Chlorophyll a Fluorescence, an Alternative Method for Estimating Primary Production

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Abstract

The in vivo chlorophyll a fluorescence index \( (F_{+DCMU} - F_{-DCMU})/F_{+DCMU} \) of natural waters was compared to the \(^{14}C\)-determined primary production, and the fluorescence intensity in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea \( (F_{+DCMU}) \) was studied as a function of extracted and spectrophotometrically determined chlorophyll concentrations. Samples were taken every second week from May through October, 1979, at the station “Systrarna” situated in a coastal area of the Bottnian Sea. In addition, samples from the Archipelago Sea of the Baltic were collected on board the Finnish research vessel R/S “Aranda” during the September cruise 1979. The correlations between the fluorescence index and the \(^{14}C\)-determined primary production and between \( F_{+DCMU} \) and total chlorophyll concentration were good when samples taken over short time intervals were compared. The shortcomings of both the fluorescence and the \(^{14}C\) methods are discussed. It is concluded that the fluorescence method is useful if it is desirable to follow with high time resolution any changes in the potential for photosynthesis (or primary production) in a water mass over relatively short time periods; e.g. during an algal bloom. The fluorescence method can furthermore be technically developed for automatic monitoring with a high time resolution. Efforts are being made in our laboratory to develop the method further to give information about the in situ rates of photosynthesis rather than the potential for photosynthesis in a phytoplankton population.

Introduction

For the past three decades, primary production in aquatic systems has generally been estimated by using the \(^{14}C\) method, originally introduced by Steemann-Nielsen (1952). The method has been improved both methodologically and technically since its introduction and efforts have been made to standardize measurement procedures in order to facilitate the comparison of data obtained by different research groups. In a recent review, Peterson (1980) discussed problems connected with the use of the \(^{14}C\) method for primary production estimations. He reported that the \(^{14}C\) method very often appears to underestimate primary production when compared to measurements obtained by alternative methods such as oxygen measurements, ATP changes, and number and volume of particles. Results obtained in our laboratory indicate that results of the \(^{14}C\) method depend on the physiological state of the algae, and that both over and underestimates of primary production can occur (K. Richardson et al., in preparation).

The importance of primary production estimates in aquatic ecology and the facts that the \(^{14}C\) method is both labor intensive and still possesses inherent methodological weaknesses, encouraged us to investigate whether measurements of the variable chlorophyll a fluorescence can be used for estimating the photosynthetic capacity of laboratory cultures of unicellular algae (Samuelsson and Öquist, 1977; Samuelsson et al., 1978). According to the theory, the variable chlorophyll a fluorescence yield is proportional to the electron flow through photosystem II during photosynthesis. Our laboratory studies (Samuelsson and Öquist, 1977) showed that there was a very good correlation between the amount of the 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) induced increase in the chlorophyll a fluorescence and the rate of photosynthesis measured with \(^{14}C\). The results have been confirmed by others (Kulandaivelu and Daniell, 1980). It has furthermore been shown that the in vivo chlorophyll a fluorescence can be used for estimation of the chlorophyll content in natural waters provided that strong (photosynthesis saturating) excitative light is used or that photosynthesis is inhibited by DCMU (Lorenzen, 1966; Halldal and Halldal, 1973; Kiefer, 1973; Samuelsson and Öquist, 1977; Tolstoy, 1980).
The reliability of the fluorescence method for chlorophyll determination is dependent on the accuracy of the calibration. For optimal use of the method, calibration should be made for each algal population studied in the laboratory or in the field.

The objective of the present work has been to investigate the usefulness of the fluorescence method for the estimation of at least relative values of the photosynthetic capacity (or primary production capacity) in naturally occurring phytoplankton populations. Although shortcomings of the $^{14}$C method may influence a comparison, correlations with this method have been made. Field studies by others have shown good correlations between values obtained with the variable chlorophyll $a$ fluorescence method and the $^{14}$C method (Ray, 1978; Roy and Legender, 1979; Przelen and Ley, 1980), although Roy and Legender stress the importance of proper calibration for the practical use of the fluorescence method. Tranter et al. (1979) have furthermore used the fluorescence method for estimating a relative “photosynthetic quantum efficiency” index in natural sea water.

