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# Reversible conversion of aggregated bacteriochlorophyll *c* to the monomeric form by 1-hexanol in chlorosomes from *Chlorobium* and *Chloroflexus*

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**When isolated chlorosomes from *Chlorobium limicola* or *Chloroflexus aurantiacus* are suspended in a solution saturated with 1-hexanol, the far-red absorption band of bacteriochlorophyll *c* at 750 or 740 nm is converted completely to a band peaking at 670 nm. The cooperation of 9 to 15 hexanol molecules is required to effect this change. This conversion corresponds to a change of the pigment molecules from the aggregated form to the monomeric form in vitro and suggests that hexanol destroys the strong interaction between the chlorosome pigments by the ligation of the hydroxyl oxygen of hexanol to the magnesium atom of the chlorophyll. However, fluorescence from the monomer in the treated chlorosomes is very small in comparison to that from monomer in organic solvent or detergent treated chlorosomes and efficient energy transfer from bacteriochlorophyll *c* to bacteriochlorophyll *a* in the hexanol-treated chlorosomes is still observed. When the treated chlorosomes are diluted slowly with buffer by a factor of two or more, the hexanol effect is reversed completely. These results suggest that the red-shifted far-red bands of bacteriochlorophyll *c* at 740 or 750 nm are largely due to strong pigment-pigment interactions rather than pigment-protein interactions and that the far-red bands are not necessary for energy transfer to the bacteriochlorophyll *a* in chlorosomes.**

## Introduction

Organization of pigment molecules in the photosynthetic apparatus is essential for efficient energy transfer of absorbed light energy to the photochemical reaction center. In green photosynthetic bacteria the major light-harvesting pigment, BChl *c* (in some case *d* or *e*) is present in the chlorosome, an elongated bag-like structure appressed to the cytoplasmic side of the plasma membrane [1,2]. In addition to BChl *c* and carotenoid molecules a small amount of BChl *a* is present in chlorosomes of the green sulfur bacterium, *Chlorobium* [3], and the green filamentous bacterium, *Chloroflexus* [4]. Although the two green bacteria are widely separated phylogenetically [5] and their photochemical reaction centers are quite different from each other [6], the characteristics of the chlorosomes are very similar in the two groups [1,2].

The far-red absorption spectrum of BChl *c* in the chlorosome is red-shifted some 70–80 nm with respect to that of the monomeric form in organic solvents. BChl *c* in non-polar solvents can form aggregates whose spectra resemble that of the chlorosome [7–10]. BChl *c* has the possibility to form oligomers through the ligation of an oxygen atom in one molecule to the central magnesium atom in another molecule [8,9]. Linear dichroism and fluorescence polarization studies are consistent with such a structure in the chlorosome [11–13]. The direct binding of BChl *c* molecules to protein has also been suggested as a possible cause of the in vivo red-shift of the absorption band [14] but this explanation has been severely weakened by Griebenow and Holzwarth [15,16] because the proteins of *Chloroflexus* apparently can be extracted by detergent without affecting the spectral properties of the BChl *c*. Olson et al. [17] also pointed out that the proposed binding sites for BChl *c* are not conserved in 6.3 kDa proteins from chlorosomes of various green sulfur bacteria.

Brune et al. [9] reported that the 740-nm absorbing form of BChl *c* in the *Chloroflexus* chlorosome can be converted to the 670-nm absorbing form by the addition of 1% 1-hexanol to the chlorosome suspension.

Abbreviation: BChl, bacteriochlorophyll.

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This effect was partially reversible. After dilution of the chlorosomes and several washes by ultracentrifugation the pigment band shifted to 733 nm. In this paper we report the complete reversibility of the hexanol effect in chlorosomes from *Chlorobium* and *Chloroflexus* based on both absorption and fluorescence spectra. We also demonstrate that energy transfer from BChl *c* to BChl *a* is maintained even when BChl *c* is converted to the 670-nm form by hexanol.

## Materials and Methods

Cells of *Chlorobium limicola* f. *thiosulfatophilum* and *Chloroflexus aurantiacus* were grown as described previously [18]. Chlorosomes were prepared in 2 M NaSCN, 10 mM potassium phosphate (pH 7.4) and 10 mM sodium ascorbate by the method of Gerola and Olson [3]. The light fraction, which came at the top of the sucrose gradient after ultracentrifugation, was used without further purification. No membranes or water-soluble BChl *a* proteins were detected in this fraction.

