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Volume II

edited by

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PREFACE

The Sixth International Congress on Photosynthesis took place from 1 to 6 August 1983, on the Campus of the "Vrije Universiteit Brussel", in Brussels, Belgium. These Proceedings contain most of the scientific contributions offered during the Congress.

The Brussels Congress was the largest thus far held in the series of International Congresses on Photosynthesis. It counted over 1100 active participants. The organizers tried to minimize the disadvantages of such a large size by making maximum use of the facilities available on a university campus. Most contributions were offered in the form of posters which were displayed in a substantial number of classrooms. The discussion sessions, twice a day, four or five in parallel, took place in lecture rooms in the very vicinity of these classrooms. In this way it was attempted to generate the atmosphere of a small meeting. The unity of the subject Photosynthesis was preserved in the ten plenary lectures, organised in such a way that a general overview of two diverse topics was given every day. In addition, there were the five times four parallel symposia dealing with some sixteen general topics.

Every editor of proceedings of a congress is faced with the problem of editing and arranging the contributions, a problem compounded by the wide diversity and the large number of the 753 manuscripts. This editor did very little in the way of editing the papers: all papers were prepared, camera-ready, by the authors themselves and there was no proof-reading. The main reason for this was the need to ensure speedy publication. The contributions are arranged in four volumes but the Proceedings form one set. Although some attempts were made to bring related topics together in one volume, the volumes I to IV should be seen as a succession of chapters, rather than as volumes in their own right. Thus, artificial and arbitrary subdivisions were avoided. A page limit was imposed in order to prevent oversized volumes.

The contributions are arranged in chapters which have no direct relation to the sessions or symposia in which they were presented. The sole criterium for putting a contribution into a certain chapter was its contents. The contributions offered during the Round Table Discussion on Light-Controlled Development of the Photosynthetic Apparatus, July 29 to 30, 1983 in Antwerp, are also included in these Proceedings. They comprise most of the contents of Chapter 7 of Volume IV.

The early publication date of these Proceedings could not have been realised without the efforts of, and the pleasant cooperation with, Mr. Ad Plaizier of Martinus Nijhoff Publishing House. Thanks are due to all Congress members, whose active participation made the Congress a success and these volumes an important document on the state of photosynthesis research. The very much needed assistance of the Local Organizing Committee is gratefully acknowledged. The Photosynthetic Community is indebted to the "Vrije Universiteit Brussel" for making available its premises, facilities and staff. Thanks are also due to the administrative staff of the Congress: secretaries, hostesses, technicians and the two diligent computer programmers, Mr. W. Dierickx and Mr. B. Philips. Special appreciation goes to Ms Blanche van den Haute for her dedicated work in the preparation and the management of the Congress and her help in editing these volumes.

Brussels, March 1984

C. Sybesma, Editor

REVERSIBLE PH INDUCED ABSORPTION CHANGE IN THE BCHL B-LH-PROTEIN OF THE ALKALOPHILE ECTOTHIORHODOSPIRA HALOCHLORIS.

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Introduction: Ectothiorhodospira halochloris and E. abdelmalekii are extremely halophilic and alcalophilic photosynthetic bacteria (1). They belong to the few species containing bacteriochlorophyll b (bchl b). In both Ectothiorhodospira species, it is esterified with the unusual alcohol 2,10-phytadienol (2). Like other bchl b-containing species, e.g. Rhodopseudomonas viridis, it has an infrared absorption maximum around 1020 nm. Unlike Rp. viridis, however, the Ectothiorhodospira species contain a second, structured infrared band peaking at 800 and 830 nm. It had originally been suspected (1) to originate from the presence of bchl a besides bchl b, which is, however, disproved by its pigment analysis (2). The 800/830 nm bands must then originate as well from bchl b. Here, we wish to report the isolation of a bchl b-containing antenna complex (B1020/800) from E.halochloris, and a reversible, pH-induced absorption change of the 1020 nm component.

