PHOTOSYNTHETIC ACTIVITY OF ISOLATED CHLOROPLAST FRAGMENTS OF *SPIROGYRA*

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INTRODUCTION

In two preliminary notes[1,2] experiments on photosynthetic activity of chloroplast fragments of *Spirogyra* have been reported. Details of these studies are described in the present paper.

Until recently it was generally accepted that isolated chloroplasts are no longer capable of complete photosynthesis, but that their photochemical activity is restricted to liberation of oxygen from water in the presence of a suitable hydrogen acceptor, the HILL reaction. A number of excellent reviews[3-7] on this topic has been published.

VISHNIAC AND OCHOA[8] and, at the same time, TOLMACH[9] observed carbon dioxide fixation in illuminated chloroplast suspensions to which TPN, pyruvate, and malic enzyme were added. The authors, however, stated that this process may differ considerably from that involved in "true" photosynthesis. According to ARNON[10] this system evolves oxygen in light.

FAGER[11] stated that illumination of cell-free chloroplast preparations led to an increased fixation of $^{14}CO_2$. However, this uptake is only very low as compared with that in intact cells.

The cessation of photosynthesis as soon as green cells are crushed can be caused by chemical or physical factors, or a combination of both. Examples of possible chemical factors are oxidations, change of pH and, in general, autolysis. As physical factors may be mentioned, *e.g.* dilution, dislocation of enzymes and substrates, mechanical destruction, and swelling phenomena. Chemically injurious influences can be partly reduced by such precautions as working at low temperature, readjusting the pH and adding protective substances. Whether the effect of physical damage can be satisfactorily reduced largely depends on the location of enzymes and cofactors involved in photosynthesis; it will be much easier to succeed if all of them are concentrated in the chloroplasts than if some of them occur in the cytoplasm. Thus the substructure of the chloroplast is essential in this respect.

The structure of chloroplasts has been examined and discussed by many authors.

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These studies have been amply reviewed[12-20]. It may be recalled that, in general, two types of chloroplasts occur: grana-bearing chloroplasts and grana-free ones. The first mentioned type, prevalent in higher plants, contains green grana suspended in a colorless stroma. The grana are composed of lamellae to which most probably the photosynthetic pigments are attached[21]. Grana-free chloroplasts, occurring in some algae, are composed of lamellae throughout the whole body. Originally, electron micrographs seemed to show that, as a rule, these grana-free or lamellated chloroplasts are stroma-free. Improvement of the sectioning technique, however, made it possible to observe that in a number of such chloroplasts, among which those of *Spirogyra*, the lamellae are bundled up into portions of 4–8. In between these bundles thin layers of stroma occur[22-24].

The aqueous plant extracts generally used in earlier experiments on photochemical activity were suspensions of grana-bearing chloroplasts. These isolated chloroplasts are easily damaged; suspensions prepared without special precautions contained many free grana. Consequently, grana are more resistant to injurious experimental conditions than stroma substance.

Owing to the small size of chloroplasts it is extremely difficult, if not impossible, to decide whether isolated plastids, even though they look intact, are indeed undamaged. The stroma substance may already have lost its functional properties before visible disintegration occurs. The inability of chloroplast suspensions to bring about photosynthesis, while still capable of performing the HILL reaction, could then be interpreted to mean that the presence of intact stroma is required for the ability to photosynthesize. The stroma may either contain part of the photosynthetic enzymes and cofactors, or merely function as a protective substance with regard to enzymes located at the surface of the lamellae.

This suggestion gains probability from the results of ARNON *et al.*[25-30] who prepared chloroplast suspensions with some special precautions. Apart from the HILL reaction, they succeeded in observing light-induced carbon dioxide fixation and phosphorylation in these suspensions, provided certain cofactors were added. Mention may also be made of experiments of UEDA[31] and IRMAK[32] who observed light-induced starch formation in specially prepared isolated chloroplasts.

Consequently it seemed worth while to study lamellated chloroplasts which, as a rule, do not contain stroma in large amounts while this stroma occurs in thin layers in between lamellar bundles. Since lamellae are fairly resistant to the usual experimental conditions, the stroma may be more or less protected against external injurious influences. If lamellated chloroplasts are the sites in which complete photosynthesis occurs, photosynthetically active suspensions of fragments of these plastids may be prepared even without the special precautions needed for retaining photosynthetic activity in grana-bearing chloroplasts.

