Chromopeptides from Phytochrome and Phycocyanin.
NMR Studies of the Pfr and Pr Chromophore of Phytochrome
and $E,Z$ Isomeric Chromophores of Phycocyanin

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Z. Naturforsch. 38c, 359 – 368 (1983); received February 2, 1983

Bilipeptides, C-Phycocyanin, High Resolution NMR Spectra, Photoisomerization, Phytochrome

Chromopeptides were prepared by pepsin digestion of C-phycocyanin isolated from the cyanobacterium *Spirulina maxima* and of phytochrome isolated from seedlings of *Avena sativa* L. The chromopeptides were characterized by amino acid analysis. The ZZZ configured chromophore of the phycocyanin peptide was transformed into its ZZE configured isomer by the method of Falk *et al.* (*Mh. Chemie* 111, 159 – 175, 1980) which had previously been applied to biliverdins. The 500 MHz $^1$H NMR spectrum of the ZZE configured chromopeptides confirmed that its chromophore has the 15 $E$ configuration. Irradiation yielded the ZZZ configured isomer for which the $^1$H NMR spectrum was also recorded. Native phytochrome was irradiated at 660 nm to yield the maximum amount of the Pfr from (about 75% of total phytochrome). By digestion in the dark the previously described Pfr chromopeptide was obtained. The 500 MHz $^1$H NMR spectrum was compared with that of the ZZE phycocyanin peptide. It confirmed the 15 $E$ configuration of the Pfr chromopeptide. Irradiation yielded the 15 $Z$ configurated Pr chromopeptide. Comparison of the high resolution $^1$H NMR spectra of Pfr and Pr chromopeptides revealed that not only the chromophore resonances but also those of some amino acids are changed by the Pfr $\rightarrow$ Pr chromopeptide phototransformation. The results are discussed in terms of chromophore amino acid interaction.

Plant growth and development is effectively influenced by light. The most important photoreceptor for these processes in higher plants is the biliprotein phytochrome [*1*] which exists in a physiologically inactive form Pr ($\lambda_{max}$ = 665 nm) and a physiologically active form Pfr ($\lambda_{max}$ = 730 nm). Both forms are photo reversible, *i.e.* interconvertible by appropriate light treatments.

The chemical structure of the Pfr chromophore, phytochromobilin (1a) has been established by investigations of small chromopeptides. On the one hand, the chromophore was cleaved with HBr [*2*] and identified with phytochromobilin obtained by total synthesis [*3*]. The nature of the covalent linkage had been established before by studies of elimination reactions after oxidative degradation [*4*]. On the other hand, high resolution NMR studies of the chromopeptides allowed to deduce the same structure 1a independently [*5*]. The structure of the Pfr chromophore remained speculative in these studies. Similar studies with phycocyanin, the light harvesting chromoprotein of blue-green algae and red algae [*6*] showed, that its chromophore (Structure 1b) differs from phytochromobilin only in the side chain at C-18, which is an ethyl group in phycocyanobilin and a vinyl group in phytochromobilin [*7, 8*].

![Chromopeptide Structures](image_url)

1a: $R = CH = CH_2$

1b: $R = C_2H_5$

Reprint requests to Prof. Dr. W. Rüdiger.
0341-0382/83/0500-0359 $ 01.30/0
Although it has been known since 1971 [9] that small chromopeptides from phytochrome (Pr form) are not photoreversible we succeeded to prepare light sensitive chromopeptides from the Pfr form of phytochrome which are different from the above mentioned Pr chromopeptides [10]. Irradiation converted these Pfr chromopeptides into Pr chromopeptides without change in the composition of the peptide moiety [10]. The conclusion that the Pfr chromophore in these chromopeptides is an E isomer (structure 2a or isomers thereof) of the ZZZ configurated Pr chromophore was drawn by comparison of UV-vis spectra of chromopeptides from phytochrome and phycocyanin with an iT-configurated chromophore (structure 2b or isomers thereof) on the one hand and Pr and Z-configurated phycocyanin chromopeptides on the other hand [11]. E-configurated chromopeptides from phycocyanin were obtained from the Z-configurated components by a reaction sequence worked out by Falk et al. [12] with biliverdins. The E-configuration of the phytochrome chromophore was supported by comparison of the methine resonances of high resolution \(^1\)H NMR spectra of phytochrome and phycocyanin chromopeptides [13]. We report here the full 500 MHz \(^1\)H NMR spectrum of Pfr and Pr chromopeptides and of E and Z configurated chromopeptides from phycocyanin. These data confirm the 15 E configuration of the Pfr chromophore (structure 2a) and furthermore indicate configuration dependent interaction of the chromophore with certain amino acids.

### Materials and Methods

Small phytochrome (60 KD) was isolated from oat seedlings (Avena sativa, Pirol, from BAYWA München) and purified by brushite-chromatography as described [14] with little modifications: In all buffers mercaptetoethanol was replaced by sodium sulfite. The brushite-column was equilibrated with 15 mM potassium phosphate buffer pH 7.5. The brushite-chromatography was followed by 50% ammonium sulfate fractionation and the precipitated phytochrome was dissolved in 10 mM tris-HCl/ pH 7.4, 10 mM KCl, 1 mM EDTA, 1 mM NaN\(_3\) and dialyzed against the same buffer. The samples were stored at \(-20^\circ\)C in the dark until use. 130 mg of phytochrome were collected and applied to a DEAE-Sepharose-column (Pharmacia, 5 x 20 cm), equilibrated with the dialysis-buffer, then washed with the same buffer and eluted with the equilibration-buffer containing 250 mM KCl. The eluate was concentrated by 50% ammonium sulfate fractionation and the precipitate was dissolved with the same buffer and dialyzed against the same buffer. The resulting solution (172 ml) contains 100 mg phytochrome, purity index \(A_{665}/A_{280} = 1:20\). The solution does not contain any reducing agent which has to be avoided during pepsin digestion.

This phytochrome solution was then irradiated to saturation at 660 nm (6 min with a fluence rate of 63 Wm\(^{-2}\); Leitz Prado projector and interference filter 660.3 nm, half width 12.8 nm, Searom, Argenteuil, France). The resulting Pfr-solution (ca. 75% Pfr, 25% Pr) was acidified with concentrated HCl to a final pH of 1.5, ascorbic acid was added to the mixture and was then incubated with 100 mg pepsin (= enzyme-phytochrome ratio of 1:1) for 1 h at 37 °C under argon gas.

The resulting peptides were purified by chromatography on Biogel P-10 and on silica gel as described earlier [11]. The silica gel with the adsorbed Pfr chromopeptide was transferred into a small glass funnel. It was first washed with water and then with \(d_6\)-acetone to remove the non-deuterated water. The Pfr chromopeptide was desorbed from the silica gel with a mixture of \(d_6\)-acetone/H\(_2\)O/CF\(_3\)COOH (85:10:5, v:v:v) and stored in this solution under argon gas at \(-18^\circ\)C until it was used for NMR measurements. Aliquots were taken for UV-Vis spectra and for amino acids analysis (see Tab. III).
The peptide concentration in the NMR tube was 9.4 × 10^{-5} M calculated from the UV-Vis spectrum with $\varepsilon_{660} = 32,000$ [9].

C-Phycocyanin from *Spirulina maxima* was isolated and digested with pepsin as previously described [11]. Purification of chromopeptides by chromatograph on Biogel P-10 and silica gel and by isoelectric focusing was also performed as described [11]. The chromopeptide used in this study was the fraction which focused at pH 5.03. Servalyte was removed by washing the chromopeptide fraction adsorbed on a silica gel column with 0.5 N HCl. The chromopeptide was eluted with 50% aqueous formic acid and then lyophilized.

For photoisomerization, 2.6 × 10^{-3} mol purified chromopeptide were dissolved in the mixture of 0.1 M Tris-HCl (pH 8.5)/mercaptoethanol (3:2, v:v), illuminated and separated from mercaptoethanol by chromatography on Biogel P-10 and silica gel as previously described [11]. The ZZE-configurated chromopeptide which was adsorbed at silica gel was then treated in the same way as described for the Pfr chromopeptide, but desorption was achieved with a mixture of $d_6$-acetone/H$_2$O/CF$_3$COOH (80:10:10, v:v:v) at -18°C until it was used for NMR measurements. Aliquots were taken for amino-acid analysis (see Table III). The peptide concentration in the NMR tube was 1.3 × 10^{-4} calculated from the UV-Vis spectrum with $\varepsilon_{665} = 35,500$ [15]. The 500 MHz $^1$H NMR spectra were recorded with a Bruker WM 500 FT NMR (quadrature detect.) with Aspect 2000 data system at 10°C at Fa. Bruker, Analytische Meßtechnik, Karlsruhe. The H$_2$O peak was reduced with the gated decoupling method.

The chemical shift values are in ppm sodium trimethylsilyl propansulfonate (TPS) and acetone was used as the internal standard (2.050 ppm). Several tests verified that chemical shift from acetone is independent from temperature and concentration of the components of the solvent system used.

**Results and Discussion**

High resolution $^1$H NMR spectra had already been obtained for a ZZZ phycocyanin peptide [7] and for a P$_r$ peptide [5]. Both measurements were performed in pyridine-$d_5$ and in pure D$_2$O. These solvents are unsuitable for our purpose because P$_r$ peptides require an acid medium for stabilization [10, 11]. As outlined earlier [13] the solvent system acetone-$d_6$/water/trifluoracetic acid was designed for our measurements. It meets the requirements for stability of P$_r$ peptides (2a) and ZZE phycocyanin peptides (2b) and overcomes the problem of protoncatalyzed exchange of H-5 and, furthermore, elutes the chromopeptides from silica gel columns.

At first, $^1$H NMR spectra of 2a and 2b were recorded. Since these compounds are light sensitive, they were handled, and their spectra recorded in the dark or under dim green safelight. Furthermore, the spectra were recorded at 10°C to avoid too much of temperature dependent reversion during measurement [10]. Subsequently, the compounds were irradiated in the NMR tubes with white light to form 1a and 1b, respectively. Their $^1$H NMR spectra were recorded immediately after this phototransformation.

For easier comparison the NMR spectra are arranged in the following manner: Fig. 1 displays the $\delta$ range downfield from the H$_2$O solvent peak at 5.33 ppm, namely 9.05 to 5.70 ppm, Fig. 2 contains the $\delta$ range upfield from the H$_2$O solvent peak namely 4.50 to 0.70 ppm. In Fig. 2 the $\delta$ range of the acetone solvent peak from 2.20 to 1.90 ppm was spared out. In each figure the spectra labelled A and A' represent non-irradiated and irradiated phytochrome peptides, the spectra labelled B and B' represent the corresponding samples of phycocyanin peptide. The $^1$H NMR resonance frequencies assigned to the chromophores are found in Table I, the resonances assigned to the peptide moities are listed in Table II.

**Bilin moiety of phycocyanin peptide**

The $^1$H NMR spectrum of the ZZZ configurated peptide (1b) is shown in Figs. 1–2B', that of the EZZ configurated peptide (2b) is shown in Figs. 1–2B. Since our preparation of 2b contains some 1b due to dark reversion of 2b [10] its $^1$H NMR spectrum contains resonances of both 1b and 2b. The assignment of the methine resonances 5, 10, and 15 was achieved by comparison with suitable model compounds as described in detail in [13] and [16]. The assignment of the other resonances was based on chemical shifts and characteristic...
Fig. 1. Partial 500 MHz $^1$H NMR spectra of phytochrome and phycocyanin chromopeptides in acetone-$d_6$/H$_2$O/CF$_3$COOH at 10°C in the range of 9.05 to 5.70 ppm. A) P$_r$ chromopeptide (2a) containing some P$_r$ chromopeptide (1a) due to dark reversion. A') P$_r$ chromopeptide (1a) obtained from A) by irradiation with white light. B) $E$-phycocyanin peptide (2b) containing some $Z$-phycocyanin peptide (1b) due to dark reversion. B') $Z$-phycocyanin peptide (1b) obtained from B) by irradiation with white light.
Fig. 2. Partial 500 MHz $^1$H NMR spectra of phytochrome and phycocyanin chromopeptides in acetone-d$_6$/H$_2$O/CF$_3$COOH at 10 °C in the range of 4.50 to 0.70 ppm. The $\delta$ range of the acetone solvent peak from 2.20 to 1.90 ppm is spared out. Increase of the base line in the region upfield from about 1 ppm is due to a specific impurity in the probe head. A) $P_r$ chromopeptide (2a) containing some $P_l$ chromopeptide (1a) due to dark reversion. A') $P_r$ chromopeptide (1a) obtained from A) by irradiation with white light. B) $E$-phycocyanin peptide (2b) containing some $Z$-phycocyanin peptide (1b) due to dark reversion. B') $Z$-phycocyanin peptide (1b) obtained from B) by irradiation with white light.
multiplicities (Table I). Furthermore, the differences in the \(^1\)H NMR spectra of the E and Z-configurated phycocyanin peptides were compared with those of reported 15 E and all Z configured model chromophores (structures 3 and 4; [17, 18]). The final assignment would require special NMR techniques which are not available to us. These techniques had, however, been previously applied to a Z-configurated phycocyanin peptide [7]. Comparison of these data with our measurements of 1b shows a good correlation (see Table I). Differences between our data and the data reported for D$_2$O are within ± 0.1 ppm with the exception of 10-H, for which a difference of −0.15 ppm is observed.

Since only ZZE compounds are light sensitive, the resonances which are specific for 2b disappear upon irradiation, whereas the resonances of 1b increase (Compare Figs. 1—2 B and B'). This facilitates the assignment of ZZE and ZZZ specific NMR resonances.

This is especially true for the methine resonances (see Figs. 1B and B'). Their assignment has been reported earlier [13] and is shown in Table I. The triplet at 1.006 ppm was assigned to the methyl protons of the 18-ethyl group of 2b. It disappears upon irradiation whereas the triplet at 1.059 ppm (18-CH$_2$—CH$_3$ of 1b) increases. The difference of chemical shifts (\(\Delta \delta\) 2b minus 1b = −0.053 ppm) corresponds to the differences reported for model compounds 3 (\(\Delta \delta\): −0.05 ppm) and 4 (\(\Delta \delta\): −0.03 ppm, 17, 18). The mixture of 1b and 2b exhibits four doublets in the region of 1.40 to 1.20 ppm, which overlap partly (and partly with a solvent resonance at 1.24 ppm) and coincide upon irradiation into two doublets of 1b at 1.345 and at 1.295 ppm by a parallel shift (\(\Delta \delta\): 0.04 ppm). Earlier NMR measurements [7] had revealed only one doublet for 2-CH$_3$. Contrary to those measurements, our ZZE configurated chromopeptide 2b was prepared photochemically from 1b according to the method of Falk et al. [12]. If such a reaction would be accompanied by an epimerisation at C-2, one would expect two resonances for the 2-CH$_3$ protons. The presence of four doublets in the spectrum of the mixture of 1b and 2b implicates, that the splitting of the 2-CH$_3$ resonance is present in the E-compound 2b, too, and does not occur during reversion to the Z-compound 1b. This question has to be investigated further.

The multiplet at 1.44 ppm yields a doublet at 1.448 ppm upon irradiation. The doublet was assigned to 3'-CH$_3$ protons (Table I). The multiplet most likely consists of the two overlapping doublets of 1b and 2b for 3'-CH$_3$ protons. The difference of the chemical shift is only about −0.01 ppm. Irradiation also leads to disappearance of the resonance at 3.14 ppm with corresponding increase of a resonance at 3.19 ppm. We have provisionally assigned these resonances to 3-H of 2b and 1b (Table I) although it cannot be excluded that they belong to cysteine $\beta$-CH$_2$ or arginine $\delta$-CH$_2$ [7] (see Table II).

Upon irradiation, the singulet at 1.849 ppm disappears and is, therefore, a resonance specific for 2b. It is tentatively assigned to the 17-CH$_3$ protons, because with respect to 7, 13, and 17-CH$_3$ resonances in model compounds 3 and 4, the 17-CH$_3$ resonance shows the most pronounced shift (\(\Delta \delta\): −0.16 and −0.07, respectively) upon transfor-
formation of the 15 E to the all Z compound. The corresponding resonance of 1b is possibly covered by the solvent peak in our spectrum.

The resonances of the other aromatic methyl protons of 1b and 2b are also very close to the solvent peak in the region around 2 ppm. Their exact position and assignment is, therefore, uncertain.

Another resonance which disappears upon irradiation is that at 2.29 ppm. It was tentatively assigned to methylene protons of the 18 ethyl group of 2b because the intensity of the multiplet at 2.352 ppm (18-CH2—CH3 of 1b) is increased upon irradiation. However, the decrease in intensity at 2.29 ppm seems to be larger than the increase at 2.352 ppm. Furthermore, our assignment would mean a  δ of -0.06 ppm, whereas values of +0.11 ppm were reported for 3 and +0.01 ppm for 4. If we suppose a similar shift of the methylene protons in our peptide their resonance would be hidden by the solvent peak in the Z-compound. Their final assignment, therefore, needs further experimentation. No shift can be detected in the resonance of the methylene protons of propionic acid side chains (2.61 and 3.07 ppm), and 3'H (3.58 ppm). However, the pattern of these resonances is clearly changed by irradiation. The resonance for the 2-H is probably covered by α-CH2 resonances of the propionic acid side chains in both compounds (cf. [7]) and is therefore, probably only slightly shifted during the light induced reversion of 2b and 1b. The reaction sequence for Z → E isomerisation of phycocyanin peptide could theoretically lead to the 4 E or 15 E isomer [11]. The reaction sequence with 2.3 dihydrobilindiones, however, yields only 15 E compounds ([16] and H. Falk, personal communication). The 1H NMR data of the E and Z-configurated phycocyanin peptide, especially the chemical shifts of the methine protons [13] clearly demonstrates that only the 15 E compound was obtained (structure 2b).

**Table I. 500 MHz 1H NMR assignments of the bilin moiety of chromopeptides from phycocyanin and phytochrome in acetone-d6/H2O/CF3COOH.**

<table>
<thead>
<tr>
<th>Chemical Shift*, Multiplicity and J [Hz]</th>
<th>Assignment</th>
<th>Chemical Shift Multiplicity and J [Hz] of Phycocyanin Peptide in D2O [7]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phycocyanin Peptide</strong></td>
<td><strong>Phytochrome Peptide</strong></td>
<td><strong>Pr(1a)</strong></td>
</tr>
<tr>
<td>ZZZ (1b)</td>
<td>ZZE (2b)</td>
<td>P1 (1a)</td>
</tr>
<tr>
<td>1.295 d (7.5)</td>
<td>1.255 d (7.5)</td>
<td>1.29 m</td>
</tr>
<tr>
<td>1.345 d (7.5)</td>
<td>1.304 d (7.5)</td>
<td>1.417 d (6.8)</td>
</tr>
<tr>
<td>1.448 d (6.8)</td>
<td>1.44 m</td>
<td>&lt;2.084 s&gt;</td>
</tr>
<tr>
<td>n.d.</td>
<td>n.d.</td>
<td>&lt;2.089 s&gt;</td>
</tr>
<tr>
<td>&lt;2.085 s&gt;</td>
<td>1.849 s</td>
<td>&lt;2.098 s&gt;</td>
</tr>
<tr>
<td>n.d.</td>
<td>n.d.</td>
<td>2.221 s</td>
</tr>
<tr>
<td>1.059 t (7.5)</td>
<td>1.006 t (7.5)</td>
<td>–</td>
</tr>
<tr>
<td>&lt;2.35 m&gt;</td>
<td>&lt;2.29 m&gt;</td>
<td>18-CH2—CH3</td>
</tr>
<tr>
<td>2.61 m</td>
<td>2.61 m</td>
<td>2.58 m</td>
</tr>
<tr>
<td>3.07 m</td>
<td>3.07 m</td>
<td>3.04 m</td>
</tr>
<tr>
<td>3.58</td>
<td>3.58</td>
<td>&lt;2.61&gt;</td>
</tr>
<tr>
<td>&lt;(2.61)</td>
<td>&lt;(3.19 m)</td>
<td>&lt;(3.20)</td>
</tr>
<tr>
<td>5.999 s</td>
<td>5.943 s</td>
<td>5.995 s</td>
</tr>
<tr>
<td>6.239 s</td>
<td>6.434 s</td>
<td>6.538 s</td>
</tr>
<tr>
<td>7.601 s</td>
<td>7.619 s</td>
<td>7.618 s</td>
</tr>
</tbody>
</table>

* The chemical shifts in acetone-d6/H2O/CF3COOH are reported in parts per million from acetone-d6 (2.050 ppm); n.d. = not detected; ppm values in < ): assignment uncertain.
dine-d$_5$ [5, 7], we were able to assign the chromophore resonances of 1a (Table I) by comparison with 1b in our solvent mixture, because the reported similarities of both chromopeptides are apparently also found in our solvent system. In particular, methine protons at C-5, C-10 and C-15 were assigned by comparison with phycocyanin peptide and other model compounds [13, 16]. In the same way, other resonances of the Pr chromophore were assigned: The multiplets at 2.58 and 3.04 ppm to the methylene protons of propionic acid side chains, the doublet at 1.417 ppm to the 3'-CH$_3$ protons. The singulet at 2.221 ppm which increases upon irradiation is provisionally assigned to the 13-methyl protons because it is the most downfield of all aromatic methyls in pyridine-d$_5$ [5]. The resonances of the 17- and 7-CH$_3$ protons are only detected as singulets on the steep flank of the hugh acetone peak (not included in Fig. 2).

The 3'H is not detected because of the high noise level of the NMR spectrum. For the 2-CH$_3$ protons, we observe a multiplet at 1.29 ppm instead of the expected doublet [5]. This could be due either to splitting of the 2-CH$_3$ resonance (see under phycocyanin peptide) or to overlapping with another, unidentified resonance (for example the $\beta$-CH$_3$ protons of alanine). We consider resonance splitting due to epimerization of 2-CH$_3$ the less likely possibility: (i) The distance of doublets would be smaller in Pr peptide than in ZZZ phycocyanin peptide, (ii) the described photochemical reaction sequence was only applied to the phycocyanin peptide but not to the Pr peptide.

In accordance with the results of Lagarias and Rapoport [5], we do not find any resonance which could be attributed to an ethyl group. Since the ethyl group of phycocyanobilin is substituted by a vinyl group in phytochromobilin, we expect corresponding resonances in the range 5—7 ppm [5].

The assignment of resonances in this region of the spectrum (Fig. 1, A') to the vinyl group is not unequivocal because of the high multiplicity of these resonances and the high noise level in the NMR spectrum.

Our preparation of Pr chromopeptides (2a) contains some 1a due to dark reversion. Therefore, the $^1$H NMR spectrum (Fig. 1—2, A) contains resonances of 2a and 1a. Upon irradiation, the Pr specific resonances disappear whereas Pr specific resonances increase. This was reported earlier [13] for the methine protons at C-5 (singulet of 2a at 5.938 ppm), C-15 (singulet at 6.533 ppm), and C-10 (singulet at 7.630 ppm), and clearly observed for the 3'CH$_3$ protons (multiplet at 1.39 ppm).

The expected doublet of 2-CH$_3$ protons partially overlaps with a solvent peak at 1.23 ppm which was also detected in phycocyanin peptides. However, disappearance of part of the 2-CH$_3$ resonance at 1.243 ppm upon irradiation can be observed. This is accompanied by a corresponding increase of the Pr specific resonance at 1.29 ppm. Also the small multiplet at 3.12 ppm disappears upon irradiation. It is assigned to 3-H of 2a but could also be assigned to cysteine or arginine protons (see discussion of phycocyanin peptide). The corresponding signal of 1a is probably covered by a resonance at 3.20 ppm (not identified). The resonance of methylene protons of propionic acid side chains (2.58 and 3.04 ppm) and 2-H (2.58 ppm) are not shifted but change their pattern upon irradiation.

In summary, our NMR data demonstrate a striking similarity between Z phycocyanin peptide (1b) and Pr peptide (1a) on the one hand and between 15E phycocyanin peptide (2b) and Pr peptide on the other hand. It is, therefore, evident that the Pr chromophore is the 15E compound (structure 2a). According to Lagarias and Rapoport [5], the aromatic methyl protons are downfield shifted by 0.01—0.04 ppm in 1a versus 1b. Such a shift could explain why solvent peaks (which are at the same position) can overlap with different resonances in phycocyanin and phytochrome peptides. This is apparently true for the singulet at 1.849 ppm in 2b for which the counterpart in 2a cannot be detected and for the singulet at 2.221 ppm in 1a for which no corresponding resonance in 1b is detected. The singulet at 2.25 ppm in the latter peptide is assigned to amino acid resonances (for instance the $\beta$-CH$_2$ of Glx, see below).

**Amino acid moiety of phytochrome and phycocyanin peptides**

For the assignment of the amino acid resonances in the $^1$H NMR spectrum, knowledge of the amino acid composition of the investigated chromopeptide fractions is essential. Table III contains the data on the amino acid analyse of those chromopeptide fractions from phycocyanin and phytochrome which were used for NMR. Cysteic acid is derived by
The assignment of the peptide resonances (Table II) is based on comparison with the known data of the chromopeptides in other solvents [5,7] and on control measurements of corresponding free amino acids in our solvent system. The \(^1\)H NMR data of the amino acids correspond throughout with the results of amino acid analysis (Table III) with only a few exceptions: whereas the resonances for 2 alanines are present in the \(^1\)H NMR spectrum of the phycocyanin peptide, alanine signals are not detected with certainty in the spectrum of phytochrome peptide. Other resonances which are uncertain in the spectrum of phytochrome peptide (but detected in the spectrum of phycocyanin peptide) are those of glutamic acid \(\beta-\text{CH}_2\), aspartic acid \(\beta-\text{CH}_2\), glycine \(\alpha-\text{CH}_2\), arginine \(\beta,\gamma-\text{CH}_2\) and the \(\alpha-\text{CH}\)'s of all amino acids. The expected position of the latter resonances is very close to a solvent peak and is, therefore, eventually hidden by this strong peak. The resonances at 8.81 and 7.42 ppm have been provisionally assigned to the ring protons of histidines. We observe rather broad resonances (contrary of free histidine) because of slightly different chemical shifts of both histidines in our peptide. Both resonances show similar shifts upon irradiation (see Fig. 1 A and 1 A'). The resonance at 7.016 and 6.677 ppm are assigned to the ring protons of tyrosine. They also behave similarly during the \(P_{fr}\) peptide \(\rightarrow\) \(P_r\) peptide transformation. Interestingly, no significant differences in the shifts of amino acid residues were found between the \(E\) and \(Z\) configurated chromopeptides from phycocyanin except the resonance at 2.25 ppm in the phycocyanin spectrum which appears upon irradiation. It seems rather to be an amino acid resonance (for instance the \(\beta-\text{CH}_2\) of Glx) than that of aromatic methyl protons which give sharp singlets. Minor but significant differences were found in the chemical shifts of \(P_{fr}\) and \(P_r\) resonances assigned to histidine and tyrosine (see above). If the \(E \rightarrow Z\) isomerisation of the chromophore changes the environment of the amino acid residues, the chromophore must be very close to the amino acid side groups (most pronounced with respect to histidine). We have recently demonstrated [19] that interaction between histidine and tetrapyrrol chromophores can occur. This interaction is probably of charge-transfer type [19]. Our presented data agree with such an interaction in the phytochrome peptide. The histidine residues are good candidates to play a role in the photosisation \(P_{fr} \rightarrow P_r\). This possibility is presently being investigated further.

Acknowledgements

Our thanks are due to Firma Bruker, Karlsruhe, Division Analytische Meßtechnik, especially to Dr. V. Formacek and Dr. G.-J. Wolff for the kind cooperation concerning the measurement of 500 MHz spectra. We thank Professor Dr. F. Dörr for continuous interest and support and Prof. Dr. J. Otto for amino acid analysis. The work was financially supported by the Deutsche Forschungsgemeinschaft, Bonn-Bad Godesberg.