Information for Contributors

Contributions to Z. Naturforsch., Section C (biological sciences), may be (i) original papers, (ii) research notes not exceeding two pages in print and (iii) reports on current research of special interest. Two copies of each contribution should be sent to Dr. Helga Hausen, P.O. Box 2645, D-7400 Tübingen. (Section B (chemical sciences): Dr. Heide Voelter, P.O. Box 2645, D-7400 Tübingen; Section A (physical sciences): Mrs. Tamara Littmann, Lenzhalde 21, D-7082 Oberkochen).

Contributors are encouraged to suggest possible reviewers of their papers. The manuscript should be type written using double spacing throughout.

The title should be concise but informative. The names of the authors, with first names and initials, and of the institution where the work was carried out follow the title. A running title with not more than 60 characters should be indicated if the title is longer than this.

In a footnote on the first page, please give an address for reprint requests.

The title, a selfconsistent abstract, suitable for direct use by the abstracting journals (no references to the main text), and five keywords, all in English, must precede the main text of each contribution.

The main text should meet the highest standards as to novelty of the material, organization and conciseness. A qualified colleague and, if the text is in a foreign language, a person who thoroughly knows it, should have been given the opportunity to check the paper before its submission for publication.

Papers, reporting mainly spectroscopic, X-ray or other data of no general relevancy should not be submitted.

References and footnotes should be numbered (e.g. [1]) and listed at the end of the paper (e.g. [1] A. Meyer, Z. Naturforsch. 30c, 633 (1976)).

Tables with the appropriate captions and a list of the figure legends should follow at the end of the paper.

Allowance should be made for the reduction in printing of the drawings (line thickness, lettering!). Original drawings larger than 21 × 30 cm should be replaced by copies of reduced size, when the manuscript is submitted and only be sent in when the manuscript is accepted. On all illustrations, the figure number and the author’s name must be written in pencil.

The authors will receive page proofs.

Changes in the text after acceptance of the paper and drawings not fit for direct reproduction cause delay and create extra costs which may be charged to the author. 50 reprints are free of charge; additional reprints cost DM .20 per page.

Information for Subscribers

The subscription prices per year are for normal subscribers:

Section A Section B Section C
DM 560.– DM 670.– DM 485.–

for authors and their institutions if they order directly from the publishers:

Section A Section B Section C
DM 448.– DM 536.– DM 388.–

plus postage and handling

Section A Section B Section C
DM 40.– DM 40.– DM 40.–

Single copies and back-numbers are available.

Subscriptions will remain standing for the following year unless cancellations are made by postage October 1st.

Information for Advertisers

The price for a small advertisement (breadth 43 mm, height 57 mm) in all three sections of the journal is DM 60.–.

For larger advertisements please order the price list from the publishers.
Invited Trends Article

Elicitor Recognition and Signal Transduction in Plant Defense Gene Activation
D. SCHEEL and J. E. PARKER 569

Original Communications

Porin of Rhodobacter capsulatus: Biochemical and Functional Characterization
D. WOITZIK, J. WECKESSER, R. BENZ, S. STEVANOVIC, G. JUNG, and J. P. ROSENBUSCH 576

The Biflavonoid Pattern of Rhytidiadelphus squarrosus (Hedw.) Warnst.
T. SEEGER, H. D. ZINSMEISTER, and H. GEIGER 583

Phenolic Composition of Bud Exudates of Populus deltoides
W. GREENAWAY, S. ENGLISH, and F. R. WHATLEY 587

Hybrid Origin of Populus × jackii Confirmed by Gas Chromatography-Mass Spectrometry Analysis of Its Bud Exudate
W. GREENAWAY, C. G. DAVIDSON, T. SCAYSBROOK, J. MAY, and F. R. WHATLEY 594

Epicuticular Waxes from Leaves of Maple (Acer pseudoplatanus L.)
R. B. N. PRASAD and P.-G. GÜLZ 599

Comparative Investigation on Formation and Accumulation of Rare Phenylpropanoids in Plants and in vitro Cultures of Pimpinella major
B. MERKEL and J. REICHLING 602

