Deprotonated 2,3-Dihydrobilindiones—Models for the Chromophore of the Far-Red-Absorbing Form of Phytochrome

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Abstract: Chromophore anions of synthetic 2,3-dihydrobilindiones can serve as models for the chromophore of the far-red-absorbing form of phytochrome because of the strong resemblance of their UV/Vis spectra. Significant red shifts of their long-wavelength maxima result from the elongation of the chromophores by deprotonation and the donor–acceptor reversal upon the conversion of the ring A lactam (N→C=O) into the lactim anion (N=C←O$^-$). The best resemblance was observed in the case of a 15-*E* configured chromophore anion. ^{13}C NMR spectroscopic investigations demonstrated that changes in chromophore structure are caused not only by regioselective deprotonation of the 2,3-dihydrolactam moiety of ring A, but also by the subsequent dipyrrin tautomerization within rings B and C. Generally, the chromophore structure can be interpreted in terms of an oxygen-donor/oxygen-acceptor-substituted polyene. The remarkable red shift of the long wavelength maximum results from the strong donor power of the oxygen atom on ring A, where the negative charge is localized. A simple model can be proposed for the interconversion of the structurally different chromophores of the red-absorbing form (Pr) and the far-red-absorbing form (Pfr) of phytochrome, based on oppositely charged chromophore ions of different geometry and stability: a stable 15*Z*-configured cation for physiologically inactive Pr and a labile 15*E*-configured anion for physiologically active Pfr. Interconversion requires only two types of reactions, namely *Z*/*E* photoisomerization and proton transfer.

Keywords: chromophores · isomerizations · photochemistry · phytochrome · tautomerism

Introduction

Phytochromes are the photoreceptors of photomorphogenesis, regulating growth and development of green plants by light-induced interconversion of two distinct forms: the red-absorbing form (Pr) and the far-red-absorbing form (Pfr) named after the different positions of their long-wavelength absorption maxima around 660 nm and 730 nm.[1] Irradiation with light of those wavelengths initiates Pr/Pfr interconversion necessary for the activation or the blocking of signaling pathways.[2] Thus, the physiological activity of phytochromes in plants is triggered photochemically and depends on the light available under the prevailing conditions.

Structurally, phytochromes are homodimers of biliproteins. Their chromophores belong to the family of 2,3-dihydrobilindiones,[3] which are also photoactive in the light-harvesting phycobiliproteins phycocyanin and allophycocyanin. The structure of the Pr chromophore is similar to that of phycocyanin chromophores, which is known in detail from crystal structure analysis.[4] Covalently attached to the protein by a thioether bond, the Pr chromophore is probably fixed by ion pairs in an extended conformation in the protonated state (Figure 1). Transformation to the Pfr chromophore is initiated by a *Z*→*E* photoisomerization of the double bond exocyclic to ring D followed by a sequence of reactions in the ground state, often called dark reactions. The formation of intermediates was determined spectroscopically, but their structures and that of the Pfr chromophore remain largely unknown.[5]

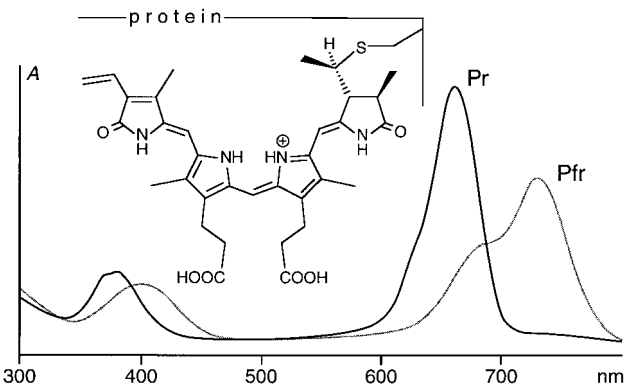

Figure 1. Absorption spectra of Pr and Pfr; structural formula of the Pr chromophore in the extended conformation.

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In this study, we report on the deprotonation of 2,3-dihydrobilindiones and on the structure of their anions, and demonstrate that the structural change of the chromophores leads to absorption spectra similar to those of the Pfr chromophore in the long-wavelength region. In spite of the different conformations between our helical model compounds dissolved in organic solvents and the extended geometry of the chromophores bound to the protein there is good agreement. Based on NMR and UV/Vis data we present a possible reaction scheme for the dark reactions of the Pr chromophore, including a structural proposal for the Pfr chromophore.

