Biochimica et Biophysica Acta, 1019 (1990) 239–244
Elsevier

BBABIO 43269

Isolation and characterisation of an unusual antenna complex from the marine purple sulphur photosynthetic bacterium *Chromatium purpuratum* BN5500

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(Received 6 March 1990)

Key words: Photosynthetic bacterium; Antenna complex; Bacteriochlorophyll; Carotenoid

The photosynthetic unit of the marinè purple sulphur bacterium *Chromatium purpuratum* has been fractionated into two components. It consists of a normal LH1-reaction centre 'core' complex together with a spectrally unusual B830 LH2 complex. The polypeptide composition and spectral properties of the B830-complex have been characterised.

Introduction

Chromatium purpuratum BN5500 is a small marine *Chromatium* species first described in 1980 [1]. It is bright purple and belongs to a small group of the Chromatiacae which contain the unusual carotenoid okenone [2].

When bacteriochlorophyll *a* (Bchl *a*) is non-covalently bound to antenna apoproteins, to form native light-harvesting complexes, its NIR absorption band is shifted to lower energy. Typically this yields complexes with absorption maxima at 800, 850 and 875 nm [3]. However, even though the purple bacterial antenna complexes are now an extremely well-studied group of pigment-proteins the origin of these spectral shifts is still a matter of debate [4].

We were intrigued, therefore, to investigate the light-harvesting complexes in *Chr. purpuratum* since the room temperature absorption spectra of its whole cells showed a single strong NIR absorption peak at 830 nm. This is a very unusual feature that has only been reported previously for *Chr. okenii* [5], but has never been studied.

Abbreviations: NIR, near infra-red, LDAO, lauryldimethylamine *N*-oxide; Bchl *a*, bacteriochlorophyll *a*.

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Materials and Methods

Cells of *Chr. purpuratum*, BN5500, were grown anaerobically in the light at 30 °C in Pfennig's medium for phototrophic bacteria, with the addition of 2.5% sodium chloride, 0.1% sodium acetate, 0.2% sodium bicarbonate, 0.05% yeast extract and 0.15% sodium thiosulphate instead of sodium sulphite [1]. The cells were harvested by centrifugation and resuspended in 20 mM Mes, 100 mM KCl (pH 6.8). Chromatophores were isolated by breaking the cells in a French pressure cell (at 156 MPa) and subsequent differential centrifugation. A low speed spin at $12000 \times g$ for 15 min removed any unbroken cells and cell debris, while a second high speed centrifugation at $150000 \times g$ for 1 h pelleted the chromatophores.

The chromatophores were initially fractionated into LH1/reaction centre 'core' complexes and their peripheral LH2 complexes by non-denaturing gel electrophoresis using the Deriphat-polyacrylamide gel system of Peter and Thornber [6]. The Bchl *a* concentration of the chromatophore sample was assayed following extraction into 7 : 2 (v/v) acetone and methanol using an extinction coefficient at 772 nm of 76 $cm^{-1} \cdot mM^{-1}$ [7]. Samples of the chromatophores were then solubilised with a detergent mixture of 50/50 lauryl maltoside and β-octyl glucoside (at a total detergent concentration of 1% (w/v)) using a range of detergent/Bchl *a* (w/w) ratios from 10 : 1 to 40 : 1. The solubilised samples were

briefly centrifuged to remove any unsolubilised material and then electrophoresed at 4° C in a 6.4% acrylamide gel as described by Peter and Thornber [6]. The gel running buffer contained the detergent Deriphat 160. After about 45 min the pigment-protein complexes were clearly resolved as purple bands. In this way the optimum detergent/Bchl *a* ratio was determined, whereby all the complexes entered the gel and were well resolved with negligible free-pigment produced. A preparative gel was then run under the optimal conditions to provide enough material for further study.