Materials and Methods

Relative photosynthetic capacities of natural phytoplankton samples were estimated by measuring the variable chlorophyll $a$ fluorescence, as described elsewhere (Samuelsson and Öquist, 1977). A fluorometer FM3 (Umeå Instrument AB, Umeå, Sweden) was used for measurement of fluorescence after having been rebuilt to allow excitation with reduced quantum flux densities of the exciting radiation (Samuelsson and Öquist, 1977). The fluorescence was measured before and after the addition of the photosynthesis inhibitor DCMU to a final concentration of $10^{-6}$ M. The DCMU-induced fluorescence increase was divided by the fluorescence intensity in the presence of DCMU and the ratio was taken as an index ($F_+/Dcmu-F_-/DCMU/F_+/DCMU$) of the photosynthetic capacity of the phytoplankton population; i.e. a high ratio is typical for a population with high photosynthetic capacity and vice versa.

The fluorescence index was correlated with the primary production estimated on whole water samples with the $^{14}$C method described before by Larsson and Hågström (1979). Different filtration-separated fractions of phytoplankton were not considered because mechanical stress of algae affects the fluorescence yield. A further reason for choosing not to consider the phytoplankton fractions separately is that the rate of $^{14}$C-estimated primary production of the sum of the fractions was much lower than that of the whole water sample phytoplankton populations. This is at least partly due to losses in the filter holders (Larsson and Hågström, 1982) and was not corrected for in this work. The fluorescence intensity in the presence of DCMU ($F_+/DCMU$) was also correlated with the total chlorophyll content of the sample. Chlorophyll $a$ and $b$ were for this purpose extracted in methanol for 3 min on a boiling waterbath, after the algae had been harvested on glass filters (Whatman GF/F filter). The equations of MacKinney (1941) for chlorophyll determination were used.

Water samples were taken at regular intervals (May through October 1979, about every second week) from the depth of 2 m at “Systrarna”, a station in the coastal area of the Bothnian Sea (Lat. 63$^°$32′N, Long. 19$^°$51′E). The fluorescence measurements were performed on board immediately after the sampling and primary production was estimated in situ by incubating samples in 125-ml flasks at the 2-m depth for 4 h. The quantum flux density at 2 m was measured with a L1-188 quantum meter equipped with a L1-193SB underwater sensor (Li-Cor Ltd, Lincoln, Nebraska 68504, USA). From September 10 to September 20, 1979, a series of experiments were performed in the Archipelago Sea North latitude 60° on board of the research vessel R/S “Aranda”. Integrated water samples (0–15 m) were obtained by mixing equal amounts of water sampled at the 0-, 5-, 10- and 15-m depths. Fluorescence was measured as described above, and the primary productivity was determined using the $^{14}$C method but with samples incubated in an incubator on board. This consisted of a rotating perspex disc (12 revolutions min$^{-1}$) to which 125 ml sample flasks were attached. Samples were incubated for 2–4 h. The quantum flux density of 300–350 μmol m$^{-2}$ s$^{-1}$ was provided by fluorescence tubes (Philips TL 20W/55) and the temperature was set to 15$^°$C.

Results

Fig. 1 shows a plot of the relative fluorescence index versus the $^{14}$C-determined primary productivity expressed in mg C m$^{-3}$ h$^{-1}$ of all samples taken from the 2-m depth at “Systrarna”. Poor correlation was obtained between the two methods if the whole sampling period was considered ($y=0.009x+0.30$; $r=0.79$). However, if spring/summer and autumn were considered independently the correlation increased so that $r$ was 0.97 and 0.96 for spring/summer ($y=0.014x+0.22$) and autumn ($y=0.01x+0.34$) samples, respectively. This indicates that the correlation between the two methods increases if the comparisons are performed over shorter periods of the season. The fluorescence index and the in situ rates of $^{14}$C-primary production were also measured on samples taken at a depth of 14 m. The rates of primary production were, however, so low at this depth that it was not meaningful to make a comparison between the two methods.

Fig. 2 shows all fluorescence values obtained in the presence of DCMU in relation to the total chlorophyll determined spectrophotometrically after extraction during the investigation period of 1979. The data points are quite scattered which may be explained by the earlier observation (Tolstoy, 1977) that calibrations are needed in order to adjust the measurements from the fluorescence method to the different algae populations which succeed each other during a growing season. Much better correlation