Results and Discussion

Deprotonation of 2,3-dihydrobilindiones is accompanied by a remarkable red shift of the long-wavelength absorption band.[6] In the case of the synthetic model compound **1** the absorption maximum is shifted bathochromically by 202 nm (λ_{max}: 582 [**1**], 784 nm [**1**$^-$]) with tetrahydrofuran (THF) as solvent and tetra-*n*-butylammonium fluoride (TBAF) as base[7] (Figure 2a). This shift value exceeds all others that have ever been observed upon chemical modification of the chromophore, such as protonation, complexation, or transformation into iminoesters.[8] For instance, the shift value for the ring A methyliminoester **2** is only 86 nm (λ_{max} 668 nm [**2**]). In spite of such a difference, however, the long-wavelength absorption bands of **1**$^-$ and **2** are of the same type, suggesting that the structure of the chromophores is similar. Their structures can be related to a hypothetical tautomer of **1**, resembled by the chromophore structure **II**, made by two independent tautomerizations: dipyrrin tautomerization of rings B and C and lactam–lactim tautomerization of ring A (Figure 2b). A tautomer like this is not stable per se in solution. However, its chromophore can be fixed, as shown in **2**, substituting the kinetically mobile proton of the lactim hydroxy group by a methyl carbon. In this context, the structure of **1**$^-$ simply results from deprotonation of the lactim hydroxy group, but strictly speaking the corresponding formula must be regarded as a reasonable resonance structure. The proof of that structure is based on NMR spectroscopic investigations complemented by an interpretation of the absorption spectra.

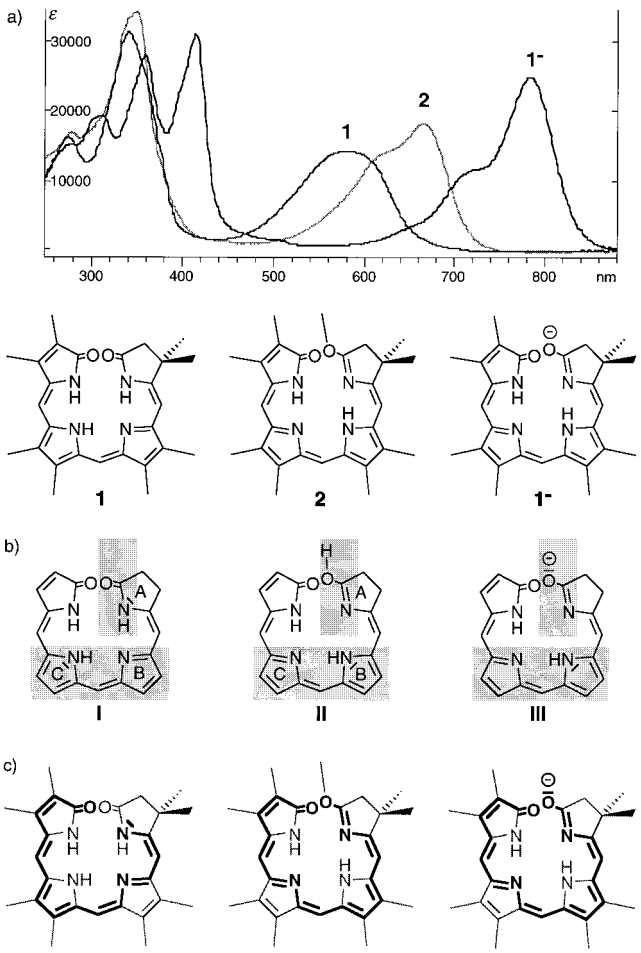

Figure 2. a) UV/Vis spectra of **1**, **2**, and **1**$^-\cdot$TBDH$^+$ in THF. b) Correlation of the chromophore structures by hypothetical tautomerization (**I**→**II**) and deprotonation (**II**→**III**). c) π-electron systems polarized in the course of the long-wavelength transition of **1**, **2**, and **1**$^-$.

Structure of deprotonated 2,3-dihydrobilindiones: Deprotonation of **1** with equivalent quantities of the bicyclic guanidine base 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD)[9] instead of TBAF was advantageous in all NMR experiments for two

Abstract in German: *Die Chromophor-Anionen von 2,3-Dihydrobilindionen wurden wegen der Ähnlichkeit ihrer UV/Vis-Spektren als Modelle für den Chromophor des Phytochroms in der physiologisch aktiven Far-red-Form spektroskopisch untersucht. Die charakteristischen Rotverschiebungen der langwellig gelegenen Absorptionsmaxima resultieren aus der Verlängerung der Chromophore durch Deprotonierung und der Donor-Acceptor-Umkehr beim Übergang vom Ring A Lactam (N→C=O) zum Lactim-Anion (N=C←O$^-$). Die beste Übereinstimmung wurde mit einem 15E-konfigurierten Chromophor-Anion beobachtet. ^{13}C-NMR-spektroskopische Untersuchungen ergaben, daß die Änderung der Chromophorstruktur nicht nur auf der regioselektiven Deprotonierung des Ring-A-Lactams, sondern auch auf der nachfolgenden Dipyrrin-Tautomerie der Ringe B und C beruht. Allgemein entspricht die Chromophorstruktur einem Sauerstoff-Donor/Sauerstoff-Acceptor substituierten Polyen. Die Lokalisierung der negativen Ladung am Ring-A-Sauerstoffatom bedingt dessen hohe Donorstärke und damit auch die ausgeprägte Rotverschiebung. Hinsichtlich der gegenseitigen Umwandlung der strukturell unterschiedlichen Chromophore der beiden Phytochromformen Pr und Pfr kann ein einfaches Modell vorgeschlagen werden, dem gegensätzlich geladene Chromophor-Ionen mit unterschiedlicher Geometrie und Stabilität zugrunde liegen: ein 15Z-konfiguriertes Chromophor-Kation für physiologisch inaktives Pr und ein 15E-konfiguriertes Chromophor-Anion für physiologisch aktives Pfr. Die Chemie der gegenseitigen Umwandlung benötigt nur zwei Reaktionstypen: Z/E-Photoisomerisierungen und Protonentransferreaktionen.*

