FEBS Letters 486 (2000) 52–56 FEBS 24342

Isolation, reconstitution and functional characterisation of the *Rhodobacter sphaeroides* photoactive yellow protein

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Received 23 October 2000; accepted 1 November 2000

First published online 16 November 2000

Edited by Richard Cogdell

Abstract We report the isolation, functional reconstitution and photophysical characterisation of *Rhodobacter sphaeroides* photoactive yellow protein (PYP), of which the gene was recently cloned. Reconstitution of the his-tagged purified apo-protein with 4-hydroxy-cinnamic acid yields the characteristic blue absorbance at 446 nm, but surprisingly also an absorbance peak at 360 nm. This additional peak is not caused by binding of a second chromophore, as confirmed with mass spectroscopy. Moreover, reconstitution with the 'locked' analogue 7-hydroxy-coumarin-3-carboxylic acid yields only a single absorbance peak at 441 nm. The 446 nm and 360 nm species are part of a temperature- and pH-dependent equilibrium. Photoactivation of the protein leads to formation of a blue-shifted intermediate as in other PYPs, with a 100-fold increased groundstate recovery rate ($k_{pB\rightarrow pG} = 500\ s^{-1}$) compared to E-PYP.

Key words: Photoactive yellow protein; *Rhodobacter sphaeroides*

1. Introduction

Photoactive yellow protein (PYP) is a small (125 amino acids) water soluble protein found in several purple bacteria. It is a blue light photoreceptor either as a single protein [1–4] or as a hybrid protein fused to other signal transduction modules [5]. A large amount of biophysical and structural data is available from a variant of this protein, isolated from *Ectothiorhodospira halophila* (E-PYP; see e.g. [6–9]). Studies on E-PYP have shown that this photoreceptor (being soluble, small and very photostable) is an excellent model protein to study the fundamental properties of light perception in biological systems.

Activation of E-PYP function is initiated through light-induced *cis*/*trans* isomerisation of the 7,8-vinyl bond of its 4-hydroxy-cinnamic acid chromophore which then leads to a more complicated multiple bond rotation [7,10–12]. The chromophore is present in the anionic form in the groundstate (pG) of E-PYP [13,14]. This anionic phenolate is buried within the hydrophobic core of PYP through stabilising hydrogen-bond formation with nearby amino acid side chains, i.e. with Tyr42 and (protonated) Glu46 [13]. Upon blue light activation, E-PYP passes through a photocycle in which at least four transient groundstate intermediates are successively formed [15–17]. Two early red-shifted intermediates appear on a ps timescale (I_0 and $I_0^{\ddagger}$), another red-shifted intermediate (pR or I_1) on a ns timescale and a blue-shifted intermediate (pB or I_2) within hundreds of μs. The blue-shifted intermediate decays to the groundstate pG with a lifetime of 140 ms.

E-PYP has also become the structural prototype [18] for a general biological signal transduction module, the so-called 'PAS' domain [19], and is therefore also an excellent model protein for studies on signal transduction. Physiological studies on the characteristics of PYP-mediated signal transduction have, however, until now been severely hampered by the lack of a suitable (i.e. genetically well accessible) model organism. To overcome this problem we initiated a study on the occurrence of a similar photoreceptor in the well known purple non-sulphur bacterium *Rhodobacter sphaeroides*. Recently we were able to clone the gene encoding the PYP from this bacterium (*R. sphaeroides* PYP: R-PYP; [4]) and have shown that although it does not show a large degree of identity to the E-PYP (48%) it does contain almost all of the characteristic amino acid residues for binding and tuning of the PYP chromophore and also models into the characteristic 3D PYP fold [4]. Furthermore we were able to extract the typical PYP chromophore, 4-hydroxy-cinnamic acid, from *R. sphaeroides* [4]. The value of a genetically well accessible organism, for studying PYP-mediated signal transfer, has recently been demonstrated [20]. It was shown that *R. sphaeroides* indeed exhibits a blue light-induced repellent tactic response, but that PYP is not the responsible photosensor; a function which has been assigned to PYP in *E. halophila* [21].

