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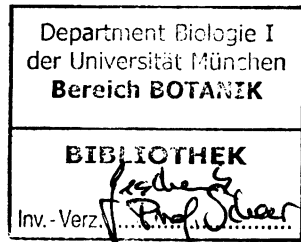
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## CONTENTS

### CONGRESS LECTURE: Japan as a theatre state

*T. Yano*

xxiii

### PHOTOSYNTHESIS

Molecular organization of the photochemical apparatus of oxygenic photosynthesis

*K. Satoh*

3

Photosystem I can control photosystem II in leaves

*U. Heber, E. Katona, G. Schönknecht and K. Asada*

13

Fucoxanthin-chlorophyll *a/c* protein in brown algae: Their molecular assembly and energy transfer

*T. Katoh*

21

Location and relaxation paths of the carotenoid S1 state and its role in energy transfer

*T. Gillbro, P.-O. Andersson, R.S.H. Liu, A.E. Asato and R.J. Cogdell*

25

Phycocyanin: A photoreceptor pigment with two faces

*K.-H. Zhao, Q. Hong, S. Siebzehnrübl and H. Scheer*

31

Blue-light regulation of Cab gene expression in the apical buds of peas and the cotyledons of Arabidopsis

*L.S. Kaufman, J. Gao, K. Bhattacharya, J. Tilghman, J. Marsh,*

*K.A. Marrs and K.M.F. Warpeha*

37

Molecular-genetic analysis of Mg-tetrapyrrole biosynthesis in bacteria and algae

*C.E. Bauer, D. Bollivar, J. Dobrowolski and J. Suzuki*

41

Phosphoenolpyruvate carboxylase for C4 photosynthesis in maize

*K. Izui, S. Yanagisawa, A. Morishima and N. Ogawa*

43

### PHOTOPHYSICS, PHOTOCHEMISTRY AND PHOTOBIOCHEMISTRY

#### Photochemistry of nucleic acids

Photooxidation reactions of nucleic acids

*J. Cadet, M. Berger, G. Buchko, J.-L. Ravanat and H. Kasai*

49

Photoinduced reactions of organic and inorganic substrates with DNA

*H. Morrison, W.M. Baird, S. Farrow, T. Mohammad and L. Paredes*

55

Photofootprinting of DNA <i>P.E. Nielsen</i>	61
Chemical aspects of DNA-protein cross-linking by UV light <i>M.D. Shetlar</i>	67
Photochemistry of 5-halouracil-containing DNA <i>H. Sugiyama, Y. Tsutsumi, E. Yamaguchi and I. Saito</i>	73
<b>Furocoumarines</b>	
The solution structures of psoralen cross-linked and monoadducted DNA oligomers by NMR spectroscopy and restrained molecular dynamics <i>H.P. Spielmann, T.J. Dwyer, J.E. Hearst and D.E. Wemmer</i>	79
C <sub>4</sub> -cycloaddition reactions between furocoumarins and unsaturated fatty acids or lecithins <i>S. Caffieri, Z. Zarebska and F. Dall'Acqua</i>	85
Photochemistry of furocoumarins <i>Sang Chul Shim and Mi Hong Yun</i>	91
Psoralen photochemotherapy and its action mechanisms <i>K. Danno</i>	97
An animal model and new photosensitizers for extracorporeal photochemotherapy <i>H.P. van Iperen and G.M.J. Beijersbergen van Henegouwen</i>	101
<b>Synchrotron-radiation photobiology</b>	
Structural analysis of antigenic variation in <i>Borrelia burgdorferi</i> using ultraviolet synchrotron radiation <i>L.L. France, B. McGrath, J.J. Dunn, B.J. Luft, J. Kieleczawa, G. Hind and J.C. Sutherland</i>	107
DNA damage induced by monochromatic vacuum-UV radiation in solids <i>K. Hieda</i>	115
<b>Rhodopsins and light receptor molecules</b>	
25 years of bacterial rhodopsins <i>W. Stoeckenius</i>	123
Structure changes of bacteriorhodopsin in the L-to-M and M-to-N conversions <i>A. Maeda</i>	135

