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FLUORESCENCE EMISSION SPECTRA OF CELLS AND SUBCELLULAR PREPARATIONS OF A GREEN PHOTOSYNTHETIC BACTERIUM

EFFECTS OF DITHIONITE ON THE INTENSITY OF THE EMISSION BANDS

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Summary

Fluorescence emission spectra were measured of intact cells and subcellular preparations of the green photosynthetic bacterium *Prosthecochloris aestuarii* in the presence and in the absence of dithionite. A 3—5-fold increase in bacteriochlorophyll *a* fluorescence at 816 nm occurred upon addition of dithionite in a membrane vesicle preparation (Complex I), in a photochemically active pigment-protein complex and in a bacteriochlorophyll *a* protein complex free from reaction centers. The pigment-protein complex showed a relatively strong long-wave emission band (835 nm) of bacteriochlorophyll *a*, which was preferentially excited by light absorbed at 670 nm and was not stimulated by dithionite. With Complex I, which contains some bacteriochlorophyll *c* in addition to bacteriochlorophyll *a*, a 3—4-fold stimulation of bacteriochlorophyll *c* emission was also observed. Emission bands at shorter wavelengths, probably due to artefacts, were quenched by dithionite. With intact cells, the effect of dithionite was smaller, and consisted mainly of an increase of bacteriochlorophyll *a* emission.

The results indicate that the strong increase in the yield of bacteriochlorophyll emission that occurred upon generating reducing conditions is, at least

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Abbreviations: BChl, bacteriochlorophyll; *P*-840, reaction center bacteriochlorophyll, the primary electron donor.

mainly, due to a direct effect on the light-harvesting systems, and does not involve the reaction center as had been earlier postulated.

Introduction

The fluorescence yield of chlorophyll *a* and of bacteriochlorophyll in intact cells and subcellular preparations depends on various conditions [1,2]. A very important factor controlling the fluorescence yield of antenna chlorophylls or bacteriochlorophylls is the oxidation-reduction state of the primary reactants. Large variations in fluorescence yield, depending on the state of the reaction center, have been observed for Photosystem II in higher plants and algae [1] and for purple bacteria [2]; for Photosystem I the yield of chlorophyll *a* fluorescence appears to be less dependent on the redox state of the reaction center [3—5].

Relatively little is known about the factors that control the fluorescence yield of BChl in green bacteria. Sybesma and Vredenberg [6] observed a small increase in BChl *a* fluorescence upon illumination of intact cells that appeared to be correlated with the oxidation state of *P*-840, the primary electron donor. On the other hand, Clayton [7] observed a light-induced fluorescence increase only under reducing conditions. Changes in the yield of BChl *c* emission were either smaller than those of BChl *a* [7], or were not observed at all [6]. Using membrane vesicle preparations, Fowler et al. [8] observed an approximately 2-fold increase in BChl *a* fluorescence upon addition of ferricyanide or dithionite. They interpreted these effects as being due to oxidation and reduction of the reaction center.

In this paper we report the effects of reducing conditions on the emission spectra of intact cells, membrane vesicles and pigment-protein complexes of the green bacterium *Prosthecochloris aestuarii*. The results indicate that the increase in the yield of BChl fluorescence observed upon lowering the redox potential is mainly due to a direct effect on the fluorescence yield of the antenna system, not involving the reaction center.

Materials and Methods

Prosthecochloris aestuarii, strain 2 K (originally known as *Chloropseudomonas ethylica* [9]), was grown in a mixed culture as described by Holt et al. [10]. The vesicle preparation Complex I and the photochemically active pigment-protein complex were prepared as described in Ref. 11. Both preparations contained 75—80 BChl *a* molecules per reaction center. Approximately half of the BChl *a* is contained in the light-harvesting BChl *a* protein complex [12], two molecules of which are presumably present per reaction center [11]. Complex I contained in addition a small amount of BChl *c* [11]. Purified BChl *a* protein complex was prepared according to Ref. 12.

