ON PHYCOCYANIN PARTICIPATION IN THE HILL REACTION OF THE BLUE-GREEN ALGA *SYNECHOCOCCUS CEDRORUM*

by

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INTRODUCTION

Originally it was believed that in chloroplast-free blue algae the photosynthetic pigments are homogeneously distributed in the cytoplasm ("chromatoplasm"), *cf.* FRITSCH[1]. However, GEITLER[2] and HYGEN[3] observed coloured grana in some of these organisms under the light microscope. CALVIN AND LYNCH[4], and THOMAS[5] published electron micrographs of such-like structures. Up till now electron microscopical investigation suggests that the occurrence of pigments involved in photosynthesis is restricted to definite structures: either chloroplasts or grana.

The photosynthetic activity of the phycobilins in coloured algae was demonstrated amongst others by EMERSON AND LEWIS[6], HAXO AND BLINKS[7], DUYSENS[8], and FRENCH AND YOUNG[9]. It is well-known that these pigments are easily extracted by grinding the cells in water, while both the chlorophyll and the carotenoids are not. CALVIN AND LYNCH[4] centrifuged macerates of cells of *Synechococcus cedrorum*. A clear blue supernatant was obtained whilst both chlorophyll and carotenoids were exclusively observed in the sediment consisting of grana-like structures. Consequently doubt may arise whether *in vivo* too the occurrence of phycocyanin is restricted to these structures. Instead of this it may be homogeneously distributed throughout the cytoplasm.

On grinding red algal cells in the presence of high molecular weight solutes, such as polyethylene glycols, dextrin, and egg albumins in suitable concentration, MCCLENDON AND BLINKS[10] prepared suspensions of plastids which retained a dark red colour instead of changing to a brilliant orange, as occurs in the absence of these solutions. According to these authors this colour change is due to the fluorescence of the phycoerythrin when it becomes released from the plastids. Light microscopical observation showed that in plastids from such an orange suspension vacuolisation often occurred; the phycoerythrin was dissolved in the vacuole while both chlorophyll and carotenoids were concentrated in a misshapen mass at one side of the plastid. Such suspensions were unable to perform the HILL-reaction in the presence of ferricyanide. If, however, the release of phycoerythrin was prevented by the procedure as outlined above, the photochemical activity of the suspensions was retained. It should be emphasized that these experiments leave no doubt that in the red algae *Griffithsia*, *Antithamnion* and *Corallina* the phycoerythrin, instead of being homogeneously distributed in the cytoplasm, is located in the plastids.

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Though no mention is made of phycocyanin, it seems probable that the same may be said of this pigment.

Statement of the problem

The fact that release of phycoerythrin from the plastids results in a loss of the ability to perform the HILL-reaction indicates that in such a case the photosynthetic apparatus is damaged. This does not necessarily mean that a causal link exists between the loss in activity and the disappearance of the phycoerythrin; the chosen experimental conditions may cause such damage that the photosynthetic apparatus itself is destroyed.

In order to study the connection between structure and function of the photosynthetic apparatus it seemed important to investigate whether in blue algae phycocyanin forms an indispensable part of this apparatus, or whether photochemical activity proceeds as well after careful removal of the phycocyanin. To answer this question it was first examined whether phycocyanin participates in the HILL-reaction, and next, whether the effectiveness quotient of the rate of oxygen liberation in light mainly absorbed by chlorophyll, and that in light mainly absorbed by phycocyanin, is changed by a careful removal of the latter pigment. The present study deals with these items.

METHODS

Cultures of varying age of *Synechococcus cedrorum* (strain ALLEN), were harvested by centrifugation. Next the cells were either suspended in a solution of 0.15 *M* phosphate buffer pH 6.2 and 0.013% potassium chloride in glass-distilled water, or they were ground with carborundum for 15 minutes, taken up in the medium desired, and centrifuged again in order to remove both carborundum and cell debris. The medium consisted of the above phosphate buffer either as such or with addition of dextrin (techn. O.P.G.) in various quantities.

Steam-distilled benzoquinone was used as a HILL reagent. For each ml of suspension, 0.05 ml of a 1% quinone solution in 0.01 *N* sulfuric acid was added in the dark, 10 minutes before the readings were started. The rate of the HILL-reaction was manometrically determined at 12° C. Due attention was given to the usual precautions with regard to the handling of the preparation in the dark, or, if necessary, in weak light, and at low temperature.

Light absorption was measured in a white sphere as described by KOK[11].

Monochromatic light was obtained either by using a sodium lamp, or by isolating light of the wave-lengths 678 and 614 mμ from one incandescent lamp by means of interference filters.

RESULTS

HILL-*reaction of intact cells*

Suspension of intact cells, even from 10 days old cultures, in buffer are highly active in photochemical oxygen liberation. At light-saturation, rates of oxygen production of 120 μl O_2/h and higher, by 2 ml of a suspension from a 4–8 days old culture, absorbing about 50% at 678 mμ, were measured.