Ring demethylated 6-s locked methano-bacteriorhodopsins <i>M. Groesbeek and J. Lugtenburg</i>	139
Photochemistry and functions of bilirubin <i>A.F. McDonagh</i>	145
Hydrophobic interaction between retinal analogues and aporetinochrome in the pigment formation <i>K. Tsujimoto, K. Iida, M. Miyazaki, T. Kinumi, Y. Shirasaka, M. Ohashi, M. Sheves, R. Hara, T. Hara and K. Ozaki</i>	147
Structure and function of the ciliate photoreceptors <i>Pill-Soon Song</i>	153
<b>Visual pigments and transduction</b>	
Vision in photobiology <i>T. Yoshizawa</i>	159
<sup>19</sup> F-NMR studies of fluorinated visual pigment analogs <i>R.S.H. Liu, L.U. Colmenares and W. Niemczura</i>	171
Excited state dynamics of retinal proteins by FTOA method <i>T. Kakitani, Y. Hatano, Y. Shichida, Y. Imamoto, F. Tokunaga and H. Kakitani</i>	173
Primary processes in rhodopsin and iodopsin <i>Y. Shichida</i>	179
N-terminal heterogeneous acylation of phototransduction proteins <i>J.B. Hurley, T.A. Neubert, A.M. Dizhoor, Ching-Kang Chen, E. Olshevskaya, R.S. Johnson and K.A. Walsh</i>	183
Regulation of signal coupling proteins in octopus photoreceptors <i>M. Tsuda, T. Iwasa, M. Nakagawa, S. Kikkawa and T. Tsuda</i>	189
Roles of lipids linked to $\alpha$ - and $\gamma$ -subunits of photoreceptor G protein <i>Y. Fukada</i>	195
Role of rhodopsin kinase and arrestin in the quenching of phototransduction <i>K. Palczewski</i>	201
<b>Ultrafast reaction by laser light and spectroscopy</b>	
Recombination dynamics of photodissociated CO of myoglobin and its E7 mutants studied by time-resolved resonance Raman spectroscopy <i>T. Kitagawa, Y. Sakan, T. Ogura, F.A. Fraunfelder, R. Mattera and M. Ikeda-Saito</i>	205
Femtosecond spectroscopy of the primary electron transfer in photosynthetic reaction centers <i>C. Lauterwasser, U. Finkeler, A. Struck, H. Scheer and W. Zinth</i>	209

DNA-protein interaction imagery using high-intensity lasers <i>D. Angelov and I. Pashev</i>	215
Recent solid state NMR studies of bacteriorhodopsin <i>J. Herzfeld, M. Auger, M.R. Farrar, K.V. Lakshmi, A.E. McDermott, J. Raap, L.K. Thompson, C.M. van der Wielen, J. Lugtenburg and R.G. Griffin</i>	221
Femtosecond studies of primary photoprocesses in octopus rhodopsin <i>T. Kobayashi, M. Taiji, K. Bryl, M. Nakagawa and M. Tsuda</i>	227
Structure and dynamics of the charge separated state $P^+Q^-$ of photosynthetic reaction centres from transient EPR spectroscopy <i>D. Stehlik, I. Sieckmann and A. van der Est</i>	233
<b>Bio- and chemi-luminescence</b>	
Bioluminescence of the $Ca^{2+}$ -binding photoprotein aequorin <i>F.I. Tsuji, S. Inouye, Y. Ohmiya and M. Ohashi</i>	239
Recent advances of chemiluminescent and bioluminescent enzyme immunoassay <i>A. Tsuji, M. Maeda, H. Arakawa, K. Itoh, M. Kitamura and N. Murakami</i>	243
The role of superoxide anion in bioluminescence <i>O. Shimomura</i>	249

## PHOTOBIOLOGY AND PHOTOIMMUNOLOGY

### Chronobiology

A circadian pacemaker in the suprachiasmatic nucleus and its interaction with light <i>S.-I.T. Inouye, K. Shinohara, K. Tominaga, Y. Otori, C. Fukuhara and J. Yang</i>	257
Circadian organization in birds: Different pacemakers for feeding and locomotor activity rhythms? <i>S. Ebihara and E. Gwinner</i>	263
Circadian photoreception in reptiles and mammals <i>R.G. Foster, S. Argamaso, W.J. DeGrip, J.M. Garcia-Fernandez and I. Provencio</i>	267

## Molecular approaches to plant development

Phytochrome-mediated light signal transduction in plants: Structure function and possible involvement of G-proteins <i>Pill-Soon Song</i>	273
Genetic engineering of phytochrome molecules <i>M. Furuya</i>	279
Control of gene expression by light, nitrate and a plastidic factor <i>H. Mohr, A. Neininger and B. Seith</i>	283
Molecular genetic approaches to plant photomorphogenesis <i>A. Pepper, T. Delaney, P. Nagpal, J. Reed, D. Poole, M. Furuya and J. Chory</i>	285
Cytoskeletal rearrangement during photomorphogenesis of fern protonemata <i>M. Wada</i>	291

