes, we tested AtCry1, AtCry2, HsCry1, and HsCry2 for autophosphorylation. The results shown in Figure 3A were quite unexpected. AtCry1 and HsCry1 exhibited kinase activities as reported and so did HsCry2; however, AtCry2 under our assay conditions lacked kinase activity. We reasoned that the failure of AtCry2 to autophosphorylate may have been a consequence of special divalent cation requirements, and therefore, we tested AtCry2 for autokinase activity in the presence of Ca^{++} and Mn^{++}, in addition to Mg^{++}. We used AtCry1 as a positive control in these assays. As is evident in Figure 3B, AtCry1 exhibits optimal activity with Mg^{++}, about 30% of the optimal activity in the presence of Mn^{++}, and no activity with Ca^{++} as the divalent cation. In contrast, AtCry2 failed to show any kinase activity under all conditions tested, and therefore, we conclude that this protein lacks kinase function.

Effect of Light and the Redox Status of FAD on the Kinase Activity of Cryptochromes. The human cryptochromes contain either no falvin or a trace amount of flavin and, therefore, were not expected to be affected by light. Indeed, light had no effect on the autokinase activity of HsCry1 and HsCry2

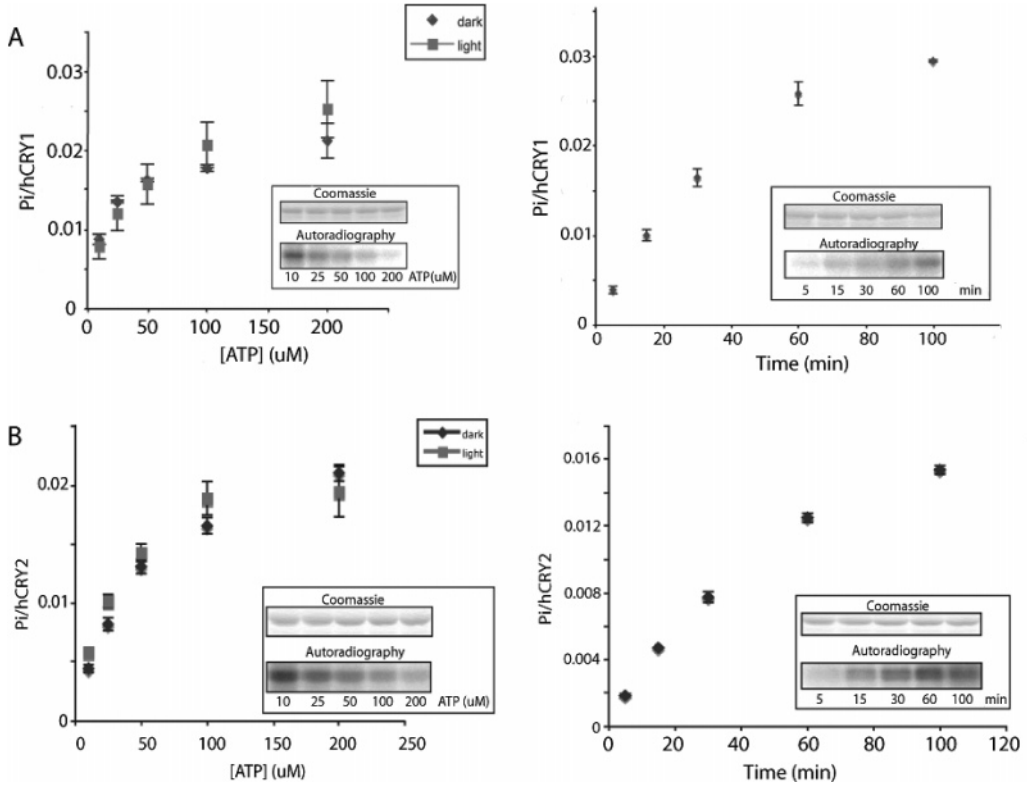

FIGURE 4: Effect of ATP concentration and light dose on the autophosphorylation of human cryptochromes. (A) HsCry1. (B) HsCry2. Left panels: Kinase assays conducted in the absence and presence of light as indicated. Right panels: kinetic reactions performed in the presence of 100 μM ATP and 31 μmol cm^{-2} s^{-1} of 366 nm light. The bars indicate standard errors of three experiments. The insets show Coomassie blue stained gels and the autoradiographic image of one of the experiments from which the graphs were generated.