In order to check phycocyanin participation in the photolysis of water, the effectiveness ratio: O_2 developed in 614 mμ light/O_2 developed in 678 mμ light, was determined at non-saturating light intensities. As a mean of 5 experiments this ratio was found to be 1.7 $\pm$ 0.2. Since absorption due to chlorophyll is much smaller at 614 mμ than at 678 mμ this result proves that light absorbed by phycocyanin is active in the HILL-reaction.

HILL-*reaction of macerates*

Macerates of cells prepared in a buffer solution without any addition were always

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inactive. As is well-known, after centrifugation the clear supernatant of such an extract shows a blue colour whilst chlorophyll and carotenoids are present in the sediment.

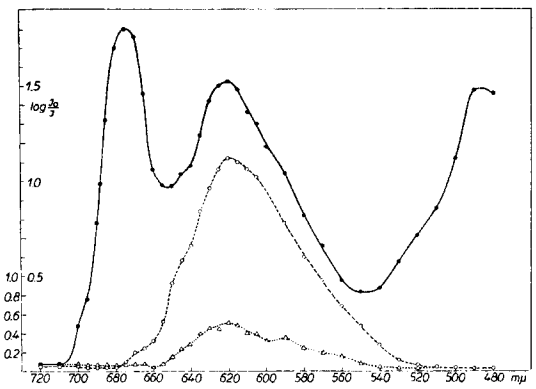

Fig. 1. Absorption spectra of intact cells and supernatants of macerates in 20% and 40% dextrin.
●——● *Synechococcus*, intact cells.
○------○ *Synechococcus*, macerate in dextrin 20%, supernatant.
△·····△ *Synechococcus*, macerate in dextrin 40%, supernatant.

In accordance with the results obtained by McCLENDON AND BLINKS[10] only a restricted release of phycocyanin was observed in macerates prepared in dextrin solutions of adequate concentration.

In Fig. 1 the absorption spectra of the supernatant of some macerates are given; the protective action of 4 g of dextrin dissolved in 10 ml of buffer is clearly demonstrated. In this case only about 25% of the total amount of phycocyanin is released. It also shows that a dextrin concentration of 2 g per 10 ml of buffer is still ineffective; in this case the supernatant contained nearly the total amount of phycocyanin.

As outlined above, it was important to study the photochemical behaviour of grana suspensions which showed only a slight release of phycocyanin. So a concentration of 4 g dextrin per 10 ml of buffer solution was used throughout the further experiments.

It was found that macerates prepared with the latter dextrin concentration were always active in the HILL-reaction, though to a lesser degree than intact cells. This is shown in Table I.

TABLE I

PHOTOCHEMICAL OXYGEN LIBERATION BY *Synechococcus* MACERATES IN DEXTRIN SOLUTION (4 g/10 ml) EXPRESSED IN PERCENTS OF PHOTOCHEMICAL ACTIVITY OF INTACT CELLS. SODIUM LIGHT SATURATION

Macerate	*Photochemical activity* %
a	21
b	14
c	89
d	38
e	68

It may be remarked by the way that the failure of PUNNETT AND FABIYI[12] to observe any photochemical oxygen liberation in preparations of the blue-green alga *Anabaena variabilis* may have been due to the absence of a protective action of high molecular weight solutes.

Attention should be drawn to the fact that, though the preparation method was standardized as much as possible, the photochemical activity rates of these macerates show considerable variation. This phenomenon will be due to the fact that it is impossible

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to grind the cells in a quite reproducible way. Since, however, this variability did not interfere with the envisaged experiments, no attempts were made to decrease the variation of the activity rates in question.

The activity decrease in *Synechococcus* macerates may be due to two causes. Firstly, part of the photochemically active light-absorbing phycocyanin may be released from all grana which would mean that per granum less light is absorbed. Consequently, the effective light intensity may drop to such an extent that light saturation conditions are no longer fulfilled. Secondly, phycocyanin may be removed from part of the grana, these grana being damaged in such a way that their ability to perform the HILL-reaction is lost.

It should be possible to discriminate between these two possibilities by determining under conditions of non-saturating light-intensities the ratio:

$$\frac{\text{oxygen liberated in light mainly absorbed by phycocyanin (interference filter 614 m}\mu\text{)}}{\text{oxygen liberated in light mainly absorbed by chlorophyll (interference filter 678 m}\mu\text{)}}$$

in both intact cells and macerates. In the following experiments intensities of about $1.8 \cdot 10^3$ ergs $cm^2 sec^{-1}$ were applied. If the first mentioned explanation holds, the above ratio should be smaller for macerates than for intact cells, whilst in the second case both ratios should be equal. The results are presented in Table II.

