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A METHOD FOR THE EXTRACTION AND QUANTITATION OF PHYCOERYTHRIN FROM ALGAE

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SUMMARY

This report describes an effective technique for the extraction of phycoerythrin from algal cells and prescribes a method for quantitating the amount of phycoerythrin pigment. A graph is shown relating concentration to fluorescence units.

INTRODUCTION

Until now no generally effective method for extraction of phycoerythrin from algal cells has been developed. One problem has been the difficulty of disrupting the cells. Phycobilin-containing cells tend to be resistant to conventional cell destruction techniques such as grinding. To ensure quantitative removal of phycoerythrin (PHE) from algal cells a technique is needed that combines physical stress with a chemical attack on the cell.

A second problem lies in distinguishing and quantitating the type of PHE in the sample. Six types of PHE are currently known to exist. These forms exhibit a variety of absorption and fluorescence emission characteristics, and different extinction coefficients. It is thus necessary to have some idea of which pigment predominates in a sample in order to use fluorescence or absorbance to estimate its abundance.

This report describes an effective technique for the extraction of PHE from algal cells. It also presents a guide for distinguishing which PHE type predominates in a sample and for quantitating the extracted PHE.

METHODS

Extraction of Phycoerythrin: A precise volume of the culture

or sample was passed through a Reeve-Angel No. 984H glass fiber filter. Duplicates were run for each sample. Each filter was thoroughly homogenized in an all-glass tissue grinder in a medium consisting of 0.25 M Trizma Base, 10mM EDTA and 2 mg/mL lysozyme (pH adjusted to 5.0 with HCl). The homogenate was incubated at 37° C. for 2 hours and then for 10 hours in the refrigerator. At the conclusion of incubation the pH was adjusted to 7.0 with 0.1 N NaOH, and the total liquid was increased to twice the original volume with distilled water. Filtration on a 5 µm Millipore filter produced a clear liquid for analysis.

Calibration and Analysis: PHE extracts prepared from several cultures were used to determine the relationship between fluorescence and absorbance for various PHE types.

The concentration of a pigment was determined from its absorbance using the following equation:

$$\frac{\text{absorbance} \times 1000\ \mu g/mg}{E^{1\%}_{1\ cm} \times \text{pathlength}} = \mu g\ \text{PHE/mL extract}$$

Absorbance was determined at the wavelength of maximum absorption of an initial PHE solution, using a Cary 17 spectrophotometer. The choice of extinction coefficient was based on the type of PHE present in the extract, as determined by its absorption characteristics. The extinction coefficients and wavelengths of maximum absorption of the algal extracts used

in this study are listed in Table 1.

Fluorescence measurements were made on a number of volumetric dilutions of each initial PHE solution using a Hitachi Perkin Elmer fluorescence spectrophotometer. It was operated in the "ratio" mode to eliminate variations in output due to fluctuation of the xenon light source. The excitation wavelength was set at 520 nm (slitwidth, 20 μm) and the material was scanned from 540 - 600 nm for PHE emission. Emission scans were made in an identical manner for extracts of seawater samples. The temperatures of all PHE solutions were between 9 and 12^{o} C. during analyses; they were kept in the refrigerator at 5^{o} C. at all other times.

RESULTS AND DISCUSSION

Relationship of Fluorescence Emission to Pigment Concentration: A graph was prepared from the dilution series to relate emission and concentration for the several types of PHE examined (Figure 1). Each PHE type produced a line with a different slope and a slightly different origin. However, in the fluorescence range encountered in the seawater samples processed thus far (0.2 - 0.7 units) there is relatively little variation in the corresponding pigment concentration (0.00025 µg/mL, maximum) among the three extracts with high fluorescence emission wavelength (BL-5, VA-70 and BL-4). BL-1, which fluoresces at a distinctly lower wavelength, has a greater slope than the three previously mentioned extracts.

It is not surprising to find variety in the fluorescence/concentration relationship. The extracts do not contain highly purified chromophores; rather, the pigments exist in much the same protein "environment" as they did within the cell. That environment varies from species to species, even for the same pigment type. With different pigment types, the variation increases.