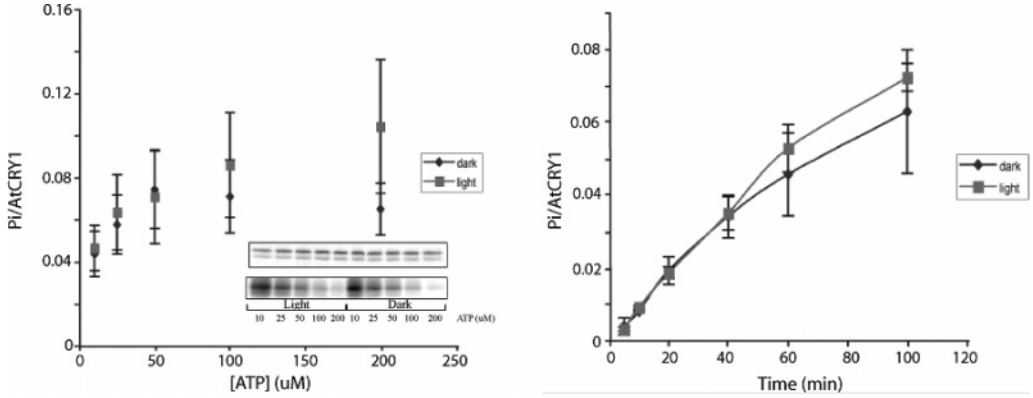

FIGURE 5: Effect of light on the autokinase activity of AtCry1. Left panel: Effect of ATP concentration. Inset: the Coomassie blue stained gel and the autoradiographic image of one of the SDS−PAGE used in the kinase assay. The graph shows the averages of three experiments with standard error. Right panel: Kinetics of autophosphorylation in the presence of 100 μM ATP, either in the dark or under 31 μmol cm^{-2} s^{-1} of light. The averages of three experiments are plotted with bars indicating standard errors.

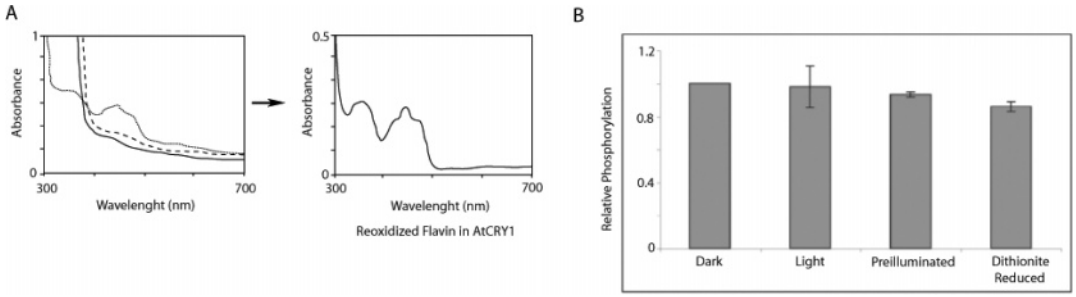

FIGURE 6: Effect of the chemical reduction of FAD and preillumination on the autokinase activity of AtCry1. (A) Spectroscopic analysis of the dithionite reduced enzyme. Left panel: (....) before dithionite reduction; (---) after reduction with equimolar dithionite concentration; (—) after the kinase reaction. Right panel: The reduced sample shown in the left panel after exposure to air reduction. (B) Effect of various treatments on AtCry1 autokinase activity. The kinase assays were performed either in the dark or under light of 31 μmol cm^{-2} s^{-1} for 15 min after being subjected to the indicated treatments. The radiolabel incorporation in the dark sample was taken as unity, and the others were expressed relative to this value. The error bars indicate the standard errors of several experiments.

and under conditions of saturating concentration of ATP, HsCry1, and HsCry2 incorporated about 0.03 mol Pi per mol and about 0.02 mol Pi per mol of protein, respectively, irrespective of light (Figure 4).

Surprisingly, light had no effect or only a minor effect on the autophosphorylation of AtCry1 as well (Figure 5). At the highest ATP concentration and light dose used in our experiments, there was a trend for higher autokinase activity under light; however, the difference even under these conditions was not statistically significant (Figure 5). We do not know the reason for the discrepancy between our result and those from previous reports (*15, 18*). However, under all reaction and illumination conditions employed, we were unable to observe a reproducible effect of light on the autokinase activity of AtCry1.