Adenylate Kinase from Maize Leaves: True Substrates, Inhibition by P$_1$,P$_5$-di(adenosine-5')pentaphosphate and Kinetic Mechanism
L. A. KLECZKOWSKI, D. D. RANDALL, and W. L. ZAHLER 607

Ascorbic Acid and Chlorophyll Content in Cell Cultures of Spruce (Picea abies): Changes by Cell Culture Conditions and Air Pollutants
B. MESSNER and J. BERNDT 614

Characterization of Conformers of D1 of Photosystem II Using Site-Directed Antibodies
R. T. BESFORD, B. THOMAS, N. S. HUSKISSON, and G. W. BUTCHER 621

Surface Charge Density Changes in Isolated Photosystem II Membranes Induced by Depletion of the Extrinsic Polypeptides of the Oxygen Evolving System
A. G. IVANOV, M. C. BUSHEVA, and M. Y. VELITCHKOVA 627

Ribulose-1,5-bisphosphate Carboxylase-Binding Chloroplast Thylakoid Membrane Proteins. In vitro Evidence that H$^+$-ATP Synthase may Serve as a Membrane Receptor
K.-H. SÜSS 633

UVA-Induced Genetic Effects of Thoridazine, Mesoridazine and Sulforidazine: an in vitro Study
S. A. SCHUONDERWOERD, G. M. J. BEIJERSBERGEN VAN HENEGOUWEN, and I. R. PANDAY 638

X-Ray Induced Inactivation of the Sulphydryl Enzyme Malate Synthase in the Presence of Various Additives. Probing the Extent of Primary and Post-Irradiation Inactivation and Repair by Rapid Screening on the Microlevel
H. DURCHSCHLAG and P. ZIPPER 645

Ribonucleosides in Milk: Characterization and Determination of the Concentration Profile of these Minor Components throughout a Lactation Period (In German)
K.-P. RAEZKE and E. SCHLIMME 655

Stimulation of Calmodulin Binding to Skeletal Muscle Membrane Proteins by 1,25-Dihydroxy-Vitamin D$_3$
V. MASSHEIMER, L. M. FERNANDEZ, and A. R. DE BOLAND 663

On the Problem of Season and Cold Dependence of Calcium Transport by Skeletal Muscle Sarcoplasmic Reticulum
B. AGOSTINI, L. DE MARTINO, and W. HASSELBACH 671

Efficacy of Different Hexacyanoferrates(II) in Inhibiting the Intestinal Absorption of Radiocaesium in Rats
B. DRESOW, P. NIELSEN, and H. C. HEINRICH 676

Continued overleaf
Bioavailability of Iron and Cyanide from $^{59}$Fe- and $^{14}$C-Labelled Hexacyanoferrates(II) in Rats
P. Nielsen, B. Dresow, R. Fischer, and H. C. Heinrich 681

Morphology and Chemical Contents of Dufour Glands of Pseudomyrmex Ants (Hymenoptera: Formicidae)
A. B. Attygalle, J. P. J. Billen, B. D. Jackson, and E. D. Morgan 691

Determination of Relative Volatility of Lepidoptera Sex Pheromones by Closed Loop Stripping (In German)
H. J. Bestmann, J. Erlter, and O. Vostrowsky 698

14-Deoxyecdysteroids in an Insect (Gryllus bimaculatus)
K. H. Hoffmann, E. Thiry, and R. Lafont 703

Ontogenetic Patterns in Amounts and Proportions of Dufour’s Gland Volatile Secretions in Virgin and Nesting Queens of Lasioglossum malachurum (Hymenoptera: Halictidae)

Cytoplasmic Vibrations Due to Flagellar Beating in Trichoplax adhaerens F. E. Schulze (Placozoa)
H. Wenderoth 715

Dominance of Celestial Cues over Landmarks Disproves Map-Like Orientation in Honey Bees
R. Menzel, L. Chittka, S. Eichmüller, K. Geiger, D. Peitsch, and P. Knoll 723

Notes

Production of β-Carboline Alkaloids in Transformed Root Cultures of Peganum harmala L.
I. N. Kuzovkina, A. Gohar, and I. E. Alterman 727