TABLE II

COMPARISON OF RATIO: O_2 EVOLVED IN 614 mμ LIGHT/O_2 EVOLVED IN 678 mμ LIGHT $= W_{614}/W_{678}$ — FOR INTACT CELLS AND FOR MACERATES IN DEXTRIN SOLUTION

Exp.	W_{614}/W_{678}	
	Intact cells	*Macerates in dextrin*
1	1.6	1.6
2	1.7	2.0
3	2.4	2.5
4	1.6	1.5

It is obvious that from these results the conclusion should be drawn that as soon as phycocyanin is released from some grana the ability to bring about photochemical evolution of oxygen of these grana is destroyed.

DISCUSSION

The above experiments demonstrate that when phycocyanin is removed as cautiously as possible from the photosynthetic pigment apparatus of *Synechococcus cedrorum*, the photolytic capacity of this apparatus is simultaneously destroyed. One may ask whether both phenomena are directly linked. This, certainly, need not be the case; it may be that damaging the grana results in a simultaneous loss of both phycocyanin and enzyme systems active in the HILL-reaction. However, since the pigment apparatus of green plants can be disintegrated to a considerable degree without total loss of the photochemical activity, *cf. e.g.* THOMAS, BLAAUW AND DUYSENS[13], the possibility in question may be deemed rather improbable. It seems much more acceptable that the activity loss is a direct consequence of the phycocyanin release.

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In a preceding paper[14] it has been pointed out that the chlorophyll molecules are probably concentrated in the lamellae of the grana. FREY-WYSSLING AND STEINMANN[15] suggested that these lamellae consist of two layers of globular (lipo)protein macromolecules. Since, as mentioned above, these lamellae are capable of performing the HILL-reaction it seems likely that the inactivation of *Synechococcus* macerates by phycocyanin extraction is due to a disarrangement of the lamellar structure. The most obvious way to explain this phenomenon may be to assume that the phycocyanin molecules are released from these macromolecular monolayers, the resulting gaps being responsible for a collapse of the lamellae. Though the experiments make it unlikely that the activity loss should be attributed to release of enzyme systems, the possibility of a linkage of these systems to the phycocyanin molecular themselves cannot be excluded with certainty.

The present study seems to indicate that the phycocyanin is located among chlorophyll-(lipo)protein complexes in the *Synechococcus* lamellae, and that, consequently, the macromolecular monolayers of these lamellae consist of at least two proteinaceous components.

Further experiments are needed to elucidate more details regarding the arrangement of the phycocyanin molecules.

SUMMARY

Release of part of the phycocyanin from grana of *Synechococcus cedrorum* results in a total loss of the ability to liberate oxygen photochemically.

The experiments favour the view that, in the blue-green alga examined, the lamellae are composed of at least two proteinaceous components.

RÉSUMÉ

Eloignement partiel de phycocyane des grana de *Synechococcus cedrorum* cause une perte totale du pouvoir de libérer de l'oxygène par voie photochimique.

Les expériences favorisent l'idée que les lamelles de l'algue bleue-verte étudiée sont constituées par, au moins, deux components protéiniques.

ZUSAMMENFASSUNG

Eine teilweise Entfernung des Phykozyans von den Granen von *Synechococcus cedrorum* veranlasst eine totale Vernichtung des Vermögens zur photochemischen Sauerstoffentwicklung.

Die Versuche deuten an, dass die Lamelle der untersuchten Blaualge am wenigsten zwei verschiedene Eiweisskomponente enthalten.

REFERENCES

1 F. E. FRITSCH, *The structure and reproduction of the Algae*. Cambridge Univ. Press, London, 2 (1945) 781.
2 L. GEITLER, *Planta*, 26 (1937) 463.
3 G. HYGEN, *Planta*, 27 (1937) 378.
4 M. CALVIN AND V. LYNCH, *Nature*, 169 (1952) 455.
5 J. B. THOMAS, *Proc. Koninkl. Ned. Akad. Wetenschap.*, C55 (1952) 207.
6 R. EMERSON AND C. M. LEWIS, *J. Gen. Physiol.*, 25 (1942) 579.
7 F. T. HAXO AND L. R. BLINKS, *J. Gen. Physiol.*, 33 (1950) 389.
8 L. N. M. DUYSENS, *Thesis*, Utrecht (1952).
9 C. S. FRENCH AND V. K. YOUNG, *J. Gen. Physiol.*, 35 (1952) 873.
10 J. H. MCCLENDON AND L. R. BLINKS, *Nature*, 170 (1952) 577.
11 B. KOK, *Enzymologia*, 13 (1948) 1.
12 T. PUNNETT AND A. FABIYI, *Nature*, 172 (1953) 947.
13 J. B. THOMAS, O. H. BLAAUW AND L. N. M. DUYSENS, *Biochim. Biophys. Acta*, 10 (1953) 230.
14 J. B. THOMAS, L. C. POST AND N. VERTREGT, *Biochim. Biophys. Acta*, 13 (1954) 20.
15 A. FREY-WYSSLING AND E. STEINMANN, *Vierteljahrsschr. naturforsch. Ges. Zürich*, 98 (1953) 20.

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