Finally, we examined the effect of the redox status of FAD on the kinase activity of AtCry1. A previous study found that preillumination of the protein, which reduces the flavin cofactor (*19*), increases the subsequent light stimulated kinase activity of the pigment (*18*). Conversely, it was reported that the presence of I_2, which quenches the excited state, and H_2O_2, which oxidizes the flavin, abolished the light stimulation of the kinase activity (*18*). We attempted to reproduce these results but were unable to observe any effect of preillumination on AtCry1 autokinase activity. We considered the possibility that our preillumunation regimen may have not been optimal for reducing the flavin cofactor and activating the kinase. Thus, we decided to reduce the flavin chemically and test AtCry1 containing reduced flavin. Figure 6 shows the results of these efforts. In Figure 6A, it is evident that sodium dithionite quantitatively reduces the FAD cofactor (dashed line) and that the cofactor remains reduced for the duration of the kinase assay (solid line). Moreover, when the enzyme is re-exposed to oxygen, the flavin is reoxidized to FAD_{ox} with the characteristic vibrational fine structure typical of enzyme bound flavin, indicating that the dithionite treatment did not adversely affect the enzyme (Figure 6A, right panel). In Figure 6B, we present the extent of AtCry1 autophosphorylation under a variety of conditions. As is apparent from this figure, neither light exposure nor preillumination of the enzyme followed by light exposure during the kinase reaction increased the level of phosphorylation. Importantly, using the enzyme with reduced flavin did not improve the kinase activity. Thus, taken together, our data indicate that not all cryptochromes have kinase activity and that of those that do have kinase activity, FAD is not required for the kinase function; for those that do contain FAD, the kinase activity is not affected by the redox status of the cofactor, nor is it stimulated by light.

DISCUSSION

Autophosphorylation is a common property of virtually all known photosensory pigments (*9*). However, its physiological role is not clear in the majority of cases. Of special relevance is recent work that has shown that the autokinase activity of phytochrome is dispensable for its function (*22*). Similarly, the autokinase activity of phototropin has no obvious effects on the phototropin photocycle (*23*).

It has been previously shown that AtCry1 is phosphorylated by PhyA in vitro and that phosphorylation was induced by red light in vivo but suppressed by far-red, consistent with it being phosphorylated by phytochrome A (*14*). In contrast, AtCry2 is hyperphosphorylated in seedlings exposed to blue light but not red light, suggesting that AtCry2 phosphorylation was mediated by a blue-light photoreceptor (*16*). More recently, it was reported that AtCry1 (*15*, *18*) and HsCry1 (*18*) carried out autophosphorylation in vitro and that the reaction was strongly stimulated by blue light. Because the only chromophore in cryptochromes purified from heterotropic sources is FAD (*1*−*3*), it was naturally assumed that FAD was the chromophore for the light stimulation of cryptochrome autokinase activity.

In the present study, we confirm that AtCry1 and HsCry1 are indeed autokinases. We also find that HsCry2 is also an autokinase. Surprisingly, however, AtCry2, which is known to be phosphorylated upon light exposure in vivo (*16*), lacked kinase activity. Furthermore, contrary to earlier reports (*18*, *19*), we find no correlation between the presence of FAD and autokinase activity, nor do we observe any effect of blue light on the kinase activity of AtCry1, which contains near stoichiometric amount of FAD. Finally, it was reported that photoreduction of AtCry1 by illuminating with blue light increased AtCry1 kinase activity and that nonphotoreducible mutants failed to exhibit such an effect, and therefore, it was concluded that the photoreduction was the primary photophysical reaction in AtCry1 (*19*). We not only fail to observe any increase in AtCry1 kinase activity under blue light but also fail to observe any measurable change in activity when the flavin of AtCry1 is chemically reduced by dithionite. It should be noted that the nonphotoreducible mutants of AtCry1 exhibited nearly 5-fold diminished kinase activity even in the dark (*19*), suggesting that the introduced mutations possibly caused an overall structural change in the enzyme that resulted in reduced activity independent of light. Clearly, further work is needed to understand the significance of the kinase activity in cryptochrome functions both in plants and in mammals and to clarify the cause of contradictory findings regarding the requirement of flavin for kinase activity and the effect, or the lack thereof, light on the cryptochrome autokinase activity.

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BI061556N