Isolation of B1020/800: E.halochloris was grown anaerobically at 35°C in a medium modified from IMHOFF and TRÜPER (1). Rhodopseudomonas viridis was grown anaerobically at 28°C in HUNTERS medium (3). After disruption by french press treatment of the cells chromatophores were isolated by the method of OKAMURA and FEHER (4). For the fractionation of the antenna pigments, chromatophores (A_{1020} = 50) were treated with 1 % Triton X-100, dialysed overnight against 0.1 % Triton and fractionated on DEAE cellulose.
According to SDS gel electrophoresis, the B1020/800 antenna pigment contains two major bands corresponding to a MW of 13500 and 13000 Dalton, if calibrated with a standard set of globular, hydrophilic proteins. When compared with the two antenna complexes of Rp. spheroides 2.4.1, the two bands move between the 6800 D of B875 and the 5400 D band of B800/850. The electrophoresis also shows a weakly stained very low molecular weight band at 4500 D, as well as two higher MW bands at 15500 and 27500 D, which may be due to impurities.

ABSORPTION SPECTRA: When whole cells, spheroplasts or chromatophores of E. halochloris or E.abdelmalekii (fig. 1a) or, isolated antenna fractions of E.halochloris are titrated with acid in the pH range between 7.5 and 5.7, the 1020 nm absorption is replaced by a new absorption peaking at 960 nm. The 830/800 nm band is essentially uneffected by this treatment. The 960 nm band is unstable and decreases again with time and especially upon addition of more acid. It is then replaced by rather broad and unstructured absorptions below 900 nm. At pH 5.7, the species absorbing at 960 nm has a half life of appx. 1 hr. If the decay of the "low pH" form is avoided by an immediate back titration with base, the 1020 nm form is at least partially restored. (fig. 1b). The losses are probably due to the instability of the 960 nm form during the titration. Neither of the two forms, nor their

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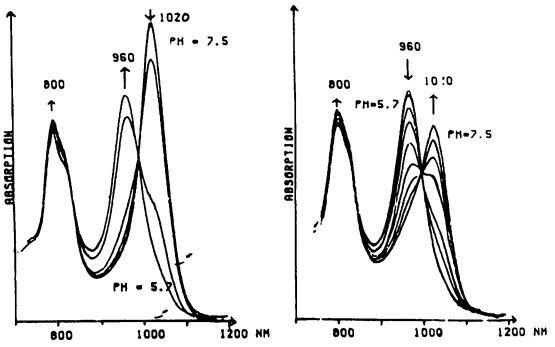
interconversion is effected by α-bromopropionate or its methyl-ester, or by high salt.

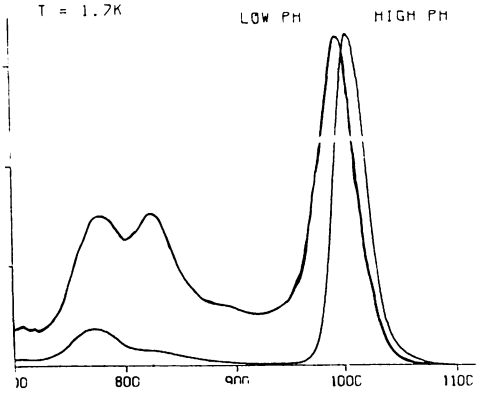
FLUORESCENCE SPECTRA: The natural "high pH" form has a single emission band peaking at 1018 nm (ambient temperature) or 1000 nm (1.7K). The acid induced "low pH" form has its emission maximum blue-shifted to 975 nm (992 nm at 1.7K), but additional emission bands are also observed at 803 nm (298K) or 824 nm (1.7K). The short wavelength emission of the "low pH" form is much more pronounced at low temperature. This data indicate an energy transfer from the chromophores absorbing at 800 nm to the ones absorbing at 1020 nm in the native "high pH" form, which is uncoupled in the "low pH" form.

CIRCULAR DICHROISM SPECTRA: The CD spectrum of the natural "high pH" form shows a complex pattern at the 1020 (limited in our machine to 1010 nm) and the 800/830 nm bands (fig. 2), which is indicative of exiton coupling between more than two chromophores. The spectrum of the "low pH" form is less complex, with only a doublet in the region of the 960 nm band, and a strongly decreased optical activity in the 800/830 nm band region.