For the absorbance and fluorescence measurements, 10 μl of concentrated chlorosomes ($A_{750}$ or $A_{740} = 400$) were suspended in 10 mM potassium phosphate (pH 7.4) or in the same buffer saturated with 1-hexanol. To make a saturated solution of 1-hexanol, it was added to 10 mM potassium phosphate (pH 7.4) to a final ratio of 0.8% (v/v) and shaken vigorously. The mixture then stood at least 30 min at room temperature (20–25 °C). Small drops of 1-hexanol appeared at the surface, but the clear aqueous solution was separated and mixed with chlorosomes immediately. The concentration of 1-hexanol in the saturated solution was assumed to be 59 mM [19]. Dilution of the chlorosomes in fresh buffer was performed gradually and slowly (over about 30 s) unless otherwise stated. Diluted samples stood at least 10 min at room temperature in the dark before any measurements. Sodium dithionite was added before the fluorescence measurements were made.

Extraction of BChls into 1-hexanol was performed by adding 3 ml of 1-hexanol to 3 ml of aqueous chlorosome suspension and shaking vigorously for a few minutes. After 1 h in the dark the separated hexanol phase contained all the pigments.

Absorbance spectra were measured with a Perkin Elmer 330 spectrophotometer, and fluorescence spectra were taken with a Spex Fluorolog 111A fluorometer.

## Results

Absorption spectra of *Chlorobium* chlorosomes before and after the hexanol treatment are shown in Fig. 1. When chlorosomes were suspended in 10 mM phosphate buffer (solid line), absorption peaks of BChl *c* were apparent at 750 nm and 459 nm. After dilution into 59 mM hexanol, the 750-nm band completely disappeared and a new band appeared at 670 nm. The peak heights of the two bands were similar, but the bandwidth of the 670-nm band was only about half that of the 750-nm band. In the blue region the peak wavelength changed from 459 nm to 438 nm and the peak height was more than doubled. The chlorosome spectrum in 59 mM hexanol was similar to that of the pigments extracted into hexanol (data not shown) except for a slight broadening in the long wavelength tail of the 670-nm band in the chlorosomes. A small shoulder around 800 nm was observed in the spectrum of chlorosomes in 59 mM hexanol, and from the second derivative of the spectrum the peak wavelength was identified as 795 nm, the same as for BChl *a* in *Chlorobium* chlorosomes reported by Gerola and Olson [3]. This indicates that the BChl *a* absorption band was not affected by 59 mM hexanol. This BChl *a* peak shifted to 770 nm after extraction by hexanol.

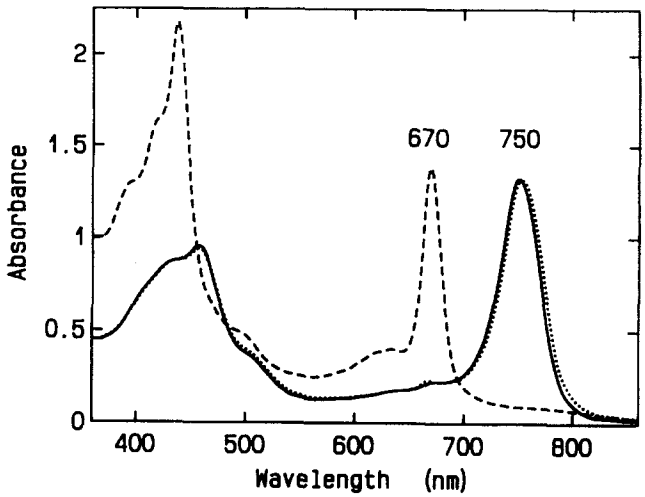


Fig. 1. Absorption spectra of *Chlorobium* chlorosomes in 10 mM potassium phosphate (pH 7.4) (solid line), in 59 mM hexanol in buffer (dashed line) and in 29.5 mM hexanol in buffer after the 59 mM hexanol treatment (dotted line).