In this paper we describe the purification, reconstitution and initial functional characterisation of the *R. sphaeroides* PYP after heterologous expression. Although displaying specific PYP properties, R-PYP also shows several interesting differences with E-PYP. Studies towards a more biological/functional characterisation of PYP function are now facilitated by substantiating the presence of a functional PYP in this well characterised model organism.

2. Materials and methods

2.1. Cloning of the pyp gene from R. sphaeroides

Cloning of the *pyp* gene from *R. sphaeroides* RK1 (*r-pyp*) into the overexpression vector pQE30 (Qiagen, Hilden, Germany) was performed using PCR and the oligonucleotides RBNTB (gcggatccgatgacgatgacaaaatggaaatcattccctttgg) and RBCTS (gcgcgagctctcagacccgtttgacgaagag), which are homologous to the DNA encoding the R-

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Abbreviations: PYP, photoactive yellow protein; E-PYP, *Ectothiorhodospira halophila* PYP; R-PYP, *Rhodobacter sphaeroides* PYP

0014-5793/00/$20.00
PII: S0014-5793(00)02242-0

PYP N- and C-termini. RBNTB contains a *Bam*HI restriction site and encodes a peptidase cleavage site, RBCTS contains a *Sac*I site and a translational stop codon. As template the vector pAMRO was used. This vector contains a 2.2 kb *Aat*II chromosomal DNA fragment encoding *r-pyp* [4]. PCR was performed using *Pwo* polymerase (Boehringer Mannheim, Almere, The Netherlands): 30 cycles, 0.5 min denaturation at 96°C, 1 min annealing at 55°C and 2 min elongation at 72°C. The product was isolated from a 2% agarose gel using a glasmilk kit (QuiaexII, Qiagen), restricted with *Bam*HI and *Sac*I and cloned into the *Bam*HI and *Sac*I site of pQE30 yielding pRPYP. The sequence of *r-pyp* was confirmed by commercial sequencing (Eurogentec, Seraing, Belgium).

2.2. Purification and reconstitution of R-PYP

Apo-R-PYP was produced and purified as described previously for apo-E-PYP [22] from *Escherichia coli* M15 containing pRPYP. Apo-R-PYP was reconstituted using activated 4-hydroxy-cinnamic acid as described in [23] or 7-hydroxy-coumarin-3-carboxylic acid as described in [24] and subsequently purified by Ni-affinity chromatography. The poly-histidine-tag was removed by cleaving with enterokinase (from calf intestine, Boehringer Mannheim, Almere, The Netherlands). Samples in 50–100 mM Tris–Cl, pH 7.5–8.5, were either kept in the dark, at 4°C (for immediate use), or stored at −20°C for longer periods. Purification was monitored using SDS–PAGE [25]. The gels were stained using Coomassie brilliant blue.

2.3. Spectroscopic measurements

Absorption spectra were recorded using a Hewlett Packard 8453 single beam spectrophotometer and a 'Kraayenhof vessel' as described in [26]. The temperature was varied using the Pelletier element, the pH during the experiment was 8.2 ± 0.2. The pH titrations were carried out as described in [26] at 20°C in 10–100 mM Tris–Cl/100 mM KCl. The data of the pH titration were analysed using the Henderson–Hasselbalch equation, in which A is the absorption at 446 nm and where $A_{1,2,3}$ correspond to the plateaus in the fitted curve at approximately pH 2 (A_1), pH 5 (A_2) and pH 9 (A_3), and an additional parameter n, describing the steepness of the transition [29]. In addition, two transitions with pK_1 and pK_2 are considered.

$$A = A_1 + \frac{A_2 - A_1}{1 + 10^{n_1(\mathrm{p}K_1 - \mathrm{pH})}} + \frac{A_3 - A_2}{1 + 10^{n_2(\mathrm{p}K_2 - \mathrm{pH})}}$$

Laser-induced time-resolved absorption spectroscopy was carried out as described in [9]. No major differences between R-PYP with and without the histidine-tag were found.

2.4. Mass spectrometry

Desalted R-PYP was analysed with electrospray ionisation using a Micromass Q-Tof mass spectrometer. The Q-Tof was fitted with a Z-spray ion source, the spray was delivered by a pneumatic pump system using a flow of 5 μl/min. The samples were diluted in the mobile phase (50% (v/v) acetonitrile, 0.2% (v/v) formic acid) to a final concentration of about 1 pmol/μl. Spectra of the positive ions were collected and combined in the mass/charge (m/z) range of 600–2600 Da. As an external calibrant lysozyme was used. Data were recorded and processed using MassLynx software, which includes MaxEnt deconvolution software.