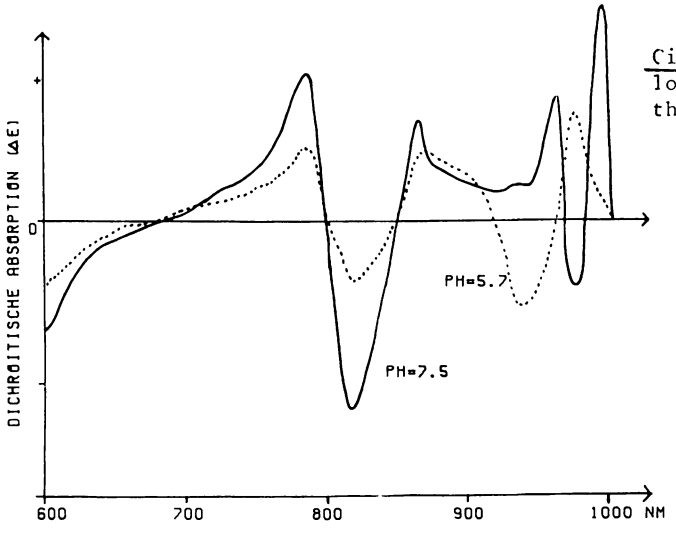
DISCUSSION: A bchl b-containing antenna complex (B1020/800) has been isolated from Ectothiorhodospira halochloris. These absorptions correspond to the ones seen in whole cells. This native form is stable at pH 8, but is reversibly transformed to a form absorbing at 960 and 800 (B960/800) at lower pH, with an apparent pK of 6.3. This value is close to the pK of histidine (6.8), or possibly carboxylic acid side chains (pK $\simeq$ 4.7). Attempts to modify histidine residue(s) with α-bromopropionate or its methyl ester did neither affect the 1020 nor the 960 nm form, which argues against the participation of histidine. High salt is also ineffective. Since the changes are observed in whole cells, spheroplasts (= "outside out" vesicles) and chromatophores (="inside out" vesicles), whichever group is affected is accessible from either side of the membrane, or the membrane is readily permeable to protons.
The fluorescence data indicate for the natural, "high pH" form a good energy transfer from the chromophores absorbing at 800/830 nm to ones absorbing at 1020 nm. This transfer is (partly) disrupted in the "low pH" form, as evidenced by the second emission band in the 800 nm region. The CD spectra would also support a transformation from a tightly coupled to a weakly coupled state. One possible explanation would be the assignment of the 1020 and the 800/830 nm band to two bchl b-polypeptides, which are in close association in the "high pH" form. There are at two strongly interacting chromophores absorbing in the 800 nm region, and more than two in the 1020 nm region, if judged from the cd data.



Titrattion of the B800/1020 antenna complex of E.halochloris with acid (left and base (right Absorption spectra in the near infrared spectral range.



Low-temperature fluorescence emission spectrum of the low pH and high pH forms of the B800/1020 antenna complex. Excitation at 599 nm. We thank K. Angerhofer in the group of Prof. H.Wolf (Stuttgart) for providing these spectra to us.



Circular dichroism of the low pH and high pH forms of the antenna complex

Bchl a-containing bacteria contain an antenna fraction B800/850. It has also two absorption maxima, which can be shifted by various treatments. (5). The B1020/800 antenna complex might then be the bchl b-containing analogue. However, the B800/850 contains carotenoids, and does not show such pronounced CD-couplings. We have also shown, that the absorption maxima (although not the relative absorption) of the two bands are acid-insensitive down to pH 1.7. It has also a similar polypeptide composition, if one disregards the very low molecular weight components present in E. halochloris. It should be noted, however, that a similar peptide has been found in another bchl b-containing organism, e.g. Rp. viridis.
The increased sensitivity of E.halochloris to positive charges may reflect a specific adaptation to the high pH ($\simeq$11) prevailing in its natural environment.

ACKNOWLEDGEMENTS: This work was supported by the Deutsche Forschungsgemeinschaft, Bonn. We thank W. Angerhofer in the group of Prof. H.C. Wolf (Stuttgart) for the fluorescence measurements.

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