## DNA damage, repair and UV mutagenesis

Analysis of DNA excision repair genes in XP <i>K. Tanaka</i>	293
DNA repair genes and proteins of yeast and human <i>S. Prakash and L. Prakash</i>	303
Recent biochemical studies of the xeroderma pigmentosum group A complementing protein involved in nucleotide excision repair of DNA <i>C.J. Jones and R.D. Wood</i>	309
Symposium: Human DNA repair diseases <i>J.E. Cleaver, L.H.F. Mullenders, M. Stefanini, A.R. Lehmann and H. Takebe</i>	315
Isolation of <i>Arabidopsis thaliana</i> mutants hypersensitive to UV-B light or ionizing radiation <i>G.R. Harlow, M.E. Jenkins, C. Davies and D.W. Mount</i>	319
SOS-inducible recombination repair genes: Products of the <i>ruvA</i> , <i>ruvB</i> , and <i>ruvC</i> genes are Holliday junction specific enzymes <i>H. Shinagawa, H. Iwasaki, M. Takahagi, T. Shiba and A. Nakata</i>	323
Regulation of DNA repair genes in <i>Saccharomyces cerevisiae</i> <i>G.B. Sancar and J. Sebastian</i>	329
Two types of photoreactivation enzyme identified in <i>Drosophila melanogaster</i> <i>T. Todo, H. Takemori and H. Ryo</i>	333



Biological role of (6-4) photoproducts and cyclobutane dimers <i>D.L. Mitchell, G.P. Pfeifer, J.-S. Taylor, M.Z. Zdzienicka and O. Nikaido</i>	337
Photoreactivation in human skin in situ <i>B.M. Sutherland, H. Hacham, R.W. Gange and J.C. Sutherland</i>	345
Photoreactivation: Perspectives and dimensions <i>B.M. Sutherland and T. Ohnishi</i>	347
<i>Neurospora crassa</i> photolyase: Diversity in photoreactivation action spectra <i>A.P.M. Eker, H. Yajima and A. Yasui</i>	349
Regulation of photorepair in fish cells <i>H. Mitani, S. Yasuhira, T. Funayama, M. Kondo and A. Shima</i>	351
Functional analysis of <i>Escherichia coli</i> DNA photolyase <i>K. Yamamoto, M. Ihara and T. Ohnishi</i>	353
Primary structures of photolyases from microorganisms <i>A. Yasui</i>	355
Understanding spectra of UV-induced mutations: Studies with individual photoproducts <i>P. Gibbs, M. Horsfall, A. Borden, B.J. Kilbey and C.W. Lawrence</i>	357
Molecular analysis of UV and EMS induced mutations in a human tumor cell line <i>M. Meuth and A. Tachibana</i>	363
Two <i>umuDC</i> -like operons in <i>Salmonella typhimurium</i> : Their roles in UV mutagenesis <i>T. Nohmi, M. Yamada, M. Watanabe, M. Matsui, S. Y. Murayama and T. Sofuni</i>	369
Gene specific DNA repair and molecular mutation spectra <i>A.A. van Zeeland, L.F.H. Mullenders, M.Z. Zdzienicka and H. Vrieling</i>	375
Symposium - Historical background of DNA repair <i>C.S. Rupert, R.B. Setlow, K.C. Smith, J.E. Cleaver, S. Kondo and J. Jagger</i>	379
<b>Photoimmunology</b>	
The role of Interleukin-10 in the induction of systemic immunosuppression following UV exposure <i>S.E. Ullrich and J.M. Rivas</i>	389
Effects of ultraviolet radiation on granuloma formation <i>H. Okamoto, K. Danno, Z.-P. Guo and S. Imamura</i>	395

## PHOTOMEDICINE

### Photocarcinogenesis

- Photocarcinogenesis: Past, present and future  
*F. Urbach* 403
- Sunlight exposure and melanoma - Is timing and character of exposure important?  
*R.P. Gallagher* 415
- Action spectrum for melanoma induction  
*R.B. Setlow and E. Grist* 421

### Advances in photodermatology

- Boundary between UVA and UVB  
*M.J. Peak and J.C. van der Leun* 425
- Human skin photoprotection and advances in sunscreens  
*M.A. Pathak* 429
- Changes of elastic fiber according to ageing process in human skin  
*C.H. Oh* 437
- Oxidative stress in cutaneous photoaging  
*Y. Miyachi* 443
- The pathogenesis of solar urticaria  
*T. Horio* 447
- The porphyrias  
*M. Kondo, Y. Yano, G. Urata and M. Shirataka* 449
- Skin photosensitivity reactions in porphyrias  
*M.A. Pathak* 455
- Biological significance of photosensitized lipid peroxidation in drug-induced phototoxicity  
*I. Matsuo, M. Ohkido, H. Fujita and M. Sasaki* 461
- Recent advances in polymorphous light eruption  
*E. Hölzle* 463
- Chronic actinic dermatitis  
*Y. Funasaka, M. Kondoh, A. Itoh, M. Ueda and M. Ichihashi* 469
- Drug-induced photosensitive skin diseases  
*R. Kamide* 475
- Protective effect of  $1\alpha,25$ -dihydroxyvitamin  $D_3$  against UVB injury - Possible role of the vitamin  $D_3$ -induced metallothionein  
*K. Hanada, T. Sugawara, Y. Ohishi and I. Hashimoto* 479