In order to exclude the possibility that the observed hexanol effect on whole chlorosomes was the result of hexanol extracting the BChl *c* from the chlorosomes and then the BChl *c* aggregating outside the chlorosomes, the density of untreated and hexanol-treated chlorosomes was compared by sucrose-gradient centrifugation. Both had exactly the same density, whereas an aggregate prepared from extracted BChl *c* had a lower density (P. Gerola and M. Miller, unpublished).

When the *Chlorobium* chlorosomes in 59 mM hexanol were diluted slowly by a factor of two with 10 mM potassium phosphate buffer almost complete recovery of the chlorosome absorbance spectrum was observed (Fig. 1). A small difference of the final spectrum from the original spectrum is a small red shift of the peak wavelength (750 nm to 752 nm). When the dilution was performed rapidly, the recovery was less complete with

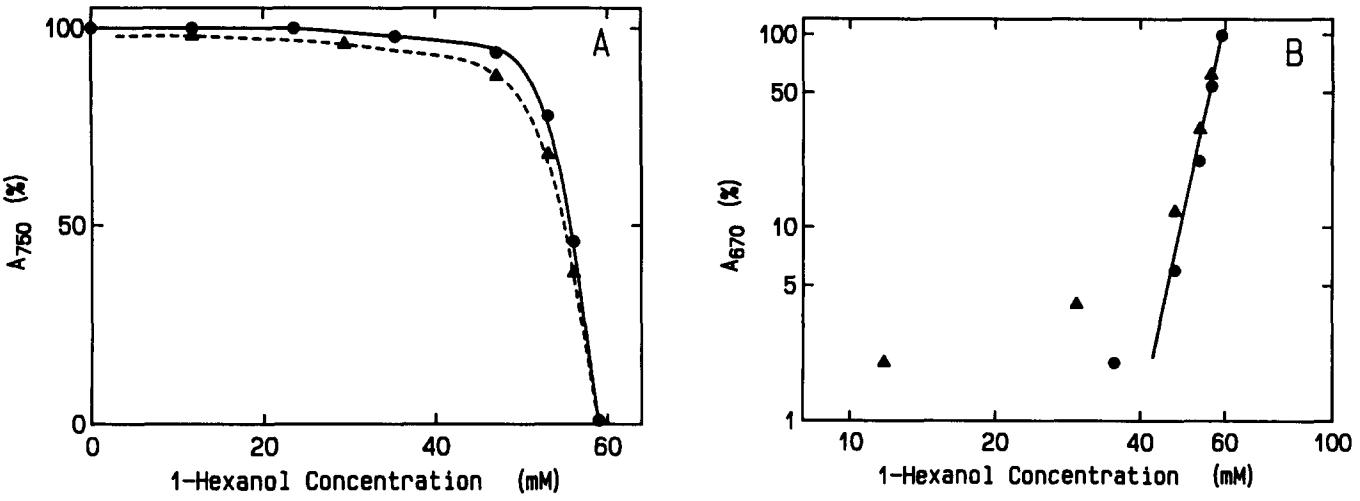


Fig. 2. Dependence of the spectral conversion on 1-hexanol concentration in *Chlorobium* chlorosomes. Chlorosomes were suspended in various concentrations of 1-hexanol in 10 mM potassium phosphate (pH 7.4) before (circles) and after (triangles) the 59 mM hexanol treatment. (A) Absorbance of B750 was plotted against the hexanol concentration of a linear scale. (B) The same set of data was replotted as log $A_{670}$ versus log[1-hexanol].

the peak wavelength around 742 nm; additional dilution did not affect this peak wavelength (data not shown). This dependence on the speed of the dilution may be related to the process of reorganization of BChl *c* molecules in the chlorosome.

Fig. 2A shows the dependence of the peak height of the 750-nm band on the hexanol concentration in *Chlorobium* chlorosomes. The values at each hexanol concentration (circles, solid line) and after dilution following 59 mM hexanol treatment (triangles, dashed line) were plotted. The conversion curve rose steeply above 45 mM hexanol, and the reversibility was almost complete after dilution. The data were replotted as log $A_{670}$ versus log[1-hexanol] in Fig. 2B. The data points for hexanol concentrations between 45 and 59 mM could be fitted by a straight line with a slope of about 11 (limits are 9 to 15). This indicates that 9–15 hexanol molecules must cooperate to convert the 750-nm form to the 670-nm form.