The graph produced in this study is a plot of Span 10 fluorescence units versus PHE concentration (Figure 1). It is important to note that these fluorescence units are unique

to the fluorescence spectrophotometer, recorder and conditions used during the study, and cannot be directly applied to any other setup.

Taxonomic Significance of the Phycoerythrin Types: The four extracts prepared in this study represent three different PHE types (Table 1). BL-4 and BL-5 are both cryptomonads and contain cryptomonad-PHE 545. The 545 refers to the wavelength of maximum absorption by this pigment, 545 nm. Supposedly, there are two other types of cryptomonad PHE, 555 and 565 (Siegelman and Kycia, 1978), but there are no organisms in the collection which contain these pigments. Cryptomonad-PHE 545 fluoresces at 580 nm.

BL-1, a cyanophyte, contains a variant of b-PHE. This pigment has two absorption peaks (500 and 545 nm) and it fluoresces maximally at 560 nm. According to Moreth and Yentsch (1970), this variant of R-PHE is typical of open ocean cyanophytes.

VA-70, a rhodophyte, contains 6-PHE, which absorbs maximally at 545 nm and fluoresces at 578 nm. b-PHE is confined to rhodophytes. However, rhodophytes generally represent a very minor component of the phytoplankton. They are more common in estuarine waters than in the open ocean.

Selection of Correct Curve for Calculation of Pigment Concentration: The choice of curve may be made on the basis of

emission wavelength. If the sample fluoresces maximally around 560 nm, the BL-1 curve should be used. This case would be most likely if the sample were collected from oceanic waters.

If, however, the sample fluoresces around 575-580 nm the main PHE contribution is probably from cryptophytes, especially in estuarine waters. The use of the BL-5 curve is recommended in this case. It comes close to passing through the origin of the graph, and is similar to the VA-70 curve.

The wavelength of maximum fluorescence emission for an extracted sample can be confirmed by the laser-excited emission maximum of the sample. Laser excitation provides a very defined peak. Thus, wavelength of PHE emission will be a valuable aid in characterizing phytoplankton populations by remote fluorosensors.

CONCLUSIONS

The BL-5 curve should be used to determine the pigment concentration of samples fluorescing around 575-580 nm. Such samples are probably dominated by cryptophytes in estuarine waters. The BL-1 curve should be used to determine the PHE content of samples fluorescing around 560 nm. These samples are dominated by cyanophytes characteristic of the open ocean.