Photoprotection: Possible role of metallothionein against UVB injury <i>K. Hanada</i>	483
Topical PUVA for psoriasis <i>S. Kawara and T. Hirone</i>	487
<b>Photosensitizers and photodynamic therapy</b>	
Photo-chlorin (ATX-S10) as a new photosensitizer for PDT <i>S. Nakajima, I. Sakata, T. Takemura and H. Hayashi</i>	493
Detection of bronchial dysplasia and carcinoma <i>in-situ</i> using laser induced fluorescence <i>S. Lam, C. MacAulay, J.C. Leriche, J. Hung and B. Palcic</i>	497
Optimizing the photodetection of early cancer <i>G. Wagnières, D. Braichotte, Ph. Monnier, R. Bays, J.-M. Calmes, J.-C. Givel, S. Folli, A. Pèlegrin, J.-P. Mach and H. van den Bergh</i>	499
Modes of biodistribution of photosensitizing agents <i>D. Kessel</i>	501
Mechanism of photodynamic therapy: Exploration by photophysicochemical study <i>T. Takemura, S. Nakajima and I. Sakata</i>	503
Integral laser-photodynamic treatment of refractory multifocal bladder tumors with special reference to carcinoma <i>in situ</i> <i>K. Naito and H. Hisazumi</i>	507
Photodynamic therapy and early response gene induction <i>C.J. Gomer, M. Luna, S. Wong, P. Ziolkowski and A. Ferrario</i>	511

## SOLAR UV LIGHT AND ENVIRONMENT

### Environment and solar UV light

UV-B observation network in the Japan Meteorological Agency <i>T. Ito</i>	515
UV-exposure and ozone monitoring with a dual bandpass solar UVA-UVB meter in Stockholm since 1989 <i>U. Wester</i>	519
UV radiation in the tropics (1979-1989) <i>M. Ilyas</i>	523
UV solar spectral irradiance in New Zealand <i>R.L. McKenzie</i>	527

**Biological and medical consequences of ozone depletion**

Biological consequences of stratospheric ozone depletion <i>T.P. Coohill</i>	531
Immunological effects of UV-B radiation <i>M.L. Kripke and A. Jeevan</i>	537
Effects of elevated ultraviolet-B-radiation, temperature and CO <sub>2</sub> on growth and function of sunflower and corn seedlings <i>M. Tevini and U. Mark</i>	541
UV-B effects on phytoplankton <i>D.-P. Häder</i>	547
<b>Effects of solar UV light on plants</b>	
Harmful and beneficial effects of solar UV light on plant growth <i>T. Hashimoto, N. Kondo and T. Tezuka</i>	551
UV-induced events at plant plasma membranes <i>T.M. Murphy, Y.C. Qian, C.K. Auh and C. Verhoeven</i>	555
Solar UV and oxidative stress in algal-animal symbioses <i>J.M. Shick</i>	561
<b>Index of authors</b>	565

## Phycocerythrocyanin: A Photoreceptor Pigment With Two Faces

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### INTRODUCTION

Biliproteins are the major light-harvesting antenna pigments of cyanobacteria, red and cryptophyte algae. Two of them, viz. allophycocyanin (APC)<sup>\*\*</sup> and phycocyanin (PC) are constitutive in cyanobacteria and red algae, and many species contain in addition either phycoerythrin (PE) or phycoerythrocyanin (PEC) to improve the light-harvesting capabilities in the green spectral region. These phycobiliproteins are organized together with linker polypeptides (which may or may not contain additional chromophores) in supramolecular structures, the phycobilisomes (PBS). Light energy is absorbed strongly by them and transferred efficiently to the photosynthetic reaction center [1-4].

Besides being photosynthetic antenna pigments, biliproteins can have rather different functions. In higher plants and some algae, a biliprotein, phytochrome, is an important sensory pigment which is essential for many lightinduced developmental responses [5]. Although the transduction chain is still only poorly understood, the primary sequence of events has similarities to those found in the animal photoreceptors, the rhodopsins. In cyanobacteria and red algae, a fraction of the phycobiliproteins, or a small pool of structurally distinct biliproteins, has also been implied in complementary chromatic adaptation and photomorphogenesis, although the relationship is still only circumstantial and no such "adaptachrome" or "photomorphochrome" [6] has yet been identified [7-9].

The binding and conformation of the bilin chromophores of the antenna (APC, PC, PE, PEC) and sensory biliproteins (phytochrome) are remarkably similar. The chromophores are covalently attached via thioether linkages, and they are present in a rigid and energetically unfavorable extended conformation [9-12]. Another common feature of both classes is that their excited states show greatly reduced internal conversion to the ground state [see 9]. However, their response to light is nonetheless rather different. The sensory pigment(s) are photochemically reactive and show little to no detectable fluorescence [5]. Isolated antenna biliproteins have, by contrast, a high fluorescence quantum yield and correspondingly long lifetimes of the excited states [13,14]. A minor photochemical response can be only

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\* ) **Abbreviations:** PBS = phycobilisome, PEC = phycoerythrocyanin, PC= phycocyanin, M.= *Mastigocladus*, KPP = potassium phosphate buffer, SDS-PAGE = sodium dodecylsulfate-polyacrylamide gel electrophoresis

induced in PC and APC by a variety of mildly denaturing conditions which may "soften" the chromophore environment(s) [see 7]. While these properties can be readily rationalized as being important to their different functions, the molecular basis for their distinctive properties is still unknown.