The major antenna complex present in *Chr. purpuratum* (B830) was isolated and purified, in bulk, from the chromatophores following solubilisation with the detergent LDAO. The concentration of the chromatophores was adjusted to give an absorbance at 830 nm of 100 cm^{-1}. They were then solubilised by the addition of 1% (v/v) of LDAO, incubated at room temperature for 10 min and centrifuged at $12000 \times g$ for 5 min to remove any unsolubilised material. The supernatant was diluted five times with 20 mM Tris-HCl (pH 8.0) and loaded on to a DE 52 cellulose anion exchange column, which had been pre-equilibrated in the same buffer. The column was loaded to about one third of its capacity. The B830 complex was then eluted by washing the column with increasing concentrations of NaCl in 20 mM Tris-HCl (pH 8.0) 0.1% (v/v) LDAO. The best antenna fractions, as judged by spectrophotometry, were eluted between 75 and 100 mM NaCl. The complex was further purified by passage over a second DE52 column.

The integrity of the antenna preparation was checked spectrophotometrically in a Pye-Unicam SP8-500 spectrophotometer. The polypeptide composition of the B830-complex was determined by SDS-polyacrylamide gel electrophoresis [8]. The Bchl *a*/carotenoid ratio was determined as previously described [9]. The identity of the major carotenoids present was confirmed by mass spectrometry.

Low temperature absorption and fluorescence emission and excitation spectra were determined in a single beam spectrophotometer which has been described elsewhere [10]. The efficiency of the carotenoid to Bchl *a* singlet-singlet energy transfer was determined at room temperature in a home-built apparatus as previously described [11]. The CD spectrum of the B830-complex was recorded on a JASCO instrument at the SERC CD facility at Stirling University.

Results and Discussion

The absorption spectra of chromatophores of *Chr. purpuratum* recorded at room temperature and 77 K are shown in Fig. 1. In the NIR region of the room temperature spectrum there is a single strong absorption peak at 830 nm, with pronounced shoulders at 800 nm and 875 nm. In the visible region there is a broad absorption band between 580 and 460 nm, which is mainly due to the major carotenoid present (okenone) and then the Bchl *a* Soret band between 360–370 nm. At 77 K the Bchl *a* absorption bands in the NIR are well-resolved. The 875 nm shoulder is shifted to approx. 890 nm and is now quite prominent. The 830 nm band is sharpened and very slightly red-shifted to 835 nm, while at room temperature the 800 nm shoulder is now clearly visible as a discrete band.

The photosynthetic membrane was initially fractionated by adapting the 'Deriphat-Green gel' system. This method is commonly used to isolate pigment-protein complexes from higher plant chloroplasts [6]. A typical gel is shown in Fig. 2. Three pigmented bands are clearly visible. These bands were cut out of the gel and their absorption spectra recorded (Fig. 3). The top band

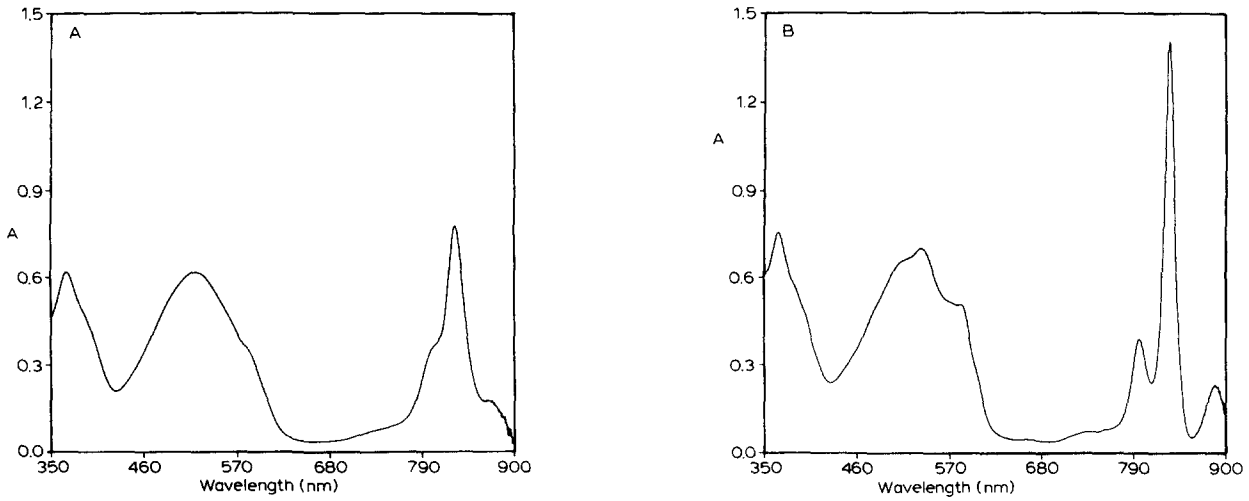

Fig. 1. The room- and low-temperature (77 K) absorption spectra of chromatophores of *Chr. purpuratum*. (A) The room temperature absorption spectrum. (B) The absorption spectrum at 77 K.