In Fig. 3 fluorescence emission spectra of chlorosomes without hexanol (Fig. 3A), in 59 mM hexanol (Fig. 3B) and in 29.5 mM hexanol after 59 mM hexanol treatment (Fig. 3C) are shown. As has been reported previously [20–22] treatment of aerated chlorosomes with dithionite resulted in a large increase of fluorescence from BChl *c*; in addition a shoulder around 800 nm became significant in the emission spectrum. By subtracting the emission spectrum before the addition of dithionite multiplied by a factor of ten (this number was chosen to minimize the contribution of the 777-nm band on the subtracted spectrum) from that after the addition, the emission spectrum from BChl *a* in the chlorosomes was obtained with a peak at 805 nm (Fig. 3A, dotted line).

In chlorosomes suspended in 59 mM hexanol (Fig. 3B) the major emission band peaked at 795 nm, possibly due to BChl *a* or a special form of BChl *c* in the chlorosome; the peak wavelength was blue-shifted with respect to the normal emission of BChl *a* about 10 nm. (The special form of BChl *c* might be an aggregate that absorbs at about 780–785 nm and that it is not converted to the 670-nm form by hexanol.) The fluorescence enhancement effect of the dithionite was approx. a factor of three. The emission from the BChl *c* band absorbing at 670 nm was very weak even though the BChl *c* was excited at 440 nm. This weak emission from BChl *c* was very different from the case of BChl *c* extracted in hexanol or chlorosomes treated with 1% Triton X-100 (data not shown). In both of these cases the BChl *c* showed a similar 670-nm absorption peak but an emission band at 680 nm, which is more than 40-times larger than the emission band of hexanol-treated chlorosomes. Fluorescence excitation spectra (data not shown) for the 680-nm emission and 795-nm emission in hexanol-treated chlorosomes both showed peaks at 435 nm, suggesting an efficient energy transfer from BChl *c* to BChl *a* or the special form of BChl *c* even after the destruction of the strong interaction between most of the BChl *c* molecules. Similar results were obtained for *Chloroflexus* chlorosomes (M. Miller, unpublished).

Fluorescence emission spectra of *Chlorobium* chlorosomes in 29.5 mM hexanol after the 59 mM hexanol treatment (Fig. 3C) were almost identical to those before the hexanol treatment (Fig. 3A). This indicates that the reversibility of the hexanol effect is complete also in terms of the full restoration of energy transfer.

Absorption spectra of *Chloroflexus* chlorosomes had peaks at 740 nm and 460 nm, which were changed completely to 670 and 438 nm by suspending in

hexanol-saturated solution. After dilution of the hexanol-treated chlorosomes by a factor of two the original absorption spectrum was completely recovered (data not shown, but very similar to Fig. 1 except for a small difference of the peak position).

Fluorescence emission spectra from *Chloroflexus* chlorosomes (Fig. 4) are somewhat different from those from *Chlorobium* chlorosomes (Fig. 3). The emission from BChl *a* with the fluorescence peak at 804–805 nm