The results of such experiments are presented below. *Spirogyra* proved to be a most suitable object. This alga contains a single large spiral-shaped chloroplast per cell. Crushing of the cells causes fragmentation of the chloroplasts. The chloroplast fragments are easily isolated from the cell debris.

The experiments were carried out in two series, each of them covering a vegetation period of the algae. During the first one metabolic activity was determined with the aid of the usual manometric technique. In the second series the experiments were repeated and extended by mass spectrometer measurements.

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METHODS

Preparation

Various species of *Spirogyra* were collected from their habitats. The freshly collected samples were successively washed with tapwater and cooled in a 0.2 *M* phosphate buffer of pH 7.2. Next, the buffer was decanted and the adhering liquid was removed by gently pressing the algae between filter paper. In weak light, about 3 g of wet algae were ground with half the amount of cooled sand, "zilverzand F" (Brocades), previously washed with the phosphate buffer. A pre-cooled porcellan mortar was used. The duration of grinding was one minute. Next, 12 ml of cooled buffer was added and the brei was briefly and gently stirred. Then, the supernatant was decanted into cooled tubes, and centrifuged at 0° C by accelerating a 1200 *g* centrifuge for 30 seconds. At the end of this period the rotor was not yet operating at full speed. During this short time the relatively large intact and broken cells completely settled down. The supernatant showed a bright green color. This suspension, which did not contain complete chloroplasts but only fragments therefrom, as checked by electron microscopy, was used. The absorption defined as log I_0/I, of chlorophyll extracts from the suspended material of these preparations in the same amount of methanol varied from 0.5 to 0.8 at 664 mμ.

After centrifugation the suspension was used instantaneously. If, however, it was necessary to remove oxygen, the suspension was placed on ice and flushed with nitrogen "extra", containing 5 % carbon dioxide, in darkness for 5 minutes. In this case the phosphate buffer used was previously flushed with this gas mixture for 15 minutes.

Manometry

Oxygen measurements. 0.4 ml Pardee carbon dioxide buffer[33], equilibrated with 3 % carbon dioxide in air, was pipetted into each of the side bulbs of Warburg flasks which had previously been flushed with the same gas mixture. Next, 2 ml of the fragment suspension was pipetted into the flasks as quickly as possible and flushing with the above gas mixture was continued for 5 minutes. Then, the flasks were closed and the manometers were mounted in a thermostat of 18° C. The flasks were allowed to equilibrate in darkness for 25 minutes.

Carbon dioxide measurements. The suspensions were made anaerobic by flushing with nitrogen "extra" containing 5 % carbon dioxide. 2 ml of the suspension were pipetted as quickly as possible into Warburg flasks. The side bulbs were provided with pieces of white phosphorus, with a volume of about 0.5 ml. The flasks had been flushed with the above gas mixture beforehand. After filling the flasks, this flushing was continued for 5 minutes. After that period, the flasks were handled as mentioned above.

In both sets of experiments 4 or 5 flasks with fragment suspension were run simultaneously with 2 flasks containing intact algae.

The sodium light intensity used was about 10^4 ergs cm^{-2}sec^{-1}.

Mass spectometry

The chloroplast fragments were taken up in the above-mentioned phosphate buffer which had been previously flushed with nitrogen "extra", containing 4.2 % oxygen and 1.0 or 0.5 % carbon dioxide, for 15 minutes. A known quantity of this suspension, as a rule 18.5 ml, was transferred into a cuvette with a content of 24.6 ml, *cf.* Fig. 1. The filling occurred as follows. A and B were