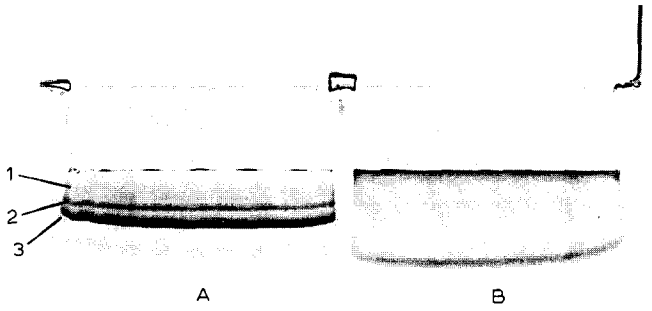

Fig. 2. Fractionation of the photosynthetic unit of *Chr. purpuratum* on a non-denaturing 'Deriphat' gel. (A) A non-denaturing sample (detergent/Bchl *a*, 25 : 1, w/w). The coloured (unstained) bands are (1) The RC LH1 conjugate (rather faint), (2) and (3) The B830-complex. (B) A sample that was denatured by boiling for 2 min prior to the electrophoresis.

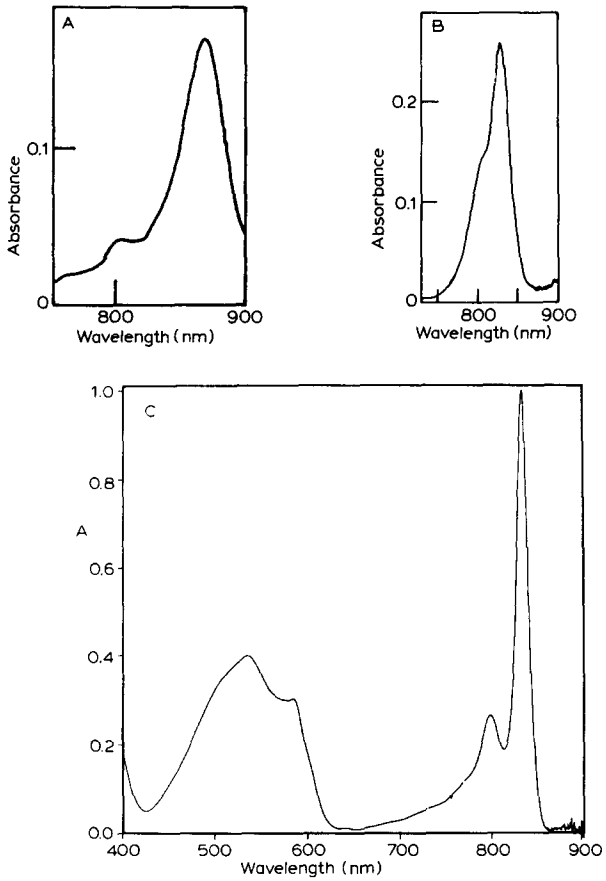

Fig. 3. The absorption spectra of the isolated pigment-protein complexes from *Chr. purpuratum*. (A) The NIR absorption spectrum of band 1 isolated from the 'Deriphat' gel. The absorption spectrum was recorded of the gel slice removed from the gel shown in Fig. 2. (B) The NIR absorption spectrum of band 3 isolated from the 'Deriphat' gel. The absorption spectrum was recorded on a gel slice. (C) The 77 K absorption spectrum of the B830-complex isolated by ion-exchange chromatography. Compare this with the spectrum shown in Fig. 1B and note the absence of the LH1 absorption band at approx. 880–890 nm.

shows the typical absorption spectrum of an LH1-reaction centre 'core' complex [12]. The absorption band at 875 nm represents the B875-complex and the absorption band at 800 and the shoulder at 760 nm arise from the reaction centre. The absorption spectrum of this complex is typical for purple bacterial 'core' complexes. The absorption spectra of bands 2 and 3 from the Deriphat gel are identical (Fig. 3B). It is clear that these bands represent the major antenna complex in *Chr. purpuratum*, the B830-complex.