Short-Wavelength Absorbing Complexes of Chlorophyll a in Micellar Solution of Cationic Detergents
A. Schmidt, J. Gottstein, H. Scheer, and A. Scherz 729
Short-Wavelength Absorbing Complexes of Chlorophyll a in Micellar Solution of Cationic Detergents

A. Schmidt, J. Gottstein, and H. Scheer
Botanisches Institut der Universität, Menzinger Straße 67, D-8000 München 19, Bundesrepublik Deutschland

and

A. Scherz
Biochemistry Department, The Weizmann Institute of Science, 15-76100 Rehovot, Israel

Z. Naturforsch. 45c, 729 – 732 (1990); received January 26, 1990

Chlorophyll, Micelles, Detergent, Aggregation, Absorption, Circular Dichroism, Molecular Orbital

Micellar aggregates have been prepared from chlorophyll a. Whereas most detergents including non-ionic, zwitterionic and anionic ones, produce aggregates with long-wavelength shifted and enhanced Q<sub>y</sub> absorption, the cationic detergents hexadecyl-trimethylammoniumbromide and hexadecyl-pyridiniumchloride produce complexes with opposite traits: The Q<sub>y</sub> absorption is shifted to shorter wavelengths, and it is hypochromic. The optical activity is increased as in the long-wavelength shifted complexes, but the cd bands are non-conservative and coincide with the absorption maxima. The complexes are most likely small aggregates of chlorophyll with a changed geometry as compared to the "standard" ones.

Introduction

Micellar complexes of chlorophylls and bacteriochlorophylls have gained attention due to the spectroscopic similarities with (bacterio)chlorophyll proteins containing the same pigments [1–8]. It has been argued that these similar properties result from the formation of aggregates with similar geometry, reflecting an inherent property of the large aromatic systems, e.g. chlorophyll. This view has been strengthened recently: Very similar types of aggregates have been found i) in the primary donor P<sub>960</sub> or P<sub>870</sub> of bacterial reaction centers as determined by X-ray crystallography [9–11], ii) in vacuo as calculated by molecular orbital methods [12], and iii) in aggregates formed in mixed organic-aqueous solvent systems as determined by the analysis of optical spectra [2, 4].

Exciton theory principally predicts both red- and blue-shifted spectra depending on the relative orientation of the monomers in chlorophyll aggregates, and concomitant hyper- und hypochromism [see 2, 4]. All chlorophyll proteins and in vitro chlorophyll aggregates investigated to date exhibit nonetheless a red-shifted Q<sub>y</sub> band. Here, we wish to report the formation of blue-shifted non-covalently linked aggregates of chlorophyll a with hypochromic Q<sub>y</sub> band, which are formed in micellar solutions of cationic detergents.

Materials and Methods

Chl<sub>a</sub> was extracted from spray-dried Spirulina geitleri and purified by dioxan precipitation [13] and subsequent chromatography on DEAE-cellulose [14]. The pigment was deposited on the walls of plastic vials by evaporating an ethereal solution in a stream of nitrogen, and then redissolved in detergent solution (0.1% in tris buffer, 10 mM, pH 7.5, if not stated otherwise) with sonification (Branson model 220) until the spectra remained constant. The final solutions had an optical density between 0.5 and 1 cm<sup>−1</sup> in the red spectral band, corresponding to Chl<sub>a</sub> concentrations of 10–20 μM. The position of the near-infrared band of the complexes was not affected by the concentration in this range.

The following detergents were used: CTAB (hexadecyl-trimethylammoniumbromide), CPC (hexadecyl-pyridiniumchloride), LiDS (Lithium dodecylsulfate), and Triton X-100 (Octylphenoxy-polyoxyethylene-glycol) from Serva, Heidelberg; RDL 203/S (dodecanoic acid-ethanolamid), Brij 30 (Polyoxyethyleneglycol-monododecylether), R-AM/V (N-3-hydroxyethyl-N-carboxymethylfatty acid amidoethylamin, Na-salt), R-AM/2L (N-3-hydroxyethyl-N-carboxymethyl fatty acid ethylamin, Na-salt), R-IPL-203 (dodecanoic acid-isopropanolamid), R-B204 (Alkylamidopropyldimethylaminoxid), all from Rewo, Steinau; Triton-H 66 (Alkylphosphatester, Na-salt), Triton-QS44 (Alkylphosphatester), from Bayrol, München. All detergents were used without further purification.