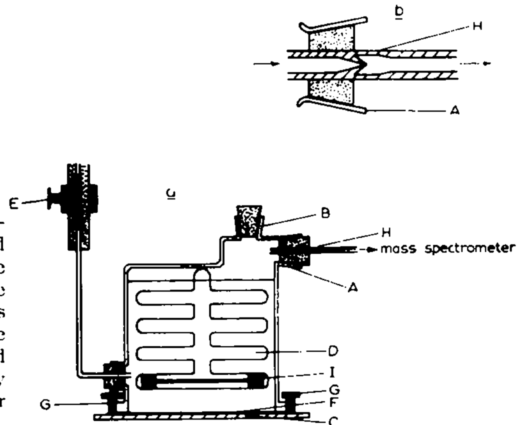

Fig. 1. a: Cuvette in mass spectrometer experiments. The brass bottom plate C, covered with a sheet of teflon F, is adjusted to the glass cuvette by means of 4 screws G. The floating stirrer D is made of small glass tubings. The lower one contains an iron core I. In operating position the cuvette is placed on an electromagnet which is interruptedly d.c.-fed. b: Gas inlet capillary enlarged. For further details see text.

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closed with rubber stoppers. After removal of the bottom plate C and stirrer D, the cuvette was placed upside down and partly filled with the fragment suspension. Next, stirrer and bottom plate were readjusted, and the cuvette was mounted in the upright working position in a water bath of about 10° C. Stirring was started immediately, A and B were opened, and the suspension was flushed with the above gas mixture via E for 5 minutes. In the meantime the gas inlet capillary of the mass spectrometer was introduced via A into the cuvette. At the end of the flushing period, first, B and, next, E were closed. Recording was started immediately. A 100 watt incandescent airplane lamp was used as a light source. Photosynthetic activity was measured at saturating light intensities. Under the experimental conditions mentioned α_{CO_2} was found to be 3.68. This high value is due to carbon dioxide retention by the phosphate buffer.

Erroneously, this retention has not been taken into consideration when calculating the carbon dioxide exchange figures presented in a preliminary note[1]. These figures should be multiplied with 1.4.

The mass spectrometer was constructed in this laboratory. It is described in detail elsewhere[34,35]. It was of the 60° sector field type with a radius of 150 mm. The maximum ion accelerating voltage was 2000. The resolving power, depending on the width of the slits installed in the ion source and collector was about 60 in the present investigation. Except for the following detail, the ion source was of the Nier type.

The electron current in the ion source was modulated by a special grid at 50 c/sec, the mains frequency, which results in an intensity modulated ion beam of the same frequency. Thus, an a. c. amplifier could be used for amplification of the ion current at the collector. In this way the amplifier drift problems were eliminated. The output of the amplifier was synchronously rectified by a fast relay, and recorded by a 12″ Honeywell-Brown recorder. This detecting system yielded a band width of 2 c/sec and, using a simple amplifier, a noise corresponding with 10^{-14} A.

The electromagnet for the analysing field was fed by an electronic current stabiliser. This stabiliser was adapted to the problem at hand in such a way the magnetic field strength could be switched over by an external relay to two preset values. Thus, the spectrometer was focussed alternately on both gas components CO_2 and O_2. At the same time an attenuator was switched into the amplifier circuit. In this way registration of two ion currents of about the same magnitude was possible. Switching over was done every 15 seconds. Thus a practically continuous recording for both gas components was obtained.

The accuracy of the determination of oxygen and carbon dioxide was 1% and 2% respectively.

The gas inlet to the ion source consisted of a glass capillary, which was directly introduced into the gas phase of the cuvette. The response time to changes in the gas phase was about 20 seconds.

Phosphorylation experiments

A few experiments on photosynthetic phosphorylation in suspensions of chloroplast fragments were carried out by Dr. ANNA MARIA WILLIAMS to whom we feel greatly indebted. Only three such experiments were made. Because of their small number, this paper will not deal with these experiments *in extenso*. Since, however, the results have been presented in a preliminary note[1] the methodical details may be mentioned here. The reaction mixture consisted of 12.3 μmoles inorganic phosphate, as potassium phosphate buffer of pH 7.0, 9 μmoles AMP, 30 μmoles $MgSO_4$, 20 μmoles tris(hydroxymethyl)aminomethane buffer of pH 7.0, 30 μmoles KF, and 2.0 ml *Spirogyra* preparation. Total volume 3.0 ml. Incubation for one hour at 18° C with constant shaking.