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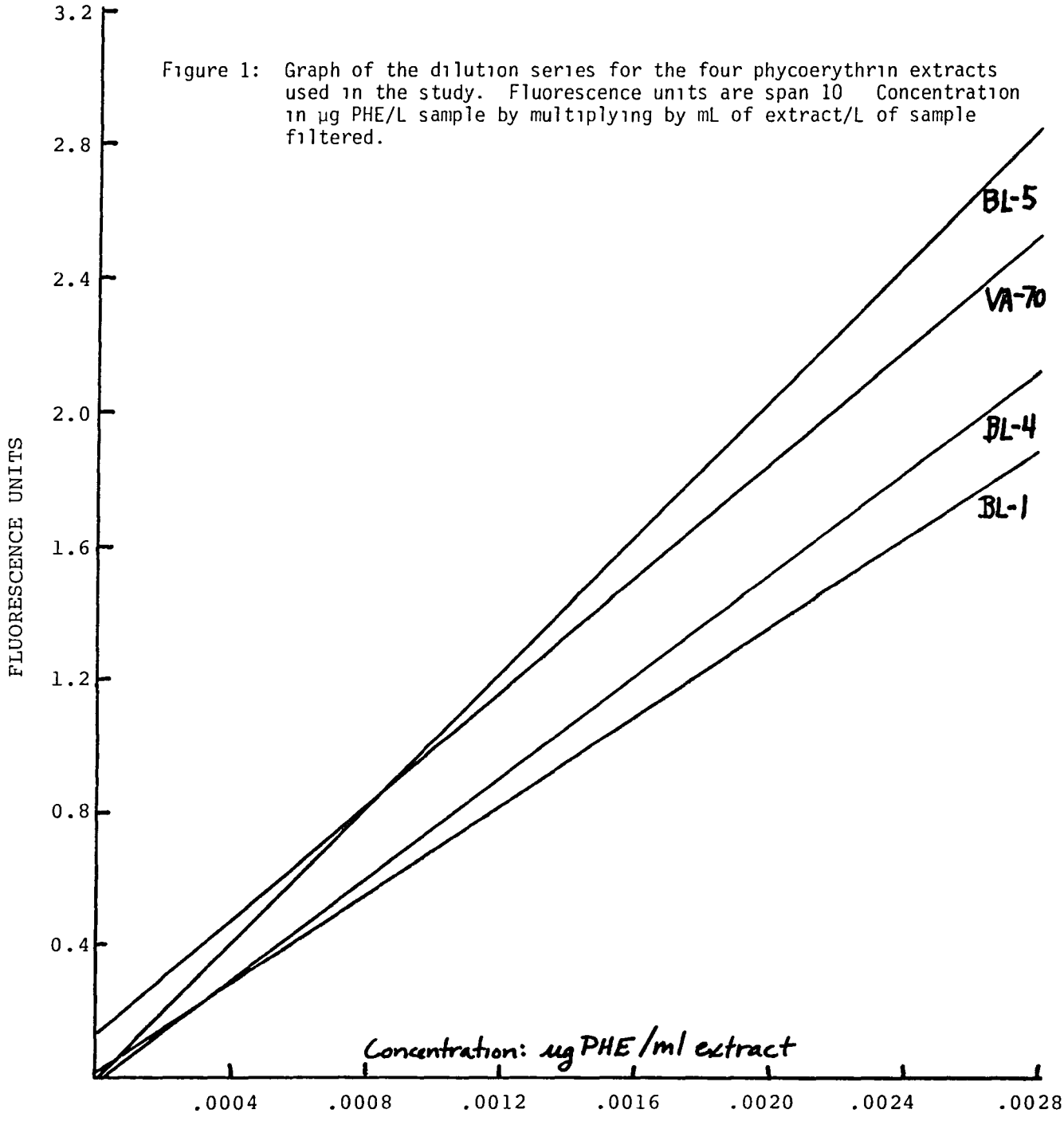
ORGANISM	SYMBOL	PHE TYPE	MAXIMUM ABSORBANCE	MAXIMUM FLUORESCENCE	EXTINCTION COEFFICIENT (1 cm, 1%)	
Unidentified cryptomonad	BL-4	Crypt.-PHE	545 nm	580	126	MacColl *et al*. 1976
Unidentified cryptomonad	BL-5	Crypt.-PHE	545 nm	580	126	MacColl *et al*. 1976
Synechococcus sp.	BL-1	R-PHE	500, 545 nm	560	74	Moreth and Yentsch, 1970
Porphyridium purpureum	VA-70	b-PHE	545 nm	578	87.8	Gantt and Lipschultz, 1972

TABLE 1: CHARACTERISTICS OF THE PHYCOERYTHRIN EXTRACTS USED TO CALIBRATE THE FLUORESCENCE SPECTROPHOTOMETER

Figure 1: Graph of the dilution series for the four phycoerythrin extracts used in the study. Fluorescence units are span 10 Concentration in µg PHE/L sample by multiplying by mL of extract/L of sample filtered.



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15 Supplementary Notes

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16 Abstract

A summary of a new technique for the extraction and quantitation of phycoerythrin (PHE) from algal samples is described. Results of analysis of four extracts representing three PHE types from algae including cryptomonad and cyanophyte types are presented. The method of extraction and an equation for quantitation are given. A graph showing the relationship of concentration and fluorescence units that may be used with samples fluorescing around 575-580 nm (probably dominated by cryptophytes in estuarine waters) and 560 nm (dominated by cyanophytes characteristic of the open ocean) is provided.

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