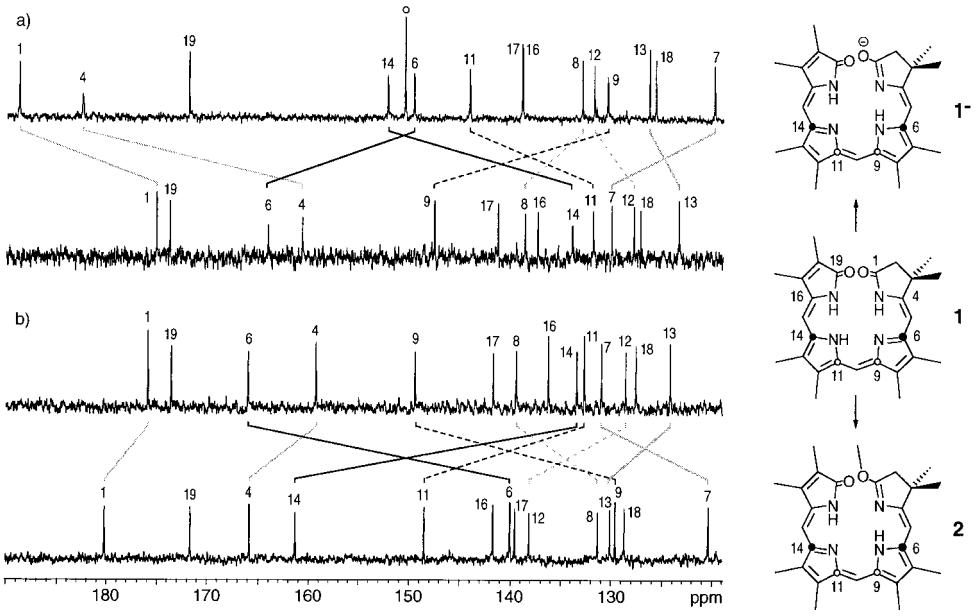

Figure 3. Correlation of ^{13}C NMR signals in the region of the quaternary carbons of a) $\mathbf{1}^-$ and **1** in [D_6]DMSO and b) **1** and **2** in $CDCl_3$. The signal of the guanidinium carbon of TBD · H^+ is indicated by ○.

reasons. First, the long-term stability of $\mathbf{1}^-$ in solution necessary for all 2D NMR techniques was achieved in this way as oxidative degradation was retarded. Second, clear spectra without signal overlap of TBD · H^+ and $\mathbf{1}^-$ were obtained and interpreted more easily in terms of structure and stereochemistry. In particular, all quaternary carbons of $\mathbf{1}^-$ were assigned by gradient-enhanced heteronuclear multiple bond correlation (HMBC) and correlated to those of **1** and **2** (Figure 3, Table 1). As for the shift differences of corresponding signals on the transformation of **1** into $\mathbf{1}^-$ and **1** into **2**, it is evident that all of these differences are equal in sign and comparable in value; this indicates the same type of chromophore for $\mathbf{1}^-$ and **2**.

Compared with the effect on **2**, the influence of the negative charge on $\mathbf{1}^-$ results in a more effective upfield shift of the C1 and C4 signals. This result implies that deprotonation takes place regioselectively, affecting the lactam proton of ring A. With respect to the four quasisymmetric pairs of carbons within the dipyrrin skeleton (C-6/C-14; C-7/C-13; C-8/C-12; C-9/C-11), the shifting of the signals of each pair in opposite directions is significant in the formation of both $\mathbf{1}^-$ and **2** starting from **1**. Seven out of eight pairs of signals even have their correlation lines crossed, the exception being that of C-8 and C-12 in $\mathbf{1}^-$, where the lines only come close to each other. This finding is in accordance with dipyrrin tautomerization causing the mutual change of pyrrolic and azafulvenic carbons of the rings B and C upon deprotonation or during the formation of ring A iminoesters.

Deprotonation and dipyrrin tautomerization do not change the overall geometry of 2,3-dihydrobilindiones in solution. In the ROESY spectra of **1** and $\mathbf{1}^-$ two clear cross-peaks correlate each of the three methine signals to the two singlets of the adjacent methyl groups, confirming the (all-*Z*,all-*syn*)-geometry. In detail, however, the exchange of the single and the double bonds of the B–C methine bridge blocks the former conformational mobility within the (9*Z*,10*syn*) range and opens a new one around the (10*Z*,9*syn*) geometry. An overall geometry like this is assumed to be stabilized by hydrogen bonds formed by the pyrrolic NH of ring B and the lactamic NH of ring D with the nitrogens of the lactimic ring A and the azafulvenic ring C. In the ^1H NMR spectrum of $\mathbf{1}^-$ the NH signals were not detected, presumably as a result of the fast proton exchange with TBD · H^+, but in **2** both NH signals can be seen slightly broadened in a region typical of hydrogen-bonding protons (δNH = 11.88 [ring B]; 9.46 [ring D]).