Accuracy and corrections

In the manometric experiments use was made of the Pardee carbon dioxide buffer. After changed metabolic conditions, equilibration of this buffer requires more than half an hour. However, in most cases, photochemical activity of the fragment suspensions is nearly lost after one hour. Since 25 minutes are necessary for temperature equilibration of the flasks in the thermostat, it was impossible to allow full equilibration of the carbon dioxide buffer during the illumination period. As a consequence, the oxygen measurements yielded too low values. In this respect it may be added that the first readings of each light or dark period were disregarded when computing the mean values published in one[1] of the preliminary notes.

Carbon dioxide exchange was measured manometrically under anaerobic conditions. These conditions, however, are unfavorable for intact *Spirogyra* cells. Since the same may hold for fragment suspensions, it seems probable that the measured values are not optimal.

Mean errors were calculated according to the formula

$$m.e. = \sqrt{\frac{\Sigma d^2}{n(n-1)}}$$

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The oxygen and carbon dioxide measurements were done in two successive series. The experiments required the whole duration of the algal vegetation period. Since towards the end of this period photosynthetic activity drops, the rates of both processes can not be mutually compared. For the above reasons the results of the manometric experiments are of only qualitative importance.

Things are different in the case of mass spectrometry. Here, no considerable temperature equilibration time was needed, while oxygen and carbon dioxide exchange determinations could be made rapidly and simultaneously. When the gas phase was replaced by air in the cuvette containing water equilibrated with 1 % carbon dioxide, the new equilibrium was reached after 6 minutes.

A slight drift of the sensitivity of the mass spectrometer occurred. For this, a correction was determined as follows. It was assumed that the R.Q. of the fragment suspension was unity, Supporting evidence was obtained from manometric experiments. Let the apparent number of μl of both gas components present at the beginning of a dark period be u_1 and u_2, and the apparent number at the end of this period be u'_1 and u'_2 respectively. Furthermore, let the sensitivity at both moments be s and s'. Then the true gas exchange will be:

$$\frac{u_1 - \frac{S}{S'} u'_1}{\Delta t} \tag{1}$$

Since it was assumed that R.Q. = 1.0:

$$u_1 - \frac{S}{S'} u'_1 = -\left(u_2 - \frac{S}{S'} u'_2\right) \tag{2}$$

or

$$\frac{S}{S'} = \frac{u_1 + u_2}{u_1 + u_2} \tag{3}$$

Substitution of (3) in (1) yields for the true gas exchange:

$$u_1 - u'_1 \frac{u_1 + u_2}{u'_1 + u'_2} \mu l/\Delta t \tag{4}$$

The same may be written for u_2 and u'_2.

In this way the sensitivity drift was determined for two dark periods and interpolated for the intermediate light period.

RESULTS

Manometric experiments

Oxygen production of illuminated intact *Spirogyra* cells and chloroplast fragments is shown in Figs. 2a and 2b respectively. Fig. 2a represents oxygen liberation by illuminated intact algae. The seemingly sustained rise of the rate of this process is caused by the slow adaption of the carbon dioxide buffer.

The dots represent means of 14 readings.

In Fig. 2b the same process is shown for chloroplast fragments. The values are means of 31 readings. No prolonged increase of the rate of oxygen production was observed here. The measured effect is a combination of slow adaptation of the buffer, and decrease of the photosynthetic activity of the fragments. The meaning of the minimum at $t = 15'$ is still obscure.

From these experiments it can be concluded that fragments of *Spirogyra* chloroplasts are able to liberate oxygen photosynthetically. In these experiments the fragment activity was roughly one third of that of intact algae.

Photosynthetic carbon dioxide uptake of intact algae and chloroplast fragments is shown in Figs. 3a and 3b respectively. In order to obtain values comparable with those of Figs. 2a and 2b on a chlorophyll base the data of Fig. 3a must be multiplied with 1.4. Because of the fact that the values in this graph are means of only 10 readings, no mean errors are given. In Fig. 3b the dots represent means of 22 readings.