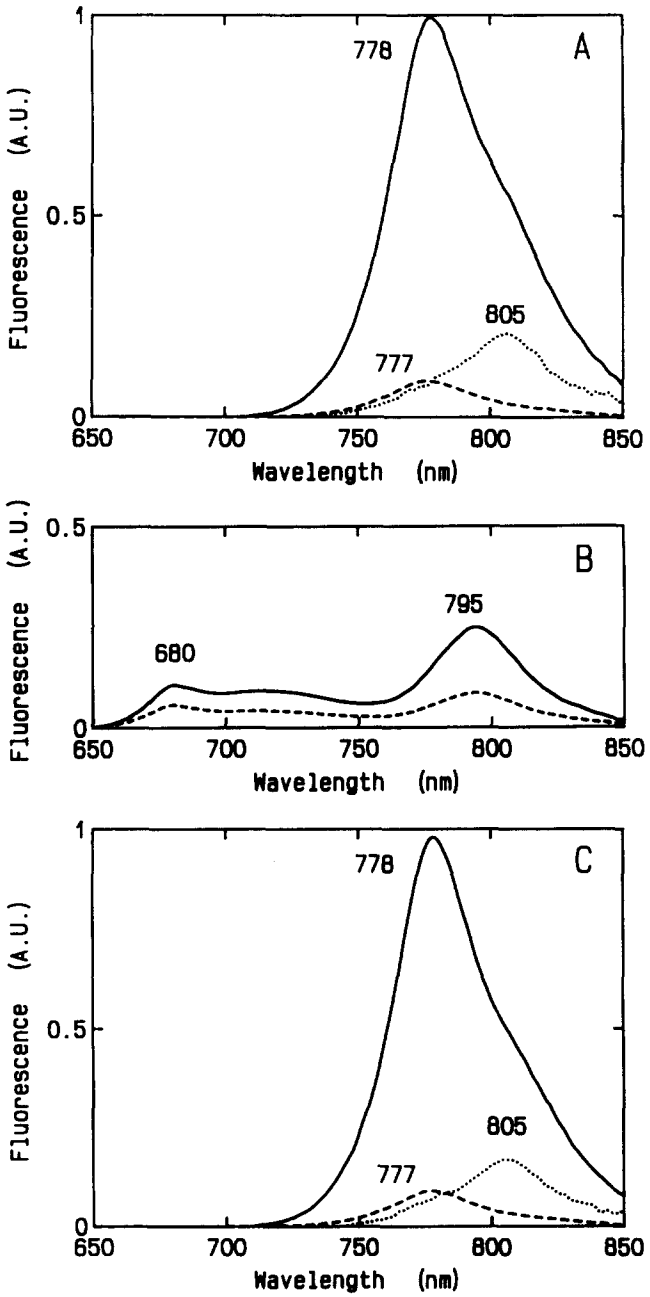


Fig. 3. Fluorescence emission spectra of *Chlorobium* chlorosomes in 10 mM potassium phosphate (pH 7.4) (A), in 59 mM 1-hexanol in buffer (B) and in 29.5 mM hexanol in buffer after the 59 mM hexanol treatment (C). Dashed line, aerobic suspension; solid line, after the addition of dithionite; dotted line in A and C, (solid line) − (dashed line) × 10. Excitation wavelengths were at 460 nm for A and C and at 440 nm for B.

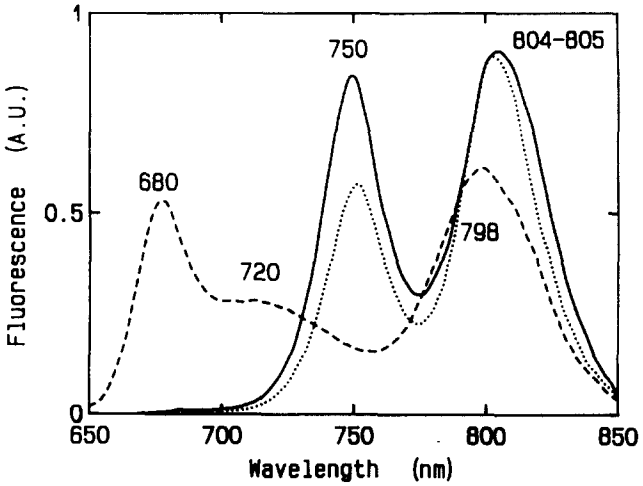


Fig. 4. Fluorescence emission spectra of *Chloroflexus* chlorosomes in 10 mM potassium phosphate (pH 7.4) (solid line), in 59 mM hexanol in buffer (dashed line) and in 29.5 mM hexanol in buffer after the 59 mM hexanol treatment (dotted line). Sodium dithionite was added before the measurements. Excitation wavelength was at 460 nm (solid line and dotted line) or 440 nm (dashed line).

is pronounced. When suspended in the hexanol saturated solution, the emission from BChl *a* shifted to shorter wavelength by 6 nm and decreased slightly in amplitude. The emission band at 750 nm disappeared upon hexanol treatment, and new emission bands appeared at 678 nm and around 720 nm. The emission spectra after the two-times dilution of the hexanol-treated chlorosomes was a little different from the original spectra, but the principal characteristics of the fluorescence emission were recovered. The effects of hexanol treatment on *Chloroflexus* chlorosomes were thus found to be very similar to those on *Chlorobium* chlorosomes.