Structurally based interpretation of the absorption spectra: The change in chromophore structure upon the conversion of **1** into $\mathbf{1}^-$ resembles that of pH indicators, for instance the phthaleins, where deprotonation initiates a chemical transformation of the chromophore, enlarging the π-electron system and shifting the absorption band bathochromically. With respect to **1** and $\mathbf{1}^-$ this enlargement can be specified as an elongation of the chromophore and has to be interpreted in terms of a donor–acceptor reversal of the ring A lactam (N →C=O) into the lactim anion (N=C ← O^-). The π-electron system polarized in the course of

Table 1. ^{13}C chemical shifts (δ) assigned by HMQC and HMBC spectroscopy.

	1 $CDCl_3$	**2** $CDCl_3$	**1** [D_6]DMSO	$\mathbf{1}^-$ [D_6]DMSO
C1	175.9	180.2	175.1	188.8
C2	44.2	45.1	43.6	46.5
C3	39.5	43.1	39.3	42.0
C3′	29.4	29.3	28.7	28.9
C4	159.3	165.8	160.7	182.5
C5	89.9	97.4	88.6	81.9
C6	166.0	140.0	164.1	149.6
C7	131.0	120.4	131.9	119.9
C7′	9.9	8.9	9.4	8.8
C8	139.5	131.3	138.6	133.0
C8′	9.9	9.5	9.5	9.2
C9	149.4	129.6	147.6	130.4
C10	111.3	115.7	111.9	111.4
C11	132.7	148.5	131.9	144.1
C12	128.6	138.2	127.8	131.8
C12′	9.5	9.9	9.2	9.7
C13	124.2	130.1	123.4	126.3
C13′	9.4	9.7	9.0	9.6
C14	133.5	161.3	134.0	152.2
C15	96.6	97.7	95.7	99.1
C16	136.3	141.7	137.4	138.9
C17	141.7	139.6	141.3	138.9
C17′	10.0	9.5	9.5	9.3
C18	127.6	128.7	127.2	125.7
C18′	8.7	8.7	8.4	8.6
C19	173.6	171.7	173.8	171.9
CH_3–O	–	56.6	–	–

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the long-wavelength electronic transition is shorter in **1** than **1**$^-$ (Figure 2c). The change of the dipole moment in **1** is caused by the fact that the polarization of the π electrons from the nitrogen donor of ring A towards the oxygen acceptor of ring D does not affect the π electrons of the ring A carbonyl group significantly. This can also be seen in the ground state of **1**, where the C=O stretching vibration of ring A is assigned to the band at 1716 cm^{-1}, which is closer to the values observed for ketones than to those for five-membered lactams. In other words, polarization of π electrons by the absorption of red light parallels the direction of the $n \rightarrow \pi^*$ delocalization of the nitrogen lone pair on ring A towards the carbonyl group of ring D in the ground state. In comparison, the chromophore of **1**$^-$ is elongated by the inclusion of the deprotonated hydroxyimino moiety of ring A. Therefore, polarization of the π electrons covers the entire chromophore from the donor oxygen of ring A to the acceptor oxygen of ring D, causing a remarkable bathochromic shift. Basically, this oxygen-donor/oxygen-acceptor concept is the same for the methyliminoester **2**, but the lower donor power of its methoxy group obviously results in a smaller bathochromic shift than in **1**$^-$, where the donor power is markedly increased by the negative charge.

Deprotonation of the (2*R*,3*R*,3′*R*,Cys*R*) configured compound **3**, which was synthesized as an optically pure model for the phytochrome chromophore, is accompanied by a clear reversal of the Δε sign in the CD spectra (Figure 4). Inversion of the chromophore helix from the *M* to the *P*-helix can be deduced by using the helicity rule, generally applied for (all-*Z*,all-*syn*)-bilindiones.[10] The signs of the Δε values are also in accordance with the CD spectrum of a similarly configured phycocyanobilin seryliminoester,[11] confirming that the chromophore structure of deprotonated 2,3-dihydrobilindiones and their iminoesters are of the same type.