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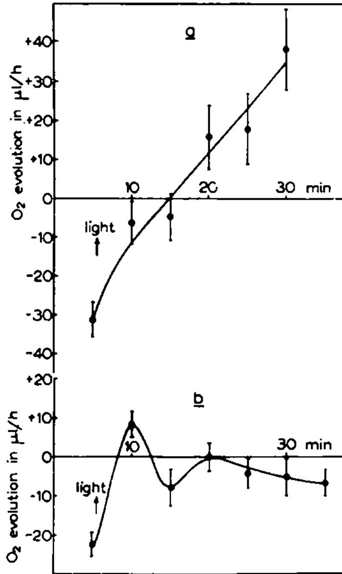

Fig. 2.

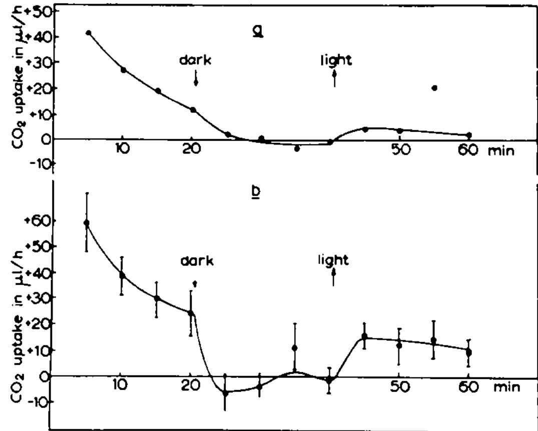

Fig. 3.

Fig. 2. Photosynthetic oxygen liberation of a: intact *Spirogyra* cells, b: fragmented *Spirogyra* chloroplasts.

Fig. 3. Photosynthetic carbon dioxide uptake of a: intact *Spirogyra* cells, b: fragmented *Spirogyra* chloroplasts.

It is striking that the rate of carbon dioxide uptake of intact algae decreased with time. This decrease apparently proceeded even somewhat more rapidly than that of the chloroplast fragments. This might be explained by assuming that, under anaerobic conditions, formation of fermentation products of an inhibiting character for photosynthesis occurred. These substances might partly diffuse out of the fragments into the medium while, in the living cell, they might accumulate.

Moreover it is notable that, in contrast to the results on oxygen liberation, the fragment activity was about as high as that of the intact cells. This may be due to the fact that during the former, aerobic, experiments oxidative inhibiting reactions within the fragments were possible, while these reactions were absent during the latter, anaerobic, measurements.

In conclusion, it can be stated that these experiments provide evidence of photosynthetic carbon dioxide uptake by fragments of *Spirogyra* chloroplasts.

Experiments with the mass spectrometer

An example of the records obtained is shown in Fig. 4. At the end of each oxygen recording an identification peak was added. In this way carbon dioxide and oxygen recordings are easily recognised.

The results are given in Table I.

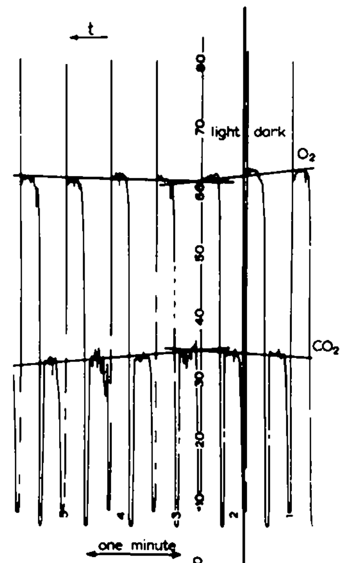

Fig. 4. Example of a mass spectrometer recording.

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TABLE I

PHOTOSYNTHETIC ACTIVITY OF FRAGMENTS FROM *Spirogyra* CHLOROPLASTS

$Q^{chl}_{O_2}$ and $Q^{chl}_{CO_2}$: maximal oxygen evolution and carbon dioxide consumption in µl/mg chlorophyll/h. A.Q.: assimilatory quotient.