In order to obtain enough of the B830-complex to study it in detail we switched to preparing it by ion-exchange chromatography. This method, however, results in the irreversible denaturation of the B875-reaction centre 'core' complex.

Fig. 3C shows the low temperature absorption spectrum of the B830-complex isolated by ion-exchange chromatography. The absorption bands at 835 nm and 800 nm are clearly visible and the peak seen at 875 nm (i.e., LH1) in the chromatophores is absent.

The polypeptide composition of the B830-complex has been determined by SDS-polyacrylamide gel electrophoresis (Fig. 4). It consists of two low-molecular-weight apoproteins, which is quite typical for purple bacterial antenna complexes [3]. The further characterisation of these polypeptides will be presented elsewhere (Hawthornthwaite and Cogdell, manuscript in preparation).

Fig. 4. An SDS-polyacrylamide gel of the isolated B830-complex from *Chr. purpuratum*. The polypeptides were separated on a 20% acrylamide gel. Lanes 1–3, Denatured B830 complex. Lane 4, Standard proteins as molecular mass markers (bovine serum albumin, 68 kDa; alcohol dehydrogenase, 41 kDa; myoglobin, 17 kDa and cytochrome *c*, 12 kDa).

In order to determine the Bchl *a*/carotenoid ratio in the B830-complex we first had to determine the carotenoid composition of the complex. We initially investigated the carotenoid composition of the chromatophores. The final extract, when run on a silica-gel thin-layer plate in a 3:7 (v/v) diethylether/40°–60° b.p. petroleum ether solvent mixture gave three well-separated bands. Each band had a very similar absorption spectrum in diethylether. The bottom band was the most abundant (87%) with a λ_{max} of 480 nm, the middle band (7.5%) had a λ_{max} of 478 nm and the top band (5.5%) had a λ_{max} of 475 nm. The mass spectrum of each of the three components was essentially the same with a molecular ion at an *m/e* ratio of 578. The absorption spectra and the mass spectra are consistent with the three bands being the carotenoid okenone [13]. The two small bands seen on the TLC plates undoubtedly represent isomers of okenone. Carotenoid extracts of the B830-complex showed the same composition as the chromatophores.

Table I shows the results of our determinations of the Bchl *a*/carotenoid ratio in the B830-complex. There is some ambiguity in the final ratio, not because the data are inaccurate, but because there is no reliable extinction coefficient for okenone. Since okenone is a keto-carotenoid its absorption spectrum is flattened by comparison with purely hydrocarbon carotenoids, such as neurosporene and so its extinction coefficient is expected to be rather lower than that of an average carotenoid. Britton reports an extinction coefficient for okenone of 134 $mM^{-1} \cdot cm^{-1}$ [15]. Using this value to Bchl *a*/Car ratio is 1.24:1. If the true value of this extinction coefficient was as low as 110 $mM^{-1} \cdot cm^{-1}$ then the ratio would be 1.05:1. However, even in the absence of a firm value for the extinction coefficient for okenone it is clear that the B830-complex has an unusual Bchl *a*/carotenoid ratio for an LH2 complex [9].

The room temperature CD spectrum of the B830-complex is shown in Fig. 5. In the region of the 830 nm absorption band there is a strong negative peak at approx. 842 nm with a slight shoulder on its blue edge at about 830 nm. Around 800 nm there is a broad positive feature. Again these spectral properties are unusual for an LH2 complex [3,14]. In the visible part of the spectrum a weak rather ill-defined peak is seen in the region where okenone absorbs.