(15*E*)-chromophore anion as a model for the Pfr chromophore: The absorption maximum of 2,3-dihydrobilindiones could best be shifted near to that of the Pfr chromophore around 730 nm by deprotonation of model compound **4**, which is the 15*E*-configured diastereomer of **1**. The preparation of the anion **4**$^-$ is conveniently achieved by the addition of TBAF to a solution of **4** in THF at a temperature around 15 °C. Under conditions like these, deprotonation followed by tautomerization is rather fast and the lifetime of **4**$^-$ is long enough to measure one absorption spectrum. The long-wavelength absorption band resembles that of Pfr. The absorption maximum is found at 752 nm. Within a few minutes **4**$^-$ is completely converted into **1**$^-$ by thermal $15E \rightarrow 15Z$ isomerization monitored spectrophotometrically in the long-wavelength region (Figure 5a). The rate of isomerization [$k(12\,^\circ\mathrm{C}) = 1.1 \times 10^{-3}\,\mathrm{s}^{-1}$] is more than two orders of magnitude greater than those of nondeprotonated 15*E*-configured chromophores.[12] Because of such instability, determined attempts to ascertain the structure of **4**$^-$ by NMR spectroscopic investigations failed. Therefore, stereochemical assignment of the (4*Z*,10*Z*,15*E*,5*syn*,9*syn*,14*syn*) geometry is based on the clear chemical correlation to **4** and **1**$^-$ complemented by the characteristic hypso- and hyperchromicity of the long-wavelength absorption maximum (λ_{max} (ε) in THF: 582 (14200) [**1**]; 535 (18200) [**4**]) also observed on $15Z \rightarrow 15E$ photoisomerization of the nondeprotonated analogues.[12b]

The absorption maximum of **4**$^-$ is blue-shifted by 32 nm compared with that of **1**$^-$. Such a difference in λ_{max} values has long been known for *E*/*Z* diastereomers of bilindiones[13] and is explained by a different twist of the planar ring moieties to each other. In **4**$^-$ steric congestion caused by the methyl group in position 17 results in higher torsion around the C14–C15 single bond, decreasing π-orbital overlap between rings C and D and shifting the absorption maximum to 752 nm. Accordingly, a further blue shift towards the λ_{max} value of the Pfr chromophore at 730 nm should be induced by higher twist angles. In principle, they can be assumed in the (14*anti*)-conformation (Figure 5b), where the increase in steric hindrance of the adjacent methyl groups in positions 13 and 17 would extend the blue shift by widening the torsion angle.

Beside the bathochromic shift of the long-wavelength absorption maximum there are two further characteristics in

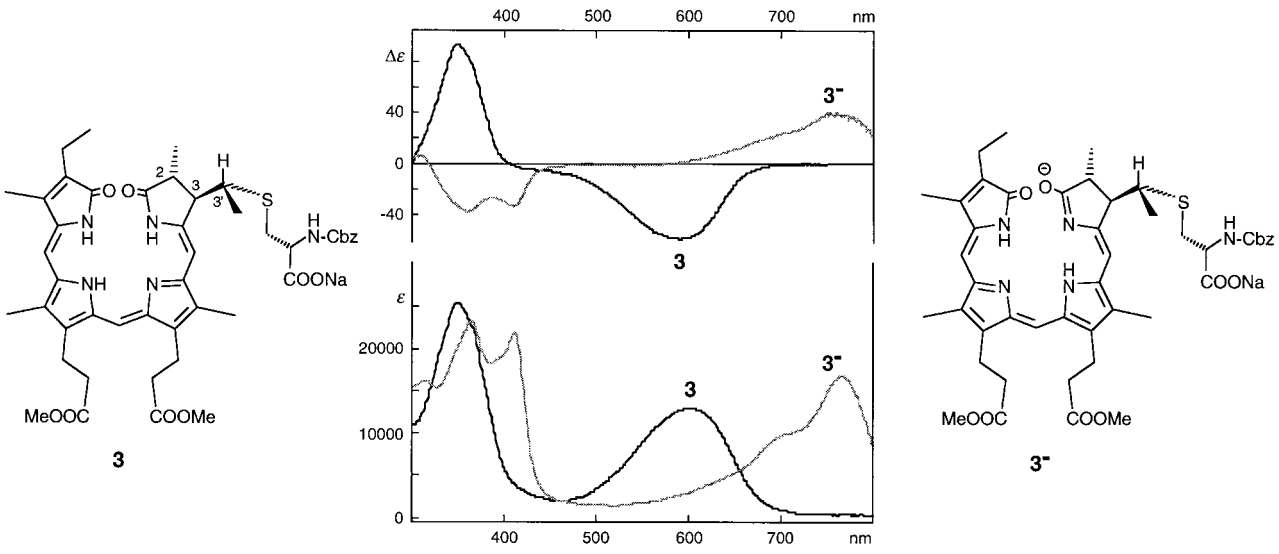

Figure 4. UV/Vis and CD spectra of **3** and **3**$^-$ in $CHCl_3$ at 25 °C.

