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ON THE QUANTITATION OF BACTERIOCHLOROPHYLL IN CHROMATOPHORE SUSPENSIONS FROM PURPLE BACTERIA

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A direct photometric quantitation of bacteriochlorophyll (BChl) at 375 nm in aqueous chromatophore suspensions from various purple bacteria is described. The assay is rapid and reproducible. It is utilized easily for processing large numbers of samples and is as sensitive as extraction methods usually applied today. Drawbacks of extraction methods, particularly not quantitative extractions, photo- and autooxidation are avoided. There is good linearity up to 20 μg BChl/ml suspension, and no interference by buffers is observed.

Laboratory practice in experiments with aqueous chromatophore suspensions from purple bacteria requires a rapid and sensitive method for the quantitation of BChl. Methods usually applied today, e.g., the photometric determination of BChl after extraction with organic solvents like ether [1], acetone/methanol (7:2, v/v) [2] or methanol [3] are time consuming and inconvenient. Chromatophores have to be ultracentrifuged to separate the aqueous phase, followed by extraction in the cold and dark to avoid photo- and autooxidation. To perform photometric determination it is necessary to centrifuge the BChl-depleted membranes again.

Extraction methods are often not quantitative and imply instability, being particularly evident in methanol. The method of Van Niel and Arnold [4], which includes conversion of BChl into bacteriopheophytin, avoids some of these drawbacks.

BChl extracted by organic solvents from purple bacteria is identical spectroscopically showing one large absorption band at 772 nm which is used for quantitative BChl determinations [2]. In aqueous chromatophore suspensions the BChl molecules can interact with each other and with membrane protein, carotenoids and lipids causing absorption peaks in the near-infrared region to occur at different wavelengths for the different species. The precise forms of the infrared absorption spectra also depend on a large variety of parameters, i.e., carbon and electron sources, growth conditions and the age of the cultures [5]. This fact has been recognized during early qualitative investigations in BChl [6], but has not been heeded adequately as far as BChl quantitation in aqueous chromatophore suspensions in the infrared region is concerned [7]. However, at 375 nm (Soret band) such suspensions from purple bacteria reveal an absorption peak which is independent of above-mentioned parameters and therefore well fitted for the quantitative photometric determination of BChl.

For this reason, the millimolar extinction coefficients (ϵ) at 375 nm of aqueous chromatophore suspensions from six species of purple bacteria were determined by direct comparison with two different extraction methods as references which are the method of Clayton ($\epsilon = 75\ mM^{-1} \cdot cm^{-1}$ at 772 nm) [2] and that of Van Niel and Arnold ($\epsilon = 9.9\ mM^{-1} \cdot cm^{-1}$ at 667.8 nm) [4]. The calculations are based on the following equation (1 cm

Abbreviation: BChl, bacteriochlorophyll.

pathway):

$$\text{millimolar extinction coefficient at 375 nm} = \frac{\text{absorbance at 375 nm}}{\text{mmol BChl per 1 determined by the reference methods}}$$

Hence, we determined for *Chromatium vinosum* (ATCC 17899), *Rhodopseudomonas acidophila* (ATCC 25092), *Rps. capsulata* (ATCC 11166), *Rps. palustris* (ATCC 11168), and *Rps. sphaeroides* (ATCC 17023) a millimolar extinction coefficient of 100 $mM^{-1} \cdot cm^{-1}$ and for *Rhodospirillum rubrum* (ATCC 11170) a millimolar extinction coefficient of 110 $mM^{-1} \cdot cm^{-1}$. These values were averaged over 10 BChl determinations from 10 different batch cultures for each organism. We found that at 375 nm the direct photometric BChl quantitations are much faster, and at least as sensitive and reproducible as extraction methods. Our assay is utilized easily for processing large numbers of samples and adaptable equally well to automatic procedures. Using these millimolar extinction coefficients at 375 nm, the BChl content of the chromatophore suspensions calculated as BChl *a* in $\mu g/ml$ can be computed from the following equation (1 cm pathway):

$$\underset{\text{suspension}}{\mu g \text{ BChl/ml}} = \frac{\text{absorbance at 375 nm} \times \text{molecular weight of BChl } a \text{ (911.5)}}{\text{millimolar extinction coefficient at 375 nm}}$$

There was good linearity in the response pattern up to 20 μg BChl/ml suspension. Higher concentrations of BChl could be measured after adequate dilution.

We consider the described method also to be applicable to chromatophore suspensions from other purple bacteria. However, attention has to be payed to the following aspects. Cytochromes likewise absorb at 375 nm. The absorbance of oxidized and reduced species differs at this wavelength and changes in cytochrome redox states have a small effect on the total absorbance at 375 nm. A few species of purple bacteria contain carotenoids which absorb at 375 nm and may vary with growth conditions, etc. In these cases a millimolar extinction coefficient determination by use of the described method is advisable to be performed for each strain and set of growth conditions.

Cell material from all species investigated, except *C. vinosum*, was grown photosynthetically on malate for 48 h at 30°C and at 10000 lx in 10-l carboys in a medium modified from this described by Knobloch et al. [8]. *C. vinosum* was grown photosynthetically on thiosulfate in a medium described by Bose [9] under the same conditions. Cells were harvested at 4°C in a continous-flow centrifuge, washed once with 50 mM Tris-HCl buffer (pH 8.0), resuspended in the same buffer and broken by sonication for 100 s at 400 W. After centrifugation at $10000 \times g$ for 30 min to remove cell debris, the supernatant was centrifuged at $20000 \times g$ for 15 min. The supernatant was ultracentrifuged at $144000 \times g$ for 100 min. Prior to the photometric measurement, the pellet (chromatophores) from this centrifugation was resuspended by means of a homogenizer (Potter S, B. Braun Melsungen) in 20 mM glycylglycine buffer (pH 7.8) or in 50 mM Tris-HCl buffer (pH 8.0), respectively. The kind of buffer applied had no influence on the results. It is necessary to homogenize the suspensions to complete transparency, since turbid suspensions cause additional absorbance during photometric measurement due to light scattering. Chromatophores prepared by the above-mentioned method involving a three-step centrifugation resulted in completely transparent suspensions after homogenization. If, due to certain circumstances, chromatophore preparations have to be quantitated which are not fully transparent, we would like to refer interested readers to the technique used by Sojka et al. [10] to reduce light scattering or we recommend using a scattered transmission accessory.

All photometric measurements were carried out in a Shimadzu UV 210 A double-beam spectrophotometer. All solvents and chemicals were of analytical grade.

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