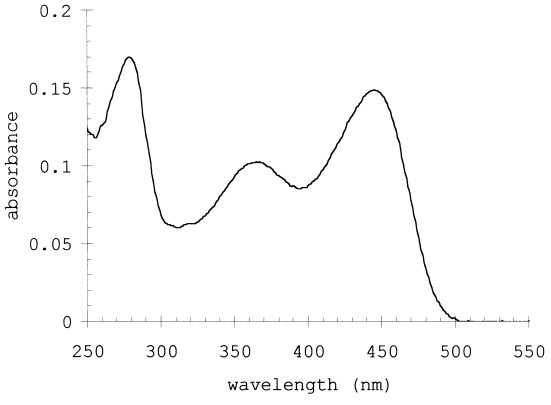

Fig. 1. Absorbance spectrum of isolated *R. sphaeroides* holo-PYP. The protein was isolated after heterologous expression in *E. coli* and reconstituted with 4-hydroxy-cinnamic acid (see Section 2 for details). The spectrum was taken from a dark-equilibrated sample at pH 8 and 20°C.

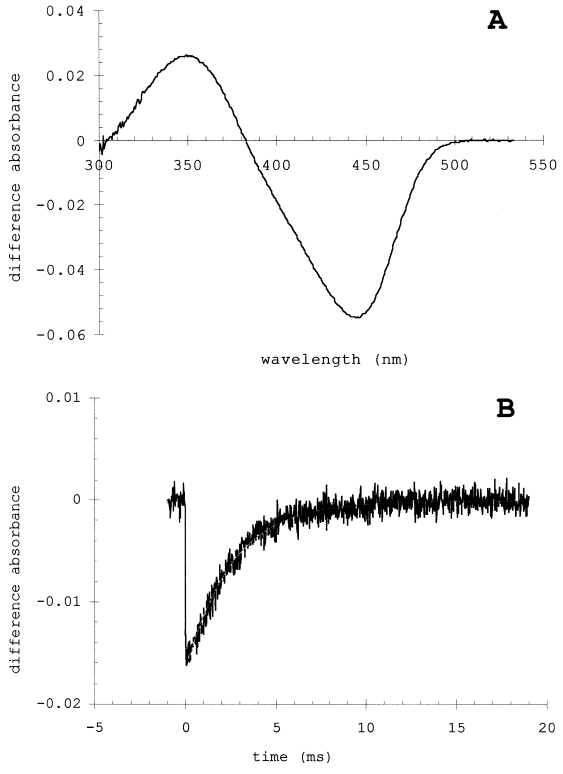

Fig. 2. Accumulation of a blue-shifted intermediate (pB) and dark recovery kinetics of R-PYP after a 465 nm laser flash. (A) Difference spectrum of R-PYP 60 μs after laser flash. (B) Time course of the absorbance transient at 450 nm (laser flash at time 0), fitted with a single exponent (grey line). Note that data from A and B were obtained from different experiments. The extent of the bleach was about 15% of the total absorption at 450 nm in both experiments (0.35 in A, and 0.11 in B).

3. Results

Recently we described the cloning of the operon encoding the *R. sphaeroides* PYP [4]. For the isolation of the encoded protein we have cloned the structural *pyp* gene in a his-tag vector and overexpressed R-PYP in *E. coli*. After reconstitution with the activated 4-hydroxy-cinnamic acid chromophore, the isolation procedure, using Ni-affinity chromatography, yields one single protein band on a Coomassie-stained SDS–PAGE gel (data not shown). Fig. 1 shows the absorbance spectrum of purified R-PYP after removal of the his-tag. Besides the characteristic 446 nm peak an additional photoexcitable absorbance band at 360 nm is present in this spectrum.

Since R-PYP, in contrast to E-PYP contains a second cysteine residue, which theoretically could bind an extra 4-hydroxy-cinnamic acid chromophore, we determined the mass of the isolated sample using a Q-Tof mass spectrophotometer. These analyses showed only one species, with a mass of 14 125 Da (13 979 Da predicted protein mass +147 Da chromophore), no doubly reconstituted species could be detected.