PEC [15] is a remarkable exception to these rules. When incorporated into the PBS, it has the properties of an antenna pigment, whereas after isolation it shows a pronounced photoreversibly photochromic response [7,8,15,16] similar to that of phytochrome [17,18]. Furthermore, considerable variations in the extent and difference spectra of the reversible photochemistry were observed [7,16,17,19-21]. Even though the evidence for a sensory function of PEC is still indirect [22], the pigment is then useful to study the factors responsible for the photochemical and photophysical properties of biliprotein chromophores. Here, a summary is given on recent studies from our laboratory with PEC from two cyanobacteria, viz. *Mastigocladus laminosus* and *Westiellopsis prolifica*.

#### DIFFERENT ISOLATES

Similar to the Füglistaller's report [23], chromatography of crude extract from *Mastigocladus laminosus* on DEAE-cellulose yielded an early eluting PEC I and a second fraction (PEC II) eluting at higher ionic strengths. Both had similar absorption spectra and SDS-Page patterns, but the extent of reversible photochemistry (measured as  $\Delta\Delta A^*$  [21]) was always distinctly lower for PEC I than for PEC II. Chromatography of a crude extract from *Mastigocladus laminosus* yielded PEC I and PEC II in a ratio of 0.8 to 1. If the same crude extract was instantly frozen with liquid nitrogen, kept for two months at  $-20^\circ\text{C}$ , and chromatographed under similar conditions, this ratio increased to 3. Either, PEC I is then derived from PEC II, or PEC II is more labile and degraded during storage.

A similar chromatography of an extract from *Westiellopsis prolifica* yielded a third fraction which is hardly adsorbed on the column at all and was termed PEC(X). It lacked the long-wavelength shoulder due to the two cyanin chromophores on the  $\beta$ -subunit, it showed the SDS-PAGE mobility of a PEC  $\alpha$ -subunit, and its  $\alpha$ -terminal sequence was very similar to that of the  $\alpha$ -subunit of PEC from *M. laminosus*. It is then concluded that PEC(X) is a free  $\alpha$ -subunit or a derivative thereof. The isolation of a free  $\alpha$ -subunit

---

\*)  $\Delta\Delta A$  has been defined similar as in phytochrome research [21]: The sample is irradiated saturatingly at one effective wavelength, generally 500 nm, and the absorption difference determined at the two maximum-response wavelengths (generally 500 and 570 nm). The sample is then irradiated saturatingly at a second effective wavelength (generally 570 nm), and the difference absorption determined again.  $\Delta\Delta A$  is the difference of these differences, normalized to the 570 nm absorption after a saturating irradiation at 500 nm.

is not without precedence: In her attempt to isolate photochromic pigments from cyanobacteria, G. Björn [24] characterized a pigment termed phycochrome b from several species, and it is most likely that this pigment is identical to the PEC  $\alpha$ -subunits.

#### AGGREGATION STATE

The aggregation state is an important factor controlling the extent of photochemistry.  $\Delta\Delta A$ -values (see above) are maximum for monomers ( $\alpha\beta$ ), they decrease upon aggregation to trimers and are close to zero for PEC in the PBS [21]. As an increased rigidity is likely upon aggregation, we see this as an argument that photochemistry requires some flexibility of the protein environment of the chromophore(s).

Possibly even more importantly, there is also an inverse effect of the photochemistry on the aggregation behavior of isolated PEC. Whereas the 570 nm absorbing form aggregates like other phycobiliproteins to trimers ( $\alpha\beta$ ), aggregation is impeded after conversion to the 500 nm absorbing forms. The  $\alpha$ -84 phycobiliviolin chromophore, which probably isomerizes from the 15Z ( $\lambda_{\text{max}} \approx 570$  nm) to the 15E-configuration ( $\lambda_{\text{max}} \approx 500$  nm), is located on the contact surface between the monomeric ( $\alpha\beta$ ) components in the torus-shaped trimer, and may be involved in two inter-monomer hydrogen bonds [11]. This situation is again reminiscent of the situation in phytochrome. The intramolecular dissociation of one domain from the chromophore domain, has been discussed as one event in the signal transducing chain following the light-induced 15Z-E isomerization of the chromophore.