Absorption spectra were recorded in a model 320 spectrophotometer (Perkin-Elmer), cd spectra

Abbreviations: Chl, chlorophyll; Bchl, bacteriochlorophyll; CTAB, hexadecyl-trimethylammoniumbromide; CPC, hexadecyl-pyridiniumchloride.

Reprint requests to Prof. Dr. H. Scheer.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen 0341–0382/90/0600–0729 $ 01.30/0
in a dichrograph model V (Jobin-Yvon), and fluorescence spectra in a fluorolog system (Spex). The fluorescence spectra are uncorrected.

**Results**

The absorption maxima for the complexes formed with the different detergents are listed in Table I. With most of the detergents, chlorophylls form two types of complexes. One has absorptions similar to chlorophyll in monomeric solution, e.g. in methanol, the other one absorbs around 740 nm in the red spectral region. A typical example is shown in Fig. 1. The oscillator strength of the Q<sub>Y</sub> band of the latter is enhanced about two-fold. The optical activity is increased by orders of magnitude, but the s-shaped cd-band is nearly conservative (not shown). The ratio of the two forms depends on the detergent concentration, with the 740 nm absorbing form favored at lower concentration, but is also dependent on the structure of the detergent used. Red-shifted complexes are also formed with pheophytin a (<i>λ</i><sub>max</sub> around 700 nm, not shown). These spectral traits and concentration dependencies are very similar to the situation found with bacteriochlorophylls [1–8].

A different situation is observed with the two cationic detergents, CTAB and CPC. Again two complexes are formed, one of which absorbs like chlorophyll in monomeric solution. The second complex formed absorbs at 646 nm (Fig. 2),

<table>
<thead>
<tr>
<th>Detergent</th>
<th>Type</th>
<th>Complex formation</th>
<th>Absorption maximum [nm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triton X-100</td>
<td>non-ionic</td>
<td>+ +</td>
<td>742</td>
</tr>
<tr>
<td>RDL203/S</td>
<td>non-ionic</td>
<td>+ +</td>
<td>742</td>
</tr>
<tr>
<td>R-IPL-203</td>
<td>non-ionic</td>
<td>+ +</td>
<td>742</td>
</tr>
<tr>
<td>Brij30</td>
<td>non-ionic</td>
<td>+</td>
<td>744</td>
</tr>
<tr>
<td>R-B204</td>
<td>non-ionic</td>
<td>+</td>
<td>742</td>
</tr>
<tr>
<td>Triton H66</td>
<td>anionic</td>
<td>+</td>
<td>742</td>
</tr>
<tr>
<td>Triton QS44</td>
<td>anionic</td>
<td>+ +</td>
<td>742</td>
</tr>
<tr>
<td>CPC</td>
<td>kationic</td>
<td>+</td>
<td>646</td>
</tr>
<tr>
<td>CTAB</td>
<td>kationic</td>
<td>+</td>
<td>646</td>
</tr>
<tr>
<td>R-AM/V</td>
<td>zwitterionic*</td>
<td>+</td>
<td>742</td>
</tr>
<tr>
<td>R-AM/2L</td>
<td>zwitterionic*</td>
<td>+ +</td>
<td>740</td>
</tr>
</tbody>
</table>

* Amphoteric between pH 4 and pH 7.
Fig. 3. Fluorescence spectra of a micellar solution of chlorophyll \( a \) with hexadecyl-trimethylammonium-bromide (0.05% in Tris buffer, 10 mM, pH 7.5) excited at 400 (—) and 450 nm (——). The absorption spectrum of this solution is shown in Fig. 2, top, dashed line.