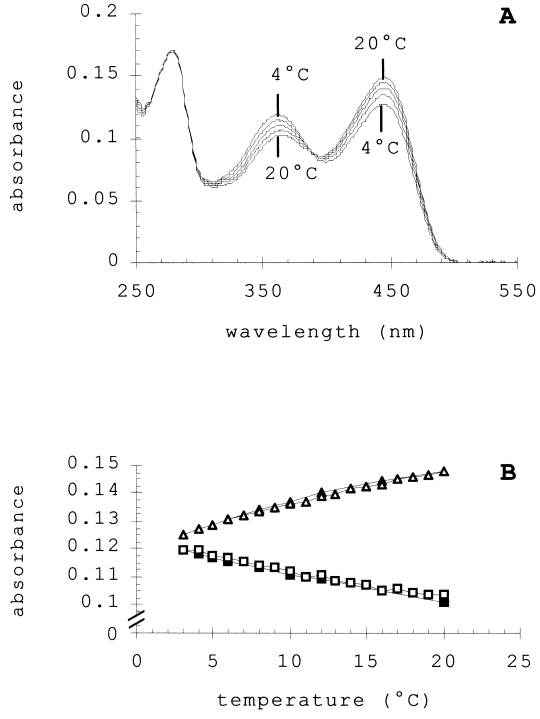

Fig. 3. Temperature dependence of the absorbance spectrum of *R. sphaeroides* PYP. The temperature of the R-PYP sample (in 50 mM Tris–HCl, pH 8.2) was decreased from 20°C to 3°C and subsequently increased back to 20°C (all within 30 min). Spectra were normalised to 0 at 550 nm. (A) Spectra are shown taken at 20, 16, 12, 8 and 4°C. (B) Absorbance at 360 nm (triangles) and 446 nm (squares). The results represented by filled and open symbols were obtained in a series of measurements with decreasing and increasing temperature, respectively.

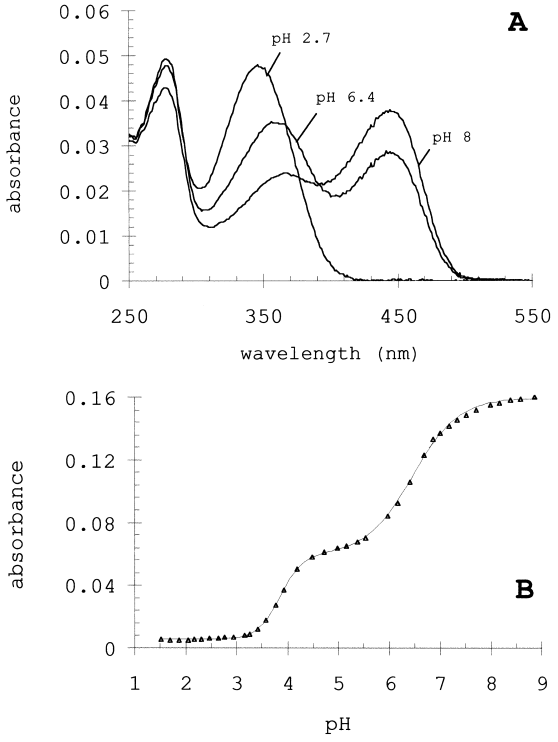

Fig. 4. pH dependence of the absorbance spectrum of *R. sphaeroides* PYP. (A) Absorbance spectra of R-PYP at pH 8, 6.4 and 2.7, normalised to 0 at 550 nm. (B) pH dependence of the absorbance at 446 nm (triangles), fitted with a Henderson–Hasselbalch model (solid line), revealing pK values of 3.8 ($n = 1.95$) and 6.5 ($n = 0.97$). Note that data from A and B were obtained from different experiments.

The photoactive properties of this novel PYP were studied using laser-induced transient absorption measurements of isolated protein. After flashing with a 465 nm laser pulse (6 ns pulse width) the 446 nm peak of the groundstate was bleached, and a blue-shifted intermediate with λ_{max} of 350 nm in the difference–absorbance spectrum was observed as shown in Fig. 2. A difference spectrum is plotted 60 μs after a 465 nm laser flash. Full recovery was observed within 20 ms after excitation. From the absorbance changes at 450 nm (pG; groundstate) a recovery rate constant of 500 ± 100 s^{-1} was determined. Fig. 2B shows the time course of the 450 nm recovery, fitted using a monoexponential function.

The absorption spectra of R-PYP at different temperatures between 3 and 20°C (Fig. 3) show large changes, unlike E-PYP (which displays no significant temperature effects at neutral pH, [27]). The two observed peaks, at 446 nm and 360 nm, can be interconverted by changing the temperature. Decreasing temperature leads to an increase in the absorbance at 360 nm and a decrease at 446 nm (with an isosbestic point at 390 nm), whereas increasing the temperature leads to the opposite behaviour. These effects are completely reversible, as shown by the identical curves in Fig. 3B, for a subsequent temperature decrease and increase.

Moreover, we have studied the spectral changes at different pH values between 9 and 1.5 (Fig. 4). At least two separate transitions could be observed; below pH 9 the absorbance at 446 nm is decreasing, whereas the absorbance at 360 nm is increasing (Fig. 4A). When pH decreases below pH 5, yet another new spectral species is formed, with a clearly blue-shifted absorption maximum at 345 nm (Fig. 4A). Fig. 4B follows the absorbance at 446 nm as a function of pH. The data were fitted with a Henderson–Hasselbalch model, revealing pK values of 3.8 and 6.5, with n values of 1.95 and 0.97, respectively.

Furthermore we reconstituted apo-R-PYP with the analogue 7-hydroxy-coumarin-3-carboxylic acid (for its structure

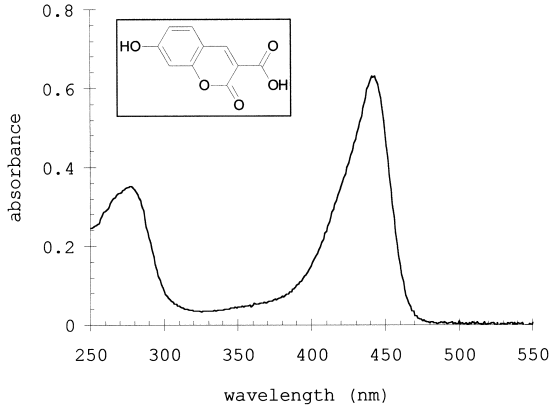

Fig. 5. Absorbance spectrum of *R. sphaeroides* PYP reconstituted with 7-hydroxy-coumarin-3-carboxylic acid. His-tagged apo-protein was purified and subsequently reconstituted with 7-hydroxy-coumarin-3-carboxylic acid. The spectrum was taken after purification on a Ni-affinity column. Inset shows the structure of 7-hydroxy-coumarin-3-carboxylic acid.

see Fig. 5 inset). Fig. 5 shows the absorbance spectrum of this R-PYP hybrid. Only the characteristic blue absorbance with a maximum at 441 nm can be observed in this hybrid.

4. Discussion

We report on the purification, functional reconstitution, and photoactive properties of PYP from *R. sphaeroides*. The absorbance spectrum in Fig. 1 clearly shows the characteristic PYP absorbance at 446 nm. As for all described PYPs this absorbance peak can be bleached by irradiation with blue light. As shown in Fig. 2 a blue-shifted intermediate is formed with a difference–absorbance maximum at 350 nm. This is slightly blue-shifted, as compared with pB from *E. halophila* PYP [16,9]. The pB form of R-PYP relaxes to pG with a rate constant of 500 s^{-1}. This process of recovery is 100-fold faster than that of E-PYP. The formation of pR is also faster than in E-PYP, characterisation of this intermediate lies at the limit of our experimental time resolution. However, the absorption maximum at 446 nm and the ability of the protein to undergo a photocycle indicate that R-PYP has the general characteristic properties of a true PYP. A more detailed investigation of the R-PYP photocycle and the photoreactivity of the 360 nm species is currently ongoing.

The R-PYP absorbance spectrum shows an additional absorption band with a maximum at 360 nm at physiological pH. We confirmed with mass spectrometry that this absorption is a feature of the purified R-PYP, which does not contain two chromophores as theoretically possible due to a second C-terminal cysteine at position 117. Because of the temperature- and pH-dependent equilibrium between the 360 nm and 446 nm forms, we can exclude that the unusual blue-shifted groundstate species is due to the binding of the chromophore at Cys117. The absorption maximum at 446 nm (identical to E-PYP) indicates the localisation of 4-hydroxycinnamic acid in a similar protein environment, thus binding at Cys68 (equivalent to Cys69 in E-PYP). While the chemical reaction between the activated chromophore and the SH group of cysteines should be non-specific, our results emphasise the importance of the pocket for the binding of the chromophore.

E-PYP at low pH, and several mutants of E-PYP have been described in literature, which have groundstate spectra which also show an additional blue-shifted absorption band:

(1) The Met100Ala mutant of PYP from *E. halophila* [28] shows a 350 nm absorbance when kept in room light due to the very slow recovery from pB to pG ($k = 2.1 \times 10^{-3}$ s^{-1}). Whereas incubation of this mutant protein in the dark leads to a full conversion of the 350 nm band into the 446 nm band, the same experiment with R-PYP did not lead to a similar conversion of the 360 nm band into a 446 nm band. Interestingly, R-PYP has, in place of a methionine at position 100, a glycine. Surprisingly, while mutation of Met100Ala in E-PYP leads to a 1000-fold decrease in recovery rate of pG from pB at pH 7, R-PYP with Gly100 has a 100-fold faster recovery to pG compared to wild-type E-PYP. Furthermore the difference–absorbance spectrum pB–pG differs from the temperature-induced difference–absorbance spectrum. Therefore the 360 nm absorption band in R-PYP cannot be caused by a fraction of the protein in the pB intermediate state of the photocycle.

(2) For PYP from *E. halophila* a pH- and temperature-dependent formation of a blue-shifted state was described. This so-called pB_{dark} (λ_{max} at 353 ± 1 nm [16,27]) is formed with a p*K* of 2.77 [29]. Moreover, this blue-shifted form of E-PYP showed an increase in absorbance upon increasing the temperature from 0 to 46°C at pH 3.4. This process is accompanied by a loss in absorption at 446 nm [27]. The interpretation of these data describes the blue-shifted intermediate as partially denatured PYP with a protonated chromophore in *trans* conformation. In contrast, our data for R-PYP show that at pH 8 a decrease of the temperature leads to formation of the blue-shifted 360 nm form (Fig. 3). Therefore, the 360 nm form of R-PYP has a lower free energy than the 446 nm form and thus cannot be explained by a similar partial unfolding as in the case of pB_{dark} in E-PYP. Moreover, by lowering the pH an even more blue-shifted state is replacing the 360/446 nm equilibrium. This low pH blue-shifted state might be a similar state as pB_{dark} of E-PYP with a slightly blue-shifted absorption maximum (345 versus 353 nm) and a p*K* of 3.8 (versus 2.77). Summarising our results on the temperature and pH dependence of the 360/446 nm equilibrium, we conclude that the 360 nm form observed in the groundstate spectrum cannot be pB_{dark}.

(3) Recently, two mutants of E-PYP were described (Glu46Asp and Glu46Ala), which have, in addition to the 444/465 nm absorbance, a blue-shifted band (345/365 nm) at neutral pH [30]. As discussed by the authors, this blue-shifted form represents a state with a protonated chromophore, which is possibly exposed to the aqueous environment. For R-PYP probably the blue-shifted form has also a protonated chromophore, which explains the large shift of about 85 nm with respect to the 446 nm peak [31]. The reason for this shift at neutral pH must be different from that in the Glu46 mutants of E-PYP because Glu46 is still present in the binding pocket of R-PYP and can stabilise the anionic chromophore.

Although several groundstate spectra with blue-shifted absorption peaks are known, none of them is likely to correspond to the 360 nm form observed for R-PYP. Holo-protein reconstituted with a locked chromophore (Fig. 5) leads to an absorption spectrum with a slightly blue-shifted maximum of 441 nm, very similar to E-PYP reconstituted with this type of chromophore [24]. No absorption band similar to the 360 nm form is observed. The lack of this blue-shifted species suggests a different configuration of the chromophore in the blue-shifted state of R-PYP.

The absorption spectra measured at different temperatures and different pH values reveal the existence of a groundstate equilibrium between a 360 nm and a 446 nm state. The relative concentration of the form absorbing at 360 nm is increasing by lowering the pH and/or the temperature (Figs. 3 and 4). From the small temperature change (16°C) necessary to convert about 10% of the protein molecules, it follows that the activation barrier for transitions between these states is low and that the differences between the free energy of the two groundstates is small. Furthermore, the equilibrium between these two states is pH-dependent. The relative concentration of the 446 nm form at pH 5 is about half the concentration at pH 8 (Fig. 4B). Nevertheless, in the whole pH range 4.5–9, both forms are present in significant amounts. This indicates that the observed pH dependence reflects the (de-)protonation of an amino acid in the chromophore binding pocket rather than the p*K* of the chromophore itself. The apparent p*K* (6.5) of this transition is very near to the recently calculated p*K* of

Glu46 (6.37) in E-PYP [32]. Since the identity between the amino acid sequences of E-PYP and R-PYP is only 48% and no crystal structure is available for R-PYP it is not possible to decide whether Glu46 is responsible for the pH-dependent absorption change in R-PYP.

For the same reason, it also still remains to be determined which amino acids are responsible for the faster recovery of pG (compared to E-PYP) and the nature of the species with the blue-shifted absorbance. The pocket for the chromophore in R-PYP has been structurally modelled and shows, besides the crucial conserved amino acids for the binding and stabilisation of the chromophore, also two cavities formed by the substitution of Thr50 and Thr70 by alanines [4]. On the other hand, the 446 nm absorption band of the pG state, with exactly the same maximum as E-PYP, indicates that amino acids for tuning of the chromophore absorbance are indeed conserved.

The absorption spectrum of R-PYP differs from that of E-PYP by the existence of an additional absorption band ($\lambda_{max} = 360$ nm) which is in coexistence with the usual (single) E-PYP absorption band ($\lambda_{max} = 446$ nm). Three major findings characterise this new spectral form: (1) a reversible thermal equilibrium exists between the two forms, (2) a pH titration of R-PYP shows a very similar form at pH 5.5 ($\lambda_{max} = 360$ nm), which is spectrally different from the pB_{dark}-like form which appears at pH values below 3.5 ($\lambda_{max} = 345$ nm) and (3) reconstitution of apo-R-PYP, with a locked chromophore (thereby forcing/locking the chromophore in the *trans* configuration), leads to a holo-protein which does not display the 360 nm absorption. Considering these arguments together we interpret this new groundstate species as an intrinsic property of the *R. sphaeroides* yellow protein.

So far no physiological function for PYP could be assigned. However, the functional characterisation of a PYP from *R. sphaeroides*, a genetically well accessible organism, opens the route towards this goal. The presence of the absorption band at 360 nm in R-PYP suggests that investigations into a physiological photoresponse in *R. sphaeroides* should be extended from blue light to near UV.

Acknowledgements: We thank Betsie Voetdijk, Henk Dekker and Dave Speijer for expert technical assistance.

References

[1] Meyer, T.E. (1985) Biochim. Biophys. Acta 806, 175–183.
[2] Meyer, T.E., Fitch, J.C., Bartsch, R.G., Tollin, G. and Cusanovich, M.A. (1990) Biochim. Biophys. Acta 1016, 364–370.
[3] Koh, M., Van Driessche, G., Samyn, B., Hoff, W.D., Meyer, T.E., Cusanovich, M.A. and Van Beeumen, J.J. (1996) Biochemistry 35, 2526–2534.
[4] Kort, R., Phillips-Jones, M.K., van Aalten, D.M., Haker, A., Hoffer, S.M., Hellingwerf, K.J. and Crielaard, W. (1998) Biochim. Biophys. Acta 1385, 1–6.
[5] Jiang, Z., Swem, L.R., Rushing, B.G., Devanathan, S., Tollin, G. and Bauer, C.E. (1999) Science 285, 406–409.
[6] Dux, P. et al. (1998) Biochemistry 37, 12689–12699.
[7] Perman, B. et al. (1998) Science 279, 1946–1950.
[8] Genick, U.K., Soltis, S.M., Kuhn, P., Canestrelli, I.L. and Getzoff, E.D. (1998) Nature 392, 206–209.
[9] Hendriks, J., van Stokkum, I.H., Crielaard, W. and Hellingwerf, K.J. (1999) FEBS Lett. 458, 252–256.
[10] Genick, U.K. et al. (1997) Science 275, 1471–1475.
[11] Kort, R., Vonk, H., Xu, X., Hoff, W.D., Crielaard, W. and Hellingwerf, K.J. (1996) FEBS Lett. 382, 73–78.
[12] Unno, M., Kumauchi, M., Sasaki, J., Tokunaga, F. and Yamauchi, S. (2000) J. Am. Chem. Soc. 122, 4233–4234.
[13] Borgstahl, G.E., Williams, D.R. and Getzoff, E.D. (1995) Biochemistry 34, 6278–6287.
[14] Kim, M., Mathies, R.A., Hoff, W.D. and Hellingwerf, K.J. (1995) Biochemistry 34, 12669–12672.
[15] Meyer, T.E., Yakali, E., Cusanovich, M.A. and Tollin, G. (1987) Biochemistry 26, 418–423.
[16] Hoff, W.D. et al. (1994) Biophys. J. 67, 1691–1705.
[17] Ujj, L., Devanathan, S., Meyer, T.E., Cusanovich, M.A., Tollin, G. and Atkinson, G.H. (1998) Biophys. J. 75, 406–412.
[18] Pellequer, J.L., Wager-Smith, K.A., Kay, S.A. and Getzoff, E.D. (1998) Proc. Natl. Acad. Sci. USA 95, 5884–5890.
[19] Taylor, B.L. and Zhulin, I.B. (1999) Microbiol. Mol. Biol. Rev. 63, 479–506.
[20] Kort, R., Crielaard, W., Spudich, J.L. and Hellingwerf, K.J. (2000) J. Bacteriol. 182, 3017–3021.
[21] Sprenger, W.W., Hoff, W.D., Armitage, J.P. and Hellingwerf, K.J. (1993) J. Bacteriol. 175, 3096–3104.
[22] Kort, R. et al. (1996) EMBO J. 15, 3209–3218.
[23] Genick, U.K., Devanathan, S., Meyer, T.E., Canestrelli, I.L., Williams, E., Cusanovich, M.A., Tollin, G. and Getzoff, E.D. (1997) Biochemistry 36, 8–14.
[24] Cordfunke, R., Kort, R., Pierik, A., Gobets, B., Koomen, G.J., Verhoeven, J.W. and Hellingwerf, K.J. (1998) Proc. Natl. Acad. Sci. USA 95, 7396–7401.
[25] Laemmli, U.K. (1970) Nature 227, 680–685.
[26] Hendriks, J., Hoff, W.D., Crielaard, W. and Hellingwerf, K.J. (1999) J. Biol. Chem. 274, 17655–17660.
[27] Van Brederode, M.E., Hoff, W.D., Van Stokkum, I.H., Groot, M.L. and Hellingwerf, K.J. (1996) Biophys. J. 71, 365–380.
[28] Devanathan, S., Genick, U.K., Canestrelli, I.L., Meyer, T.E., Cusanovich, M.A., Getzoff, E.D. and Tollin, G. (1998) Biochemistry 37, 11563–11568.
[29] Hoff, W.D., Van Stokkum, I.H.M., Gural, J. and Hellingwerf, K.J. (1997) Biochim. Biophys. Acta 1322, 151–162.
[30] Devanathan, S., Brudler, R., Hessling, B., Woo, T.T., Gerwert, K., Getzoff, E.D., Cusanovich, M.A. and Tollin, G. (1999) Biochemistry 38, 13766–13772.
[31] Hoff, W.D., Devreese, B., Fokkens, R., Nugteren-Roodzant, I.M., Van Beeumen, J., Nibbering, N. and Hellingwerf, K.J. (1996) Biochemistry 35, 1274–1281.
[32] Demchuk, E., Genick, U.K., Woo, T.T., Getzoff, E.D. and Bashford, D. (2000) Biochemistry 39, 1100–1113.