#### REGULATION OF PHOTOCHEMICAL ACTIVITY

For a long time, we have been puzzled by the poorly reproducible photochemistry of different PEC preparations. Kufer and Björn [16] published spectra for the  $\alpha$ -subunit corresponding to  $\Delta\Delta A$ -values close to 70%, whereas even the most active preparations in our hands gave a  $\Delta\Delta A$  of only <20%. The results of a systematic investigation of environmental factors other than aggregation on the photochemistry, can be summarized as follows: 1) PEC I and PEC II both showed variable photochemistry, but that of PEC II was consistently higher than that of PEC I. 2) After dialysis against increasing concentrations of KPP buffer, the photochemistry increased first (5 mM to 500 mM), but decreased again at 1 M. 3) Both PEC fractions showed higher amplitudes of the photochemical response with increasing (7 to 9) and decreasing pH (7.0-5.0). 4) The photochemistry of PEC II could be enhanced further by addition of KSCN to 1 M, which is known to dissociate phycobiliprotein aggregates to monomers [25,26]. 5) The effect of increasing pH is still present in the presence of KSCN, while that of low pH is leveled.

## A SECOND TYPE OF PHOTOCHEMISTRY

Since the photochemically active chromophore is  $\alpha$ -84, further studies concentrated on the  $\alpha$ -subunit. Upon electrofocusing under denaturing conditions, both PEC I and PEC II yielded two bands each with the spectral characteristics of the  $\alpha$ -subunit, which had pairwise the same isoelectric points. No significant difference was found among the four fractions on SDS-PAGE, but considerable differences in their  $\Delta\Delta A$  values. When irradiated alternately with 577 and 600 nm, they all showed a different type of response characteristic of a line broadening rather than a line shift (difference maxima at 504 and 594 nm, minimum at 565 nm). This reversible response, which was termed type II photochemistry, had been observed previously [19] with integral PEC, but could be studied more clearly in one of the four fractions in which the type I photochemistry was almost lacking. The response of all  $\alpha$ -subunit fractions to ionic strength was negligible, and the response to pH strongly decreased. This may indicate again, that interactions among the subunits is an important factor. However, the differences among the different fractions still remain puzzling.

## EFFECT OF SH-GROUP DIRECTED REAGENTS

Reagents known to react with SH-groups showed a remarkable effect on the photochemistry of subunits: The type I response of all four subunit fractions was increased to  $\Delta\Delta A > 70\%$  in the presence 2-mercaptoethanol (5 mM). It remained high even after 2-mercaptoethanol was dialysed out, but decreased again slowly upon storage. The type II response is barely discernible under these conditions. On the other hand, addition of p-chloro-mercury-benzenesulfonate (PCMS) resulted in the inverse effect. Type I photochemistry was strongly decreased. That of type II was clearly discernible, but it is not clear if it increased, or was only buried before under the intense type I-response. This modulation is fully reversible.

The reversibility with PCMS, and the persistent high photochemistry if 2-mercaptoethanol is dialyzed out of the sample, provide good evidence that (modification of) SH-group(s) plays a dominating role in controlling the photochemistry. Besides the cysteine which binds the  $\alpha$ -84 chromophore and does not react with PCMS, there are two free cysteines ( $\alpha$ -98 and  $\alpha$ -99) in the PEC  $\alpha$ -subunit. They are located on helix E [11] close to the loop connecting it to helix F, at a distance of  $>17$  Å from the nearest edge of the chromophores. A direct interaction is unlikely over such a distance, but since the chromophore is bound to helix E and in contact with helix F, an indirect effect can be rationalized by which e.g. the mobility of the chromophore is affected.

Irrespective of the details, the possibility to obtain reproducibly subunits with a maximum photochemical response of type I by treatment with 2-mercaptoethanol, provides for the first time an access to polypeptides



highly enriched in either the chromophore of the 570 or the 500 nm form. By the same token, the treatment of  $\alpha$ -subunits with PCMS provides samples in which the type II photochemistry maximum. If one takes into account that the two cysteines can be oxidized by air to a similar form with low type I and high type II activity, this would provide a ready explanation for the rather different photochemical signals found previously for  $\alpha$ -subunits.

Scharnagl [27] has provided recently a model for biliproteins, in which the microenvironment of the chromophores is very heterogeneous. Possibly, the line-broadening features of the type II-reponse, can be explained by a photochemically induced shift of the population equilibrium of the  $\alpha$ -84 chromophore. Changes in the orientation of nearby aromatic amino acids like His- $\alpha$ 90, may well influence ring D of the phycobiliviolin chromophore in PEC.