## Discussion

As reported previously by Brune et al. [9] for *Chloroflexus* chlorosomes, 1-hexanol dissolved in aqueous solution can change the spectral properties of BChl *c* in chlorosomes drastically. We have shown in this paper that the hexanol effect is completely reversible both in *Chlorobium* chlorosomes and *Chloroflexus* chlorosomes. In the experiment by Brune et al. the recovery was incomplete, i.e., the far-red peak of BChl *c* after the removal of hexanol was 733 nm instead of the original 740 nm. A possible reason for the incomplete recovery was the use of 1% hexanol (0.6% is saturating) and the speed of dilution with fresh medium. From our experience hexanol concentrations above 0.6% result in a hexanol phase at the surface of the aqueous phase and the extraction of some BChl *c* into the hexanol phase with shaking.

The speed of the dilution after the hexanol treatment seems to be another important factor affecting the reversibility. If the dilution is made rapidly, the position

of the far-red peak is blue-shifted up to several nanometers. Once this blue shifted form has appeared, no further change in the peak position can be effected by further dilution. This dependence on the speed of dilution for the restoration of the original configuration of BChl *c* may be related to the degree of order or the number of molecules in an aggregate of BChl *c* in chlorosomes.

To explain the conversion of the 740–750 nm absorbing band to 670 nm, we assume that hexanol dissolves in the lipid phase of the chlorosomes and the hydroxyl oxygen ligates to the magnesium atom in BChl *c*. Monomeric BChl *c* in organic solvents has a peak around 670 nm, and aggregates of BChl *c* whose spectra resemble the spectrum of the chlorosome have been shown in vitro [7–10]. Methanol and higher alcohols have been shown to convert the aggregates to the monomeric form in hexane solution [9]. Our demonstration of the complete reversibility of the hexanol effect on BChl *c* in chlorosomes supports the idea of a strong direct interaction between BChl *c* molecules rather than the interaction of BChl *c* with protein to explain the in vivo spectroscopic properties.

Since the interior of the chlorosomes is thought to be lipophilic, hexanol is thought to partition into the chlorosomes in proportion to its concentration in the aqueous phase. However, the concentration dependence on hexanol for the conversion from the 750-nm form to the 670-nm form is not linear with the concentration of hexanol; the conversion takes place abruptly above 45 mM hexanol in the aqueous phase. In their model system in hexane, Brune et al. [9] reported an almost linear dependence on methanol concentration for the conversion of the 740-nm form to the 670-nm form. We find that in *Chlorobium* chlorosome cooperativity among 9–15 hexanol molecules is required for this conversion. This cooperativity suggest that hexanol must bind to 9–15 BChl *c* molecules before the conversion to the 670-nm form can take place.

Although the absorption spectrum of BChl *c* in chlorosomes in 59 mM hexanol is similar to that of monomeric BChl *c* in hexanol or other organic solvents, the fluorescence characteristics are different. Fluorescence emission from the 'monomeric' BChl *c* in the chlorosome was much less than that from BChl *c* in organic solvents or in Triton X-100 treated chlorosomes. At the same time, an efficient energy transfer to BChl *a* or specialized BChl *c* in chlorosomes was observed in the fluorescence emission spectra. Although the peak position of the 'BChl *a*' emission was blue-shifted in 59 mM hexanol solution, it seems reasonable to assign the emission to BChl *a* at least in the case of *Chloroflexus* (Fig. 4). It is not entirely clear how to assign this emission in *Chlorobium* chlorosomes. The efficient energy transfer in the hexanol-treated chlorosomes indicates that the presence of the 740- or 750-nm absorbing band (probably due to strong chromophore interactions of BChl *c*) is not essential for the energy transfer. The absorption spectrum of 'monomeric' BChl *c* in the chlorosome has a pronounced tail around 700 nm compared to the BChl *c* spectrum in organic solvents. This may represent a small fraction of BChl *c* having absorption bands around 700 nm, which contributes to the energy transfer in the hexanol-treated chlorosomes.