however, which corresponds to a blue-shift of 20–22 nm as compared to \( e.g. \) methanolic solution. The Soret band is blue-shifted as well to 420 nm. The oscillator strength of the \( Q_Y \) band is decreased, and it is strongly optically active showing essentially a purely negative band peaking at the wavelength of maximum absorption (Fig. 2). The complex shows moderate fluorescence, and the emission band is blue-shifted as well (Fig. 3). As in the other detergents, the ratio of the two complexes depends on the detergent concentration, with the short-wavelength form dominant at low concentrations. At 0.008% CTAB, the blue-shifted form is essentially pure (Fig. 2), but already at 0.05% there is an equal absorption of the two species at 642 and 668 nm. The short-wavelength absorbing complex is also unstable towards detergents forming red-shifted complexes by themselves. As an example, the addition of LiDS (0.5%) shifts the absorption to 658 nm, and higher concentrations give a mixture of two forms absorbing at 672 and 746 nm, which is similar to the situation with LiDS alone.

Discussion

The red-shifted complexes formed with Bchl and Chl alike have been related to aggregation of the pigments (see [5, 8]). In detergent solution, aggregation numbers of the pigments are small. Using exciton theory, Scherz and Parson [2, 4] suggested a dimeric structure of the basic unit in which the two macrocycles are nearly parallel (\( \approx 15^{\circ} \) angle between the planes of the macrocycles) at a distance of \( \approx 3.5 \) Å, and in which only part of the macrocyclic system overlaps due to a lateral displacement of both macrocycles from the center of the dimer. The \( Q_Y \) axes form an angle of \( \approx 145^{\circ} \).

It is surprising that this structure, which is formed in micelles of a variety of detergents, and also in mixed aqueous-organic solvent systems, closely resembles the structure of the Bchl-special pair in reaction centers of \( R_{hodopsedomonas viridis} \) and \( R_{hodobacter sphaeroides} \), \( e.g. \) the primary donor involved in light-induced charge separation [9–11]. Moreover, a similar arrangement has been calculated by Plato \( e.t\. al. [12] \) as the minimum energy arrangement in the absence of interactions with the solvent. These similar arrangement of the two chlorophylls in very different environments—vacuum, detergent micelle, pigment micelle, or protein—points to an inherent tendency of the bacteriochlorophyll molecule in forming aggregates of this special geometry.

The present study was undertaken in part to test if the same type of complex can be formed as well with chlorophyll \( a \). This was indeed observed and further strengthens the hypothesis. Nearly independent of the detergent used, there is always the formation the same type of red-shifted complex as shown in Fig. 1 for Triton X-100, if judged from the similarities of their absorption and cd spectra. This is true but for two exceptions, \( viz \). the cationic detergents CPC and CTAB, which form blue-shifted complexes. It is principally inherent to exciton theory, that both blue and red shifts (as well as none at all) can occur in dimers (or higher aggregates) of chlorophylls, depending on the relative orientation and distance of the two pigment molecules (see \( e.g. [2] \)). The present example is to our knowledge nonetheless the first case among the many different aggregates of chlorophylls formed by association or a single covalent linkage, in which a blue-shifted species has actually been observed. A blue-shifted bis-chlorophyll-cyclophane has been reported, however, by Overfield \( e.t\. al. [16] \) in which the two pigments show little interaction.
Bchl $a$ complexes have been studied before with the same two cationic detergents [6]. Generally, the latter are strongly dissociating both to long-wavelength absorbing complexes of Bchl $a$ with other detergents, and to Bchl $a$ proteins. In our hands, of the two detergents, only CTAB does form a small proportion of Bchl $a$ aggregates (besides monomers) at all at low detergent concentrations. However, these complexes are red-shifted similar to the ones observed in any of the other non-ionic, anionic and zwitterionic detergents studied. This points to a somewhat different aggregation pattern in the Bchl as compared to the Chl chromophore, which may be related to the different oxidation states of the macrocycles, or the different substitution pattern. Differences of this kind should be important in the interactions of polypeptide and pigments in (bacterio)chlorophyll proteins as well.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (SFB 143, project A1 and AZ 140/9-1).