			Photosynthetic activity				
Date	*Series*	*Respiration in µl h*	*Oxygen evolution in µl h*	*Carbon dioxide consumption in µl h*	$Q^{chl}_{O_2}$ $\times 10^3$	$Q^{chl}_{CO_2}$ $\times 10^3$	*A.Q.*
4-14	a	187	302	316	—		0.96
4-25	a	174	405	510	—	—	0.91
5-4 A	a	435	768	607	—		1.26
5-4 B	a	180	104	682	1.1	7.4	0.15
5-7 A	a	265	522	1034	3.5	7.0	0.51
5-7 A	a	292	607	1312	4.1	8.9	0.47
5-7 B	a	228	277	1344	2.4	11.9	0.21
5-7 B	a	162	148	604	1.3	5.4	0.24
5-9	a	24	288	1310	2.3	10.8	0.22
5-17 A	b	177	49	33	0.02	0.02	1.48
5-17 A	b	186	173	143	0.1	0.1	1.22
5-31	b	140	87	774	0.1	1.3	0.11
5-31	b	126	75	147	0.1	0.2	0.51

As mentioned above, the correction for a slight sensitivity drift of the mass spectrometer was based on the assumption that the respiratory quotient was unity. Consequently, the figures of column 3 refer to both oxygen uptake and carbon dioxide evolution. Two series of experiments were done. They differ in three respects: habitat of the algae, phase of the vegetation period, and freshness. Series a refers to algae under optimal vegetation conditions and used immediately after collecting while the algae of series b were used at the end of the vegetation period and collected one day before the experiment. The activity difference is evident. However, within each series also a considerable scattering of the results occurred. This divergence may indicate that the used technique still needs improvement.

The A.Q. values scattered as well. This may also mean that the photosynthetic apparatus was injured to some extent by the experimental conditions. As a rule, the rate of photosynthetic carbon dioxide consumption surpassed that of oxygen liberation. The mean A.Q. was 0.63 ± 0.13.

In three experiments, $Q^{chl}_{O_2}$ of intact algae, suspended in Warburg No. 9 buffer, was determined. This value was found to be about $2 \cdot 10^3$, and, thus, of the same order of magnitude as those of series a. It may be added that ARNON *et al.*[29] measured $Q^{chl}_{O_2}$ values of about $1 \cdot 10^3$ for the HILL reaction of spinach chloroplast suspensions. The values of $Q^{chl}_{CO_2}$, series a, are of the same order of magnitude as those obtained with various higher plants by WILLSTÄTTER AND STOLL[36].

In conclusion, it may be stated that the mass spectrometer measurements confirm the manometric experiments by demonstrating light-induced oxygen evolution and carbon dioxide consumption in fragments of *Spirogyra* chloroplasts. As a rule, the rate of the latter process was found to be somewhat higher than that of the former one. This effect may be due to a still imperfect preparatory technique. However, the observed rates of gas exchange are of the same order of magnitude as those measured with intact cells.

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DISCUSSION

The described experiments show that fragments of *Spirogyra* chloroplasts are capable of performing two photosynthetic reactions, namely oxygen evolution and carbon dioxide uptake. In this respect it may be recalled that, in a preceding note[1], a few experiments by Dr. WILLIAMS, suggesting the ability of these chloroplast fragments to carry out photo-induced phosphorylation, were mentioned.

It is hoped that the fluctuations of the results will be diminished by improvement of the preparatory technique. For this reason it is advisable to postpone a discussion about the measured low mean value of the A.Q. until further results are obtained. At present, however, stress may be laid on two items:

1. the fragment suspensions were prepared with a buffer solution without any further additions,
2. the rates of both photosynthetic carbon dioxide consumption and oxygen evolution, on a chlorophyll base, by the fragments are roughly the same as those of chloroplasts in intact cells.

When comparing these results with those of ARNON *et al.*[25–30] the following may be remarked. First, as mentioned above, these authors demonstrated the ability of isolated chloroplasts to carry out three "photosynthetic" reactions, namely light-induced oxygen evolution, carbon dioxide uptake, and phosphorylation. The oxygen evolution, however, was measured as a HILL reaction. Thus, this procedure is based on the assumption that the mechanism of photo-induced oxygen liberation is the same for photosynthesis and HILL reaction. In the present study, however, the light-induced oxygen evolution is actually measured as a photosynthetic oxygen liberation.

Apart from this difference, the present results are apparently in contradiction with those obtained by ARNON and co-workers. These authors stated that addition of certain cofactors and enzymes is needed in order to restore the full rate of photosynthetic phosphorylation and carbon dioxide uptake in isolated chloroplasts and fragments thereof. It seems unlikely that this discrepancy should be due to different techniques.

However, the types of chloroplasts used by the Berkeley and the Utrecht groups differed, and, thus, the discrepancy may well be explained in this way. The first group worked with spinach chloroplasts. These plastids are of the grana-bearing type. ARNON *et al.* prepared three kinds of suspensions: "whole chloroplasts (P_1)", "broken chloroplasts (P_{1w})", and "fragmented chloroplasts (P_2)". "P_1" was capable of performing three photochemical reactions: the HILL reaction, carbon dioxide reduction, and phosphorylation. "P_{1w}", prepared by transferring "P_1" into ice-cold water by which treatment the chloroplasts became swollen and irregular in shape, could perform the HILL reacton and photosynthetic phosphorylation. However, addition of an aqueous extract from whole chloroplasts (soluble enzymes), ATP, and TPN was required in order to restore full capacity for photosynthetic carbon dioxide fixation. "P_2", consisting of mechanically fragmented, grinded, chloroplasts, was capable of carrying out the HILL reaction at the usual rate, but the capacity for photosynthetic phosphorylation was rather poor while that for carbon dioxide fixation was practically abolished. The capacity for both latter processes could not be restored.

In ARNONS' "P_1" preparation the total amount of stroma may be present in the

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chloroplasts in a relatively intact state. "P_{1w}" chloroplasts still contain the original quantity of stroma but in a swollen state while some soluble substances are leached out. In the "P_2" preparation a considerable part of the grana is most likely isolated from the stroma, while as far as they are not isolated, this stroma probably occurs in a highly denatured state.

In the present study fragments from lamellated chloroplasts were used. As mentioned in the introduction, the layered structure of this type of chloroplasts is likely to be more capable of protecting the stroma against injury, caused by the preparation procedure, than that of granulated chloroplasts. The capacity for photosynthetic carbon dioxide uptake and oxygen evolution roughly equals that of chloroplasts in the living cell.

A consideration of the photochemical activity of the preparations mentioned, in connection with the probable state of the chloroplasts, strongly suggests that the presence of intact stroma is one of the prerequisites for the capacity to carry out complete photosynthesis. Whether the stroma is the site of part of the photosynthetic processes or functions merely as a substance of a "protective" character with regard to the enzymes and cofactors involved remains to be elucidated.

Finally, it may be remarked that suspensions of fragments from *Spirogyra* chloroplasts seem to be an adequate tool for studying photosynthesis outside the living cell.

SUMMARY

Suspensions of fragmented *Spirogyra* chloroplasts were prepared and their capacity for photosynthetic oxygen evolution and carbon dioxide reduction was studied by means of manometry and mass spectrometry.

No special substances, enzymes or cofactors were added to the suspensions.

The rates of both photosynthetic processes were found to be of the same order of magnitude as those observed in intact cells.

The presence of intact stroma seems to be a prerequisite for the capacity to carry out complete photosynthesis.

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PHOTOPHOSPHORYLATION BY SUBCELLULAR PARTICLES FROM *CHROMATIUM*

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Light-induced phosphorylation of adenosine nucleotides has been demonstrated to be an enzymic reaction characteristic of a variety of pigmented preparations derived from photosynthetic organisms[1-3]. Several observations indicate that generation of pyrophosphate bonds in such systems may take place through coupling or a light-activated process to electron transport chains which are similar to and perhaps identical with those of known respiratory pathways. During a study of the enzymic reactions associated with submicroscopic particulate cell components derived from the obligately anaerobic, photosynthetic purple sulfur bacterium *Chromatium*, a light-activated phosphorylation of adenosine diphosphate has been observed. In previous publications we have described the isolation and quantitative determination of some of the electron transport components present in the pigmented particulate fractions from *Chromatium* extracts[4,5], and have discussed some characteristics of the anaerobic electron transport chain. In the present communication, properties of the photophosphorylation reaction are described. Some insight into the mechanism of photophosphorylation is suggested by the fact that the *Chromatium* system is stimulated by certain redox reagents having

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