Fluorescence emission spectra were recorded at 20°C by means of the spectrophotometer described in Ref. 13. The monochromator was supplemented by suitable glass cut-off filters (Schott RG 610 or RG 645) to absorb scattered excitation light; it was set at a half-width of 1.6 nm. The spectra were corrected

for the wavelength-dependent sensitivity of the apparatus and plotted in arbitrary units proportional to Watts per wavelength interval. The excitation light was filtered by Schott AL interference filters. The samples were contained in 1 mm vessels in 0.01 M phosphate buffer and 0.01 M sodium ascorbate (pH 7.4). The absorbance was less than 0.05 at the main infrared absorption band. Fluorescence was measured at an angle of 90° to the excitation beam at the front side of the cuvette.

Results and Interpretation

Emission spectra of the vesicle preparation Complex I obtained with two different wavelengths of excitation are shown in Fig. 1. Upon excitation at 420 nm, the emission spectrum showed bands near 635 and 675 nm, in addition to the near-infrared emission band at 816 nm. These bands were also observed by Fowler et al. [8] in the emission spectrum of a similar preparation from *Chlorobium limicola*. The 816 nm band, which was accompanied by a shoulder at longer wavelength of variable intensity, is presumably due to BChl *a*; the 675 and 635 nm bands may, at least partly, be due to artefacts produced during the preparation procedure, as will be discussed below. The band at 675 nm was much weaker, relative to the BChl *a* emission, upon excitation at 595 nm (Fig. 1B).

Addition of dithionite caused an about 3-fold increase in the intensity of BChl *a* emission at 816 nm, both upon 420 and 595 excitation, and strongly quenched the 635 and 675 nm bands, especially the latter one. Upon excitation at 420 nm dithionite also caused a stimulation of the emission at 750—780 nm, resulting in a clearly defined emission band with maximum at 770 nm, presumably due to BChl *c*.

Before addition of dithionite, the redox potential of the medium (which contained 0.01 M ascorbate) was approximately + 0.03 V. Addition of dithio-

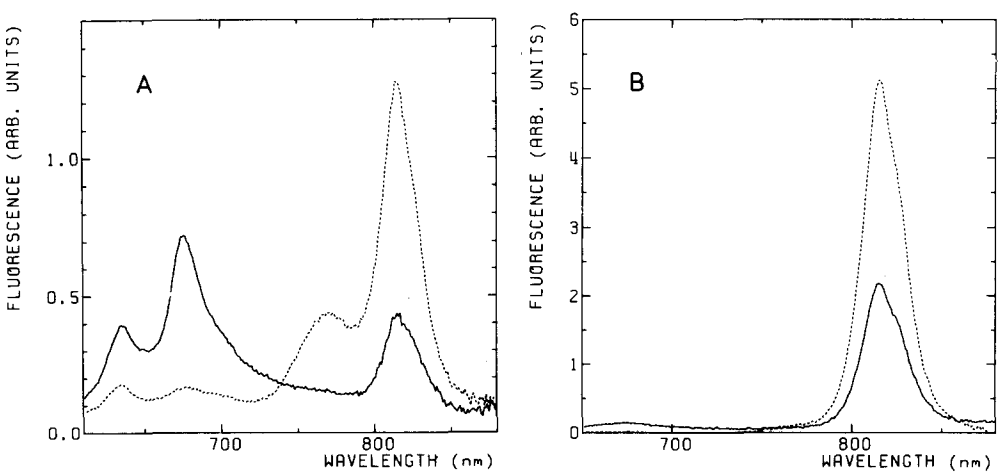

Fig. 1. Fluorescence emission spectra of Complex I in the absence (solid line) and in the presence (broken line) of 10 mM dithionite, upon excitation at 420 (A) or 595 nm (B). The vertical scales for the two parts of the figure are not comparable.

nite caused a lowering to about —0.40 V. This redox potential is probably not low enough to cause significant reduction of the primary electron acceptor [14—16]. This indicates that the fluorescence stimulation should either be attributed to the reduction of a secondary electron acceptor (as e.g. observed by Vernotte et al. [17] for Photosystem II chlorophyll in spinach chloroplasts), or to an effect of dithionite on the light-harvesting bacteriochlorophyll. In order to investigate this point further, we also measured the effect of dithionite on pigment-protein complexes and on intact cells.