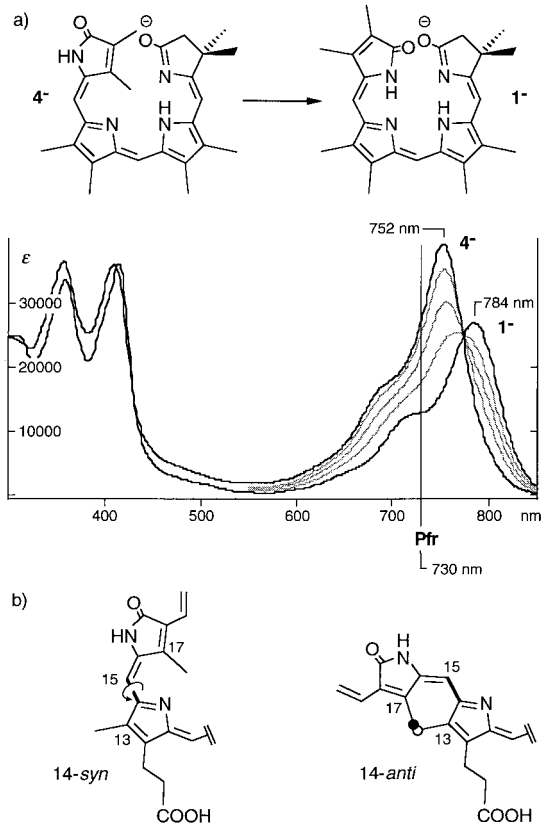

Figure 5. a) UV/Vis spectra showing the thermal 15*E* →15*Z* isomerization of **4**⁻ to **1**⁻ in THF at 12 °C. b) The C/D half of the structural formula of the 15*E*-configured phytochrome chromophore in the 14-*syn* and the 14-*anti* conformation. Additional torsion in the 14-*syn* conformer compared with **4**⁻ could induce a hypsochromic shift of the long-wavelength maximum towards 730 nm. In the 14-*anti* conformer such torsion might already be set by the additional steric hindrance of the adjacent methyl groups in positions 13 and 17.

the absorption spectra of the chromophore anions **1**⁻ and **4**⁻ that can also be observed in the spectrum of the Pfr form. Firstly, the long-wavelength maximum is accompanied by a shoulder, which should more precisely be termed a vibronic structure. The resemblance to the structured band of various donor–acceptor substituted polyenes,[14] like merocyanines, corroborates the applicability of the oxygen-donor/oxygen-acceptor concept for spectra interpretation. Secondly, the spectrum exhibits a new absorption band at about 400 nm that is significant for deprotonated chromophores. The band actually originates from one of two overlapping bands observed in the spectra of neutral uncharged or protonated 2,3-dihydrobilindiones at ca. 350 nm. The red shift to 400 nm is caused by the high donor strength of the negatively charged oxygen. The corresponding electronic transition is polarized perpendicular to that of the long-wavelength band. The intensities of the short and long-wavelength absorption maxima are balanced in **1**⁻ and **4**⁻ because of the roughly square geometry of their chromophores. In Pfr, however, the intensities are quite different, indicating a more or less rectangular geometry of the chromophore, which is characteristic of bilindiones in stable extended conformations.[15]

Conclusion

Understanding the biological functionality of phytochrome at the molecular level requires information on the structural changes of both chromophore and protein. Because of the color change from the inactive to the active state, investigations concentrate on methods using the chromophore as the reporter molecule. In organic chemistry approaches, structural changes of model chromophores by chemical transformations were classified as relevant when they were accompanied by the characteristic changes in the absorption spectra.

In this study the deprotonation of 2,3-dihydrobilindiones was found to change the chromophore structure in such a way that the UV/Vis spectra of the anions formed and that of Pfr become conformable. The best resemblance was observed with a 15*E*-configured chromophore anion. Deprotonation takes place regioselectively at the 2,3-dihydrolactam substructure and is followed by tautomerization of the dipyrrin moiety. The chromophore structure formed thereby can be interpreted in terms of an oxygen-donor/oxygen-acceptor-substituted polyene. The remarkable red shift of the long-wavelength maximum results from the strong donor power of the ring A oxygen where the negative charge is localized.

Concerning the photocycle of phytochrome, we can now present a model that is based on oppositely charged chromophore ions of different geometry and stability (Figure 6): a stable 15*Z*-configured cation for physiologically inactive Pr and a labile 15*E*-configured anion for physiologically active Pfr. To run the photocycle, two distinct types of reversible reactions are necessary: photoisomerization via the excited state and proton transfer in the ground state. In our proposed pathway for the Pr→Pfr transformation (Figure 6), the photochemical 15*Z* →15*E* isomerization is followed by two proton transfers from the chromophore and one within the chromophore. Thereby the mutual switch of opposite charges between chromophore and protein may contribute to the physiologically different states of the Pr and Pfr form. Hydrogen-bridged ion pairs can stabilize both states. The chemistry of our model is in accordance with that of two other well-studied photoactive proteins: in bacteriorhodopsin[16] and in the photoactive yellow protein[17] it is a *Z*/*E* photoisomerization of the chromophore that is likewise followed by a proton transfer.