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## Index of Authors

- Andersson, P.-O., 25  
 Angelov, D., 215  
 Arakawa, H., 243  
 Argamaso, S., 267  
 Asada, K., 13  
 Asato, A.E., 25  
 Auger, M., 221  
 Auh, C.K., 555
- Baird, W.M., 55  
 Bauer, C.E., 41  
 Bays, R., 499  
 Beijersbergen van Henegouwen, G.M.J.,  
 101  
 Berger, M., 49  
 Bhattacharya, K., 37  
 Bollivar, D., 41  
 Borden, A., 357  
 Braichotte, D., 499  
 Bryl, K., 227  
 Buchko, G., 49
- Cadet, J., 49  
 Caffieri, S., 85  
 Calmes, J.-M., 499  
 Ching-Kang Chen, 183  
 Chory, J., 285  
 Cleaver, J.E., 315, 379  
 Cogdell, R.J., 25  
 Colmenares, L.U., 171  
 Coohill, T.P., 531
- Dall'Acqua, F., 85  
 Danno, K., 97, 395  
 Davies, C., 319  
 DeGrip, W.J., 267  
 Delaney, T., 285  
 Dizhoor, A.M., 183  
 Dobrowolski, J., 41  
 Dunn, J.J., 107  
 Dwyer, T.J., 79
- Ebihara, S., 263  
 Eker, A.P.M., 349
- Farrar, M.R., 221  
 Farrow, S., 55  
 Ferrario, A., 511  
 Finklele, U., 209  
 Folli, S., 499  
 Foster, R.G., 267  
 France, L.L., 107  
 Fraunfelter, F.A., 205  
 Fujita, H., 461  
 Fukada, Y., 195  
 Fukuhara, C., 257  
 Funasaka, Y., 469  
 Funayama, T., 351  
 Furuya, M., 279, 285
- Gallagher, R.P., 415  
 Gange, R.W., 345  
 Gao, J., 37  
 Garcia-Fernandez, J.M., 267  
 Gibbs, P., 357  
 Gillbro, T., 25  
 Givel, J.-C., 499  
 Gomer, C.J., 511  
 Griffin, R.G., 221  
 Grist, E., 421  
 Groesbeek, M., 139  
 Guo, Z.-P., 395  
 Gwinner, E., 263
- Hacham, H., 345  
 Häder, D.-P., 547  
 Hanada, K., 479, 483  
 Hara, R., 147  
 Hara, T., 147  
 Harlow, G.R., 319  
 Hashimoto, I., 479  
 Hashimoto, T., 551  
 Hatano, Y., 173  
 Hayashi, H., 493  
 Hearst, J.E., 79  
 Heber, U., 13  
 Herzfeld, J., 221  
 Hieda, K., 115  
 Hind, G., 107  
 Hirone, T., 487

- Hisazumi, H., 507  
Hölzle, E., 463  
Hong, Q., 31  
Horio, T., 447  
Horsfall, M., 357  
Hung, J., 497  
Hurley, J.B., 183
- Ichihashi, M., 469  
Ihara, M., 353  
Iida, K., 147  
Ikeda-Saito, M., 205  
Ilyas, M., 523  
Imamoto, Y., 173  
Imamura, S., 395  
Inouye, S., 239  
Inouye, S.-I.T., 257  
Ito, T., 515  
Itoh, A., 469  
Itoh, K., 243  
Iwasa, T., 189  
Iwasaki, H., 323  
Izui, K., 43
- Jagger, J., 379  
Jeevan, A., 537  
Jenkins, M.E., 319  
Johnson, R.S., 183  
Jones, C.J., 309
- Kakitani, H., 173  
Kakitani, T., 173  
Kamide, R., 475  
Kasai, H., 49  
Kato, T., 21  
Katona, E., 13  
Kaufman, L.S., 37  
Kawara, S., 487  
Kessel, D., 501  
Kieleczawa, J., 107  
Kikkawa, S., 189  
Kilbey, B.J., 357  
Kinumi, T., 147  
Kitagawa, T., 205  
Kitamura, M., 243  
Kobayashi, T., 227  
Kondo, M., 351, 449  
Kondo, N., 551  
Kondo, S., 379
- Kondoh, M., 469  
Kripke, M.L., 537
- Lakshmi, K.V., 221  
Lam, S., 497  
Lauterwasser, C., 209  
Lawrence, C.W., 357  
Lehmann, A.R., 315  
Leriche, J.C., 497  
Liu, R.S.H., 25, 171  
Luft, B.J., 107  
Lugtenburg, J., 139, 221  
Luna, M., 511
- MacAulay, C., 497  
Mach, J.-P., 499  
Maeda, A., 135  
Maeda, M., 243  
Mark, U., 541  
Marrs, K.A., 37  
Marsh, J., 37  
Matsui, M., 369  
Matsuo, I., 461  
Mattera, R., 205  
McDermott, A.E., 221  
McDonagh, A.F., 145  
McGrath, B., 107  
McKenzie, R.L., 527  
Meuth, M., 363  
Mi Hong Yun, 91  
Mitani, H., 351  
Mitchell, D.L., 337  
Miyachi, Y., 443  
Miyazaki, M., 147  
Mohammad, T., 55  
Mohr, H., 283  
Monnier, Ph., 499  
Morishima, A., 43  
Morrison, H., 55  
Mount, D.W., 319  
Mullenders, L.F.H., 315, 375  
Murakami, N., 243  
Murayama, S.Y., 369  
Murphy, T.M., 555
- Nagpal, P., 285  
Naito, K., 507  
Nakagawa, M., 189, 227  
Nakajima, S., 493, 503

- Nakata, A., 323  
 Neininger, A., 283  
 Neubert, T.A., 183  
 Nielsen, P.E., 61  
 Niemczura, W., 171  
 Nikaido, O., 337  
 Nohmi, T., 369
- Ogawa, N., 43  
 Ogura, T., 205  
 Oh, C.H., 437  
 Ohashi, M., 147, 239  
 Ohishi, Y., 479  
 Ohkido, M., 461  
 Ohmiya, Y., 239  
 Ohnishi, T., 347, 353  
 Okamoto, H., 395  
 Olshevskaia, E., 183  
 Otori, Y., 257  
 Ozaki, K., 147
- Palcic, B., 497  
 Palczewski, K., 201  
 Paredes, L., 55  
 Pashev, I., 215  
 Pathak, M.A., 429, 455  
 Peak, M.J., 425  
 Pèlegri, A., 499  
 Pepper, A., 285  
 Pfeifer, G.P., 337  
 Pill-Soon Song, 153, 273  
 Poole, D., 285  
 Prakash, L., 303  
 Prakash, S., 303  
 Provencio, I., 267
- Qian, Y.C., 555
- Raap, J., 221  
 Ravanat, J.-L., 49  
 Reed, J., 285  
 Rivas, J.M., 389  
 Rupert, C.S., 379  
 Ryo, H., 333
- Saito, I., 73  
 Sakan, Y., 205  
 Sakata, I., 493, 503  
 Sancar, G.B., 329
- Sang Chul Shim, 91  
 Sasaki, M., 461  
 Satoh, K., 3  
 Scheer, H., 31, 209  
 Schönknecht, G., 13  
 Sebastian, J., 329  
 Seith, B., 283  
 Setlow, R.B., 379, 421  
 Shetlar, M.D., 67  
 Sheves, M., 147  
 Shiba, T., 323  
 Shichida, Y., 173, 179  
 Shick, J.M., 561  
 Shima, A., 351  
 Shimomura, O., 249  
 Shinagawa, H., 323  
 Shinohara, K., 257  
 Shirasaka, Y., 147  
 Shirataka, M., 449  
 Siebzehrnühl, S., 31  
 Sieckmann, I., 233  
 Smith, K.C., 379  
 Sofuni, T., 369  
 Spielmann, H.P., 79  
 Stefanini, M., 315  
 Stehlik, D., 233  
 Stoeckenhuis, W., 123  
 Struck, A., 209  
 Sugawara, T., 479  
 Sugiyama, H., 73  
 Sutherland, B.M., 345, 347  
 Sutherland, J.C., 107, 345  
 Suzuki, J., 41
- Tachibana, A., 363  
 Taiji, M., 227  
 Takahagi, M., 323  
 Takebe, H., 315  
 Takemori, H., 333  
 Takemura, T., 493, 503  
 Tanaka, K., 293  
 Taylor, J.-S., 337  
 Tevini, M., 541  
 Tezuka, T., 551  
 Thompson, L.K., 221  
 Tilghman, J., 37  
 Todo, T., 333  
 Tokunaga, F., 173  
 Tominaga, K., 257

Tsuda, M., 189, 227  
Tsuda, T., 189  
Tsuji, A., 243  
Tsuji, F.I., 239  
Tsujimoto, K., 147  
Tsutsumi, Y., 73

Ueda, M., 469  
Ullrich, S.E., 389  
Urata, G., 449  
Urbach, F., 403

Van den Bergh, H., 499  
Van der Est, A., 233  
Van der Leun, J.C., 425  
Van der Wielen, C.M., 221  
Van Iperen, H.P., 101  
Van Zeeland, A.A., 375  
Verhoeven, C., 555  
Vrieling, H., 375

Wada, M., 291  
Wagnières, G., 499  
Walsh, K.A., 183  
Warpeha, K.M.F., 37  
Watanabe, M., 369  
Wemmer, D.E., 79  
Wester, U., 519  
Wong, S., 511  
Wood, R.D., 309

Yajima, H., 349  
Yamada, M., 369  
Yamaguchi, E., 73  
Yamamoto, K., 353  
Yanagisawa, S., 43  
Yang, J., 257  
Yano, Y., 449  
Yasuhira, S., 351  
Yasui, A., 349, 355  
Yoshizawa, T., 159

Zarebska, Z., 85  
Zdzienicka, M.Z., 337, 375  
Zhao, K. -H., 31  
Zinth, W., 209  
Ziolkowski, P., 511