If the strong chromophore interactions in chlorosomes are not essential for energy transfer, the primary role of the strong interaction may be to enhance photon capture. Since, without any ordered structure, it would seem impossible to retain the efficient energy transfer from the 'monomeric' BChl *c* molecule to BChl *a* in the chlorosome, some kind of ordered structure of BChl *c* in the chlorosomes must exist even after the original strong chromophore interactions have been destroyed by hexanol. In vitro studies of the hexanol-induced reversible monomerization of BChl *c* in large aggregates and in vivo studies of linear dichroism and fluorescence polarization studies of hexanol-treated chlorosomes are now in progress to clarify these issues.

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## References

1 Olson, J.M. (1980) Biochim. Biophys. Acta 594, 33–51.
2 Blankenship, R.E., Brune, D.C. and Wittmershaus, B.P. (1988) in Light-energy Transduction in Photosynthesis: Higher Plant and Bacterial Models (Stevens, Jr., S.E. and Bryant, D.A., eds.), pp. 32–46, American Society of Plant Physiologists.
3 Gerola, P.D. and Olson, J.M. (1986) Biochim. Biophys. Acta 848, 69–76.
4 Feick, R.G. and Fuller, R.C. (1984) Biochemistry 23, 3693–3700.
5 Oyaizu, H., Debrunner-Vossbrinck, B., Mandelco, L., Studier, J.A. and Woese, C.R. (1987) Syst. Appl. Microbiol. 9, 47–53.
6 Bruce, B.D., Fuller, R.C. and Blankenship, R.E. (1982) Proc. Natl. Acad. Sci. USA 79, 6532–6536.
7 Krasnovsky, A.A. and Bystrova, M.I. (1980) BioSystems 12, 181–194.
8 Smith, K.M., Kehres, L.A. and Fejer, J. (1983) J. Am. Chem. Soc. 105, 1387–1389.
9 Brune, D.C., Nozawa, T. and Blankenship, R.E. (1987) Biochemistry 26, 8644–8652.
10 Olson, J.M. and Pedersen, J.P. (1990) Photosynth. Res., in press.
11 Fetisova, Z.G., Kharchenko, S.G. and Abdourakhmanov, I.A. (1986) FEBS Lett. 199, 234–236.

12 Van Dorssen, R.J., Vasmel, H. and Amesz, J. (1986) Photosynth. Res. 9, 33–45.
13 Fetisova, Z.G., Freiberg, A.M. and Tipmann, K.E. (1988) Nature 334, 633–634.
14 Wechsler, T., Suter, F., Futter, R.C. and Zuber, H. (1985) FEBS Lett. 181, 173–178.
15 Griebenow, K. and Holzwarth, A.R. (1990) in Molecular Biology of Membrane-bound Complexes in Photosynthetic Bacteria (Drews, G., ed.), Plenum, New York, in press.
16 Holzwarth, A.R., Griebenow, K. and Schaffner, K. (1990) Z. Naturforsch. 45c, in press.
17 Olson, J.M., Brune, D.C. and Gerola, P.D. (1990) in Molecular Biology of Membrane-bound Complexes in Photosynthetic Bacteria (Drews, G., ed.), Plenum, New York, in press.
18 Cox, R.P., Jensen, M.T., Miller, M. and Pedersen, J.P. (1988) in Green Photosynthetic Bacteria (Olson, J.M., Ormerod, J.G., Amesz, J., Stackebrandt, E. and Truper, H.G., eds.), pp. 15–21, Plenum, New York.
19 Kinoshita, K., Ishikawa, H. and Shinoda, K. (1958) Bull. Chem. Soc. Japan 31, 1081–1082.
20 Wittmershaus, B.P., Brune, D.C. and Blankenship, R.E. (1988) in Photosynthetic Light-Harvesting Systems (Scheer, H. and Schneider, S., eds.), pp. 543–554, Walter de Gruyter, Berlin.
21 Van Dorssen, R.J., Gerola, P.d., Olson, J.M. and Amesz, J. (1986) Biochim. Biophys. Acta 848, 77–82.
22 Wang, J., Brune, D.C. and Blankenship, R.E. (1990) Biochim. Biophys. Acta 1015, 457–463.