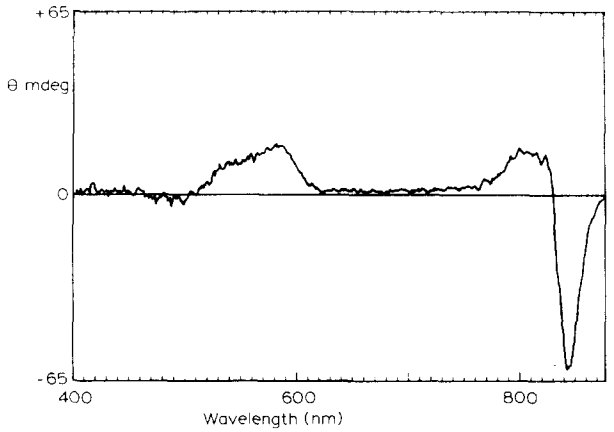

Fig. 5. The room-temperature CD spectrum of the B830-complex from *Chr. purpuratum*. The spectrum was recorded in a 1 cm pathlength with a sample whose absorbance at 830 nm was 1.1. The spectrum shown was baseline corrected.

The 77 K fluorescence emission and excitation spectra of the B830-complex are shown in Fig. 6. The major emission band is at approx. 850 nm and clearly comes from the 830 nm absorption band. There is a minor emission band at about 805 nm (see the 25 × inset in Fig. 6A). The relative intensity of this minor band compared to the major emission at 850 nm is approx. 2%. The excitation spectrum for both these emission bands has been determined (Fig. 6B). It is clear that both the Bchl *a* Q_x absorption band at approx. 590 nm and the carotenoid absorption bands between 460 and 570 nm contribute strongly to the excitation spectra for the major 850 nm fluorescence band. At room temperature the energy transfer efficiency of the carotenoid Bchl *a* singlet-singlet energy transfer is about 60–70% (Fig. 6C). The excitation spectrum for the 805 nm emission is quite different; essentially all of the excitation comes from the 590 nm Bchl *a* band and hardly any from the carotenoid. There are two explanations for this. If the emission at 805 nm originates from the 800 nm absorption band of the B830-complex then this Bchl(s) must not be in energy transfer communication with the carotenoid. However, there is also the possibility that some or all of this emission is due to some 'free' 780 nm absorbing Bchl *a* in the complex. 'Free' Bchl *a* has a much higher fluorescence yield and would swamp any emission from the 800 nm absorption band. If this was the case then the low efficiency of energy trnasfer from the carotenoids would be expected. Efficient singlet-singlet energy transfer from the carotenoids to the

TABLE I

Determination of the Bchl a/carotenoid ratio in eight samples of the isolated B830-complex

The average Bchl *a*/Car ratio = 1.24 ± 0.05.

Sample number	Absorbance at 772 nm (Bchl)	Absorbance at 480 nm (carotenoid)	Bchl *a*/Car
1	0.5	0.725	1.23:1
2	0.472	0.69	1.24:1
3	0.457	0.67	1.24:1
4	0.393	0.57	1.22:1
5	0.469	0.678	1.22:1
6	0.314	0.454	1.22:1
7	0.426	0.654	1.31:1
8	0.338	0.487	1.22:1