Experimental Section

General techniques: All chemicals were reagent grade. Solvents were generally distilled prior to use, THF was distilled from sodium benzophenone ketyl. Column chromatography was performed on silica gel (Merck, silica gel 60, 0.063–0.200 mm). NMR spectra were recorded on a Bruker Avance DRX-500 spectrometer. The assignment of ^{13}C signals is based on gradient-enhanced HMQC and HMBC experiments. IR spectra were recorded on a Perkin Elmer FT-IR spectrometer Paragon 1000 PC. UV/Vis and CD spectra were recorded on a Hitachi U-3210 spectrometer and a Jobin-Yvon Mark V circular dichrograph. Isosbestic points are indicated by λ_{ip}. Electron-impact mass spectra were measured on a Hewlett Packard MS-Engine 5989 A.

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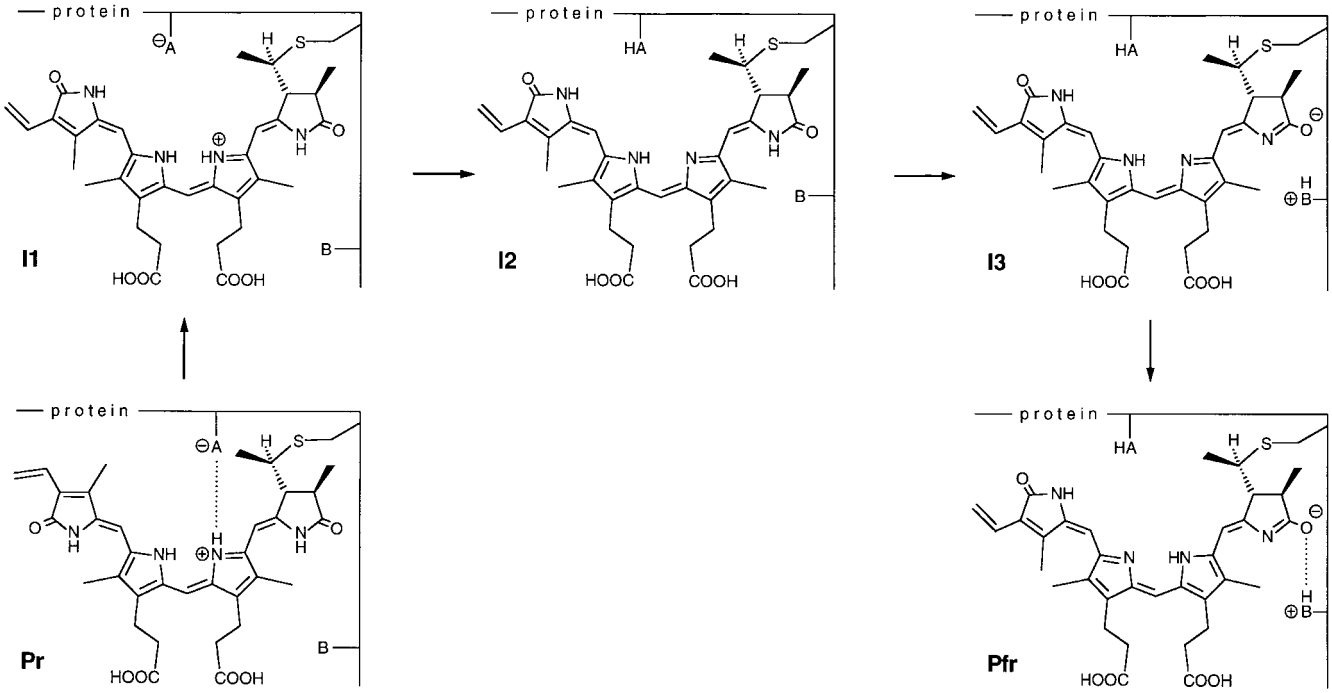

Figure 6. Proposed model for the Pr→Pfr transformation via three intermediates by $Z \rightarrow E$ photoisomerization (Pr→**I1**), two proton transfers from the chromophore to the protein (**I1**→**I2**→**I3**), and a tautomeric proton transfer within the chromophore (**I3**→Pfr).

Synthesis of model compounds: Synthesis of compounds **1**, **3,** and **4** is described in the preceding paper. Model compound **2** was synthesized according to refs. [18,19].

Spectroscopic data for 1: ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta = 6.74$ (s, 1H; H-C10), 6.00 (s, 1H; H-C15), 5.53 (s, 1H; H-C5), 2.21 (s, 2H; H$_2$-C2), 2.15 (s, 3H; H$_3$-C12′), 2.12 (s, 3H; H$_3$-C8′), 2.04 (s, 3H; H$_3$-C17′), 2.06 (s, 3H; H$_3$-C13′), 1.97 (s, 3H; H$_3$-C7′), 1.71 (s, 3H; H$_3$-C18′), 1.34 (s, 6H; 2 × H$_3$-C3′); (H,H)-ROESY NMR (500 MHz, [D$_6$]DMSO): 2↔3′, 5↔(7′, 3′), 10↔(8′, 12′), 15↔(13′, 17′), 17′↔18′.