Fig. 2 shows emission spectra of the photochemically active pigment-protein complex obtained from Complex I. This preparation did not contain BChl *c*. It showed a clear emission near 835 nm, visible as a shoulder on the main peak at 816 nm, and presumably due to long-wave absorbing BChl *a*. Again the peaks at 635 and 675 could be observed most prominently upon excitation at 420 nm. The effect of dithionite was very similar to that in Complex I and consisted of an about 3-fold stimulation at 816 nm and strong quenching of the 675 and 635 emission. In contrast to Complex I, the shape of the emission spectrum in the BChl *a* region was now clearly dependent on the wavelength of excitation, the band near 835 nm being higher relative to that at 816 nm upon excitation at 420 than at 595 nm. This effect was even more pronounced upon excitation at 670 nm, which wavelength preferentially excited emission in the 835 nm band (Fig. 3). The emission at 835 nm was not affected by dithionite. Since the long-wave absorbing chlorophyll is thought to be more closely associated with the reaction center, this observation suggests that the effect of dithionite is at least in part due to a direct action on the light-harvesting BChl *a*.

This conclusion is in agreement with experiments with the light-harvesting BChl *a* pigment-protein (Fig. 4). The shape of the fluorescence spectrum was independent of the wavelength of excitation. Except for a shoulder at the long-wave side of the main emission band at 816 nm, the spectrum was similar to that earlier reported for similar preparations [8,18]. No emission at 635 or 675 nm could be detected. Addition of dithionite caused a 4—5-fold increase in the

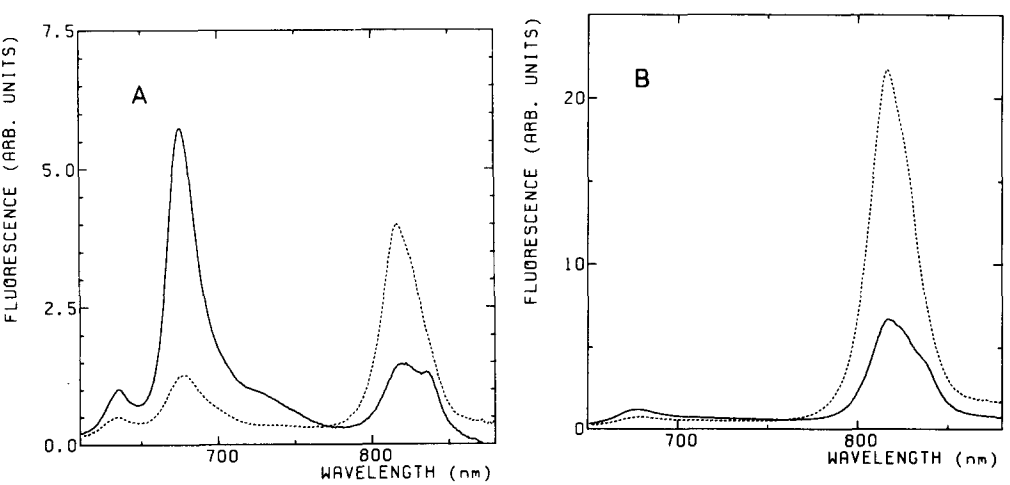

Fig. 2. Fluorescence spectra of the pigment-protein complex in the absence (solid line) and in the presence (broken line) of dithionite, upon excitation at 420 (A) or 595 nm (B).

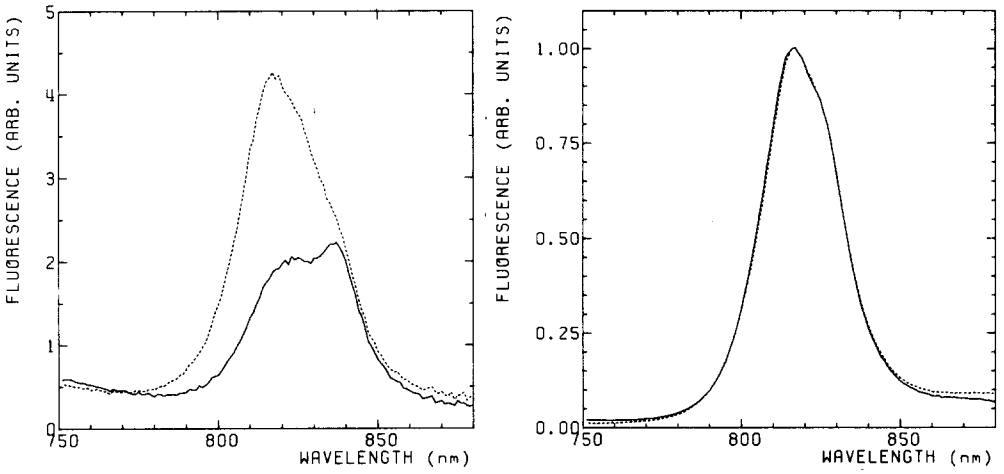

Fig. 3. Fluoresence spectra of the pigment-protein complex in the absence (solid line) and in the presence (broken line) of dithionite. Excitation at 670 nm.

Fig. 4. Fluorescence spectra of the BChl *a* protein complex in the absence (solid line) and in the presence (broken line) of dithionite upon excitation at 595 nm. The spectrum obtained with dithionite was reduced by a factor of 4.8 in order to facilitate comparison.

intensity of fluorescence, without changing the shape of the emission spectrum. This result directly demonstrates that dithionite can affect the yield of BChl *a* fluorescence by a process not involving the reaction center.

Experiments with intact cells showed that dithionite stimulated the emission by BChl *a* up to 2-fold but not or only slightly that by BChl *c*. The cells had the usual emission spectrum in the infrared region with emission bands near 770 and 815 nm [18,19]. Significant emission at 635 and 675 nm was not observed.

Interestingly, strong stimulations by dithionite (up to 8-fold) were observed when cells had been stored in buffer solution for several days, both at 770 and 815 nm. These 'old cells' now also exhibited emission bands at 635 and 675 nm upon excitation at 420 nm (cf. Refs. 18 and 19). Like in the subcellular preparations, these bands were quenched by dithionite.

Discussion

A common feature of the emission spectra of membrane fragments and pigment-protein complexes from *P. aestuarii* is a prominent band at 816 nm, due to BChl *a*. In addition, the preparations showed bands or shoulders at longer wavelengths. In the pigment-protein complex a relatively strong emission at 835 nm was observed which appeared to be selectively excited at 670 nm. This indicates that structural changes occur upon preparation of the pigment-protein complex, which affect the fluorescence yield of the bacteriochlorophylls that absorb at longer wavelengths. Light absorbed by the pigment absorbing at 670 nm, which is thought to be bacteriopheophytin *c* [11,20] appears to be transferred directly to these long-wave bacteriochlorophylls, which appear to be more closely associated with the reaction center [11,20].

Our results strongly indicate that, at least in the vesicle and pigment-protein preparations, the fluorescence stimulation caused by dithionite is at least mainly due to a direct effect on the fluorescence yield of the antenna bacteriochlorophylls. This applies both to BChl *a* and BChl *c* fluorescence emission. The effect might be due to removal of artificial quenching sites in the light-harvesting systems, by reduction of the quenching substance. The effect of dithionite on the fluorescence of BChl *a* more closely connected to the reaction center appears to be considerably smaller than on that of the other pigments.

The experiments with intact cells are compatible with the notion that an effect of dithionite involving the reaction center may also occur. Here, the fluorescence stimulation occurred mainly in the BChl *a* band, and appeared to be of similar magnitude to that observed by Clayton [7] upon illumination. The effect of dithionite on 'old cells' is particularly striking. Perhaps a partial desintegration of the structure of these cells occurs during storage, resulting in the generation of artificial quenchers in the light-harvesting pigment systems.

The emission bands at 635 and 675 nm are probably largely due to artefacts generated during the preparation procedures or during the ageing of the cells. Complex I preparations obtained from bacteria that had been stored for about a week at 0—4°C showed an unusually high absorption band near 670 nm. Addition of dithionite to such a preparation reduced the 670 nm absorption to approximately the usual value. This suggests that most of the 675 nm emission (and by inference also of the 635 nm emission) is due to small amounts of artefacts present even in the 'purer' preparations, which are destroyed by dithionite.

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