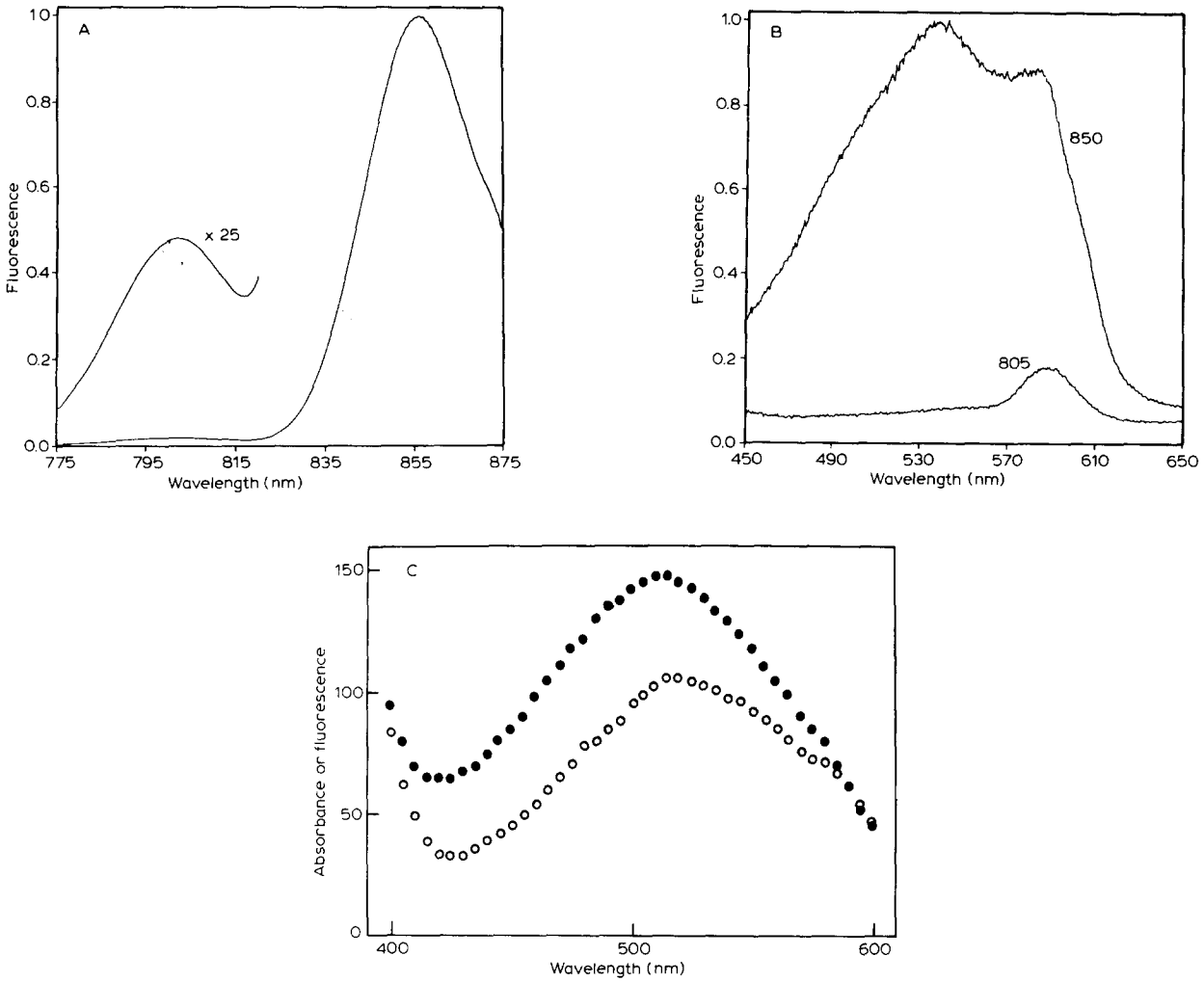

Fig. 6. The fluorescence emission and excitation spectra of the B830-complex from *Chr. purpuratum.* (A) The 77 K emission spectrum. Excitation was provided at 590 nm with an 8 nm band width. The band width of the fluorescence detection system was 6 nm. The insert was expanded 25× to show the 805 nm emission. (B) The excitation spectra at 77 K. The top spectrum is the excitation spectrum recorded while monitoring the emission at 850 nm, while the lower spectrum was recorded while monitoring the emission at 805 nm. The bandwidth of the excitation light was 6 nm and that of the detection system was 10 nm. The two spectra are not directly comparable with regard to intensity since they were recorded with different sample integration times. (C) A comparison at room temperature of the fractional absorption spectrum of the B830-complex with the fluorescence excitation spectrum. ●, fractional absorption; ○, fluorescence intensity. The two spectra were normalised at 590 nm in the bacteriochlorophyll absorption band. The fluorescence emission intensity was recorded at 850 nm with a 10 nm bandwidth.

Bchls only occurs in the antenna complexes when the pigments are correctly bound within those complexes. Further work will be required to distinguish between these two possibilities.

Acknowledgements

The authors wish to note that their use of LH terms for describing antenna complexes somewhat confuses the guidelines recommended in an earlier publication [16]. However, in the light of further developments in this field, namely the ability of some species to alter the type of LH2 complex which is synthesised, some of which absorb with differing intensities at the same NIR absorption maxima, such guidelines should be treated with a degree of compromise.

This work was supported by grants from the SERC and the E.E.C. M.B. Evans was supported by a SERC postgraduate studentship. We would also like to thank Sharon Kelly and Dr. Nick Price at Stirling University for help in running the CD spectra, and Dr. J.F. Imhoff for sending us the culture of *Chr. purpuratum.*

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