(4*Z*,10*Z*,15*Z*)-2,3-Dihydro-1-methoxy-3,3,7,8,12,13,17,18-octamethyl-22*H*-bilin-19(24*H*)-one (2): A mixture of **1** (30 mg, 67.5 μmol) in $CHCl_3$ (3 mL) and $Zn(OAc)_2 \cdot 2H_2O$ (25 mg, 209 μmol) in MeOH (1.4 mL) was stirred for 2 min under an argon atmosphere at room temperature. Acetic anhydride (0.54 mL, 5.71 mmol) was added and the reaction mixture was stirred under reflux for 7 min. After cooling the mixture was diluted with CH_2Cl_2 (50 mL), washed with aqueous $NaHCO_3$ (0.2 M, 3 × 80 mL), and dried over Na_2SO_4. After the evaporation of the solvent the residue was subjected to column chromatography (silica gel, CH_2Cl_2/MeOH = 30/1) yielding **2** (20 mg, 64%) as a bluish-green solid. M.p. 211 °C; R_f (silica gel, CH_2Cl_2/MeOH = 30/1): 0.75; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 11.88$ (s, 1H; H-N22), 9.46 (s, 1H; H-N24), 6.81 (s, 1H; H-C10), 5.94 (s, 1H; H-C15), 5.67 (s, 1H; H-C5), 3.74 (s, 3H; H$_3$-CO), 2.47 (s, 2H; H$_2$-C2), 2.20 (s, 3H; H$_3$-C8′), 2.17 (s, 3H; H$_3$-C12′), 2.09 (s, 3H; H$_3$-C17′), 2.07 (s, 3H; H$_3$-C13′), 2.06 (s, 3H; H$_3$-C7′), 1.89 (s, 3H; H$_3$-C18′), 1.39 (s, 6H; 2 × H$_3$-C3′); (H,H)-ROESY NMR (500 MHz, $CDCl_3$): 2↔3, 5↔(7′, 3′), 10↔(8′, 12′), 15↔(13′, 17′), 17′↔18′; IR ($CHCl_3$): $\tilde{\nu} = 3338$, 3002, 2861, 1687, 1599, 1572, 1511 cm^{-1}; UV/Vis (THF): λ_{max} (ε) = 668 (18200), 349 (34200), 340 (33300), 279 nm (16800); $C_{28}H_{34}N_4O_2$; MS (70 eV; EI): *m*/*z* (%): 458 (100) $[M]^+$.

Preparation of the chromophore anions: For NMR spectroscopic investigations **1**$^-$ was prepared by the addition of 1.5 equiv TBD to a solution of **1** in [D$_6$]DMSO. For UV/Vis spectroscopic investigations **3**$^-$ was prepared by the addition of TBD to a solution of **3** in $CHCl_3$ or DMSO. The anion **4**$^-$ was prepared by the addition of TBAF in excess to a solution of **4** in THF.

Spectroscopic data of 1$^-$: ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta = 6.55$ (s, 1H; H-C10), 5.89 (s, 1H; H-C15), 5.03 (s, 1H; H-C5), 2.10 (s, 3H; H$_3$-C8′), 2.07 (s, 3H; H$_3$-C12′), 2.01 (s, 3H; H$_3$-C13′), 1.98 (s, 3H; H$_3$-C17′), 1.94 (s, 3H; H$_3$-C7′), 1.94 (s, 2H; H$_2$-C2), 1.67 (s, 3H; H$_3$-C18′), 1.23 (s, 6H; 2 × H$_3$-C3′), TBD · H$^+$: 3.10 (t, $^3J = 5.9$ Hz, 4H; H$_2$-C2,C10), 2.98 (t, $^3J = 5.9$ Hz, 4H; H$_2$-C4,C8), 1.74 (tt, 4H; H$_2$-C3,C9); (H,H)-ROESY NMR (500 MHz, [D$_6$]DMSO): 2↔3′, 5↔(7′, 3′), 10↔(8′, 12′), 15↔(13′, 17′), 17′↔18′; UV/Vis (THF): λ_{max} (ε) = 784 (24900), 414 (31000), 339 (28000), 312 nm (19200); UV/Vis ($CHCl_3$): λ_{max} (ε) = 759 (16200), 405 (20900), 361 nm (24300).

Spectroscopic data for 3$^-$: UV/Vis ($CHCl_3$): λ_{max} (ε) = 765 (16700), 410 (21900), 362 (23200), 313 nm (16100); UV/Vis (DMSO): λ_{max} (ε) = 796 (16700), 419 (41000), 363 (34000), 316 nm (24800); CD ($CHCl_3$): λ_{max} ($\Delta\varepsilon$) = 762 (41), 411 (−32), 385 (−26), 361 nm (−37); CD (DMSO): λ_{max} ($\Delta\varepsilon$) = 819 (15), 747 (7), 427 (-23), 388 (−11), 364 nm (−20).

Spectroscopic data for 4$^-$: UV/Vis (THF, 285 K): λ_{max} (ε) = 752 (40800), 407 (34100), 356 (34000), 263 nm (23400); λ_{ip} [**4**$^-$ →**1**$^-$] (ε) = 774 (24800), 430 (15600), 413 (35800), 316 (23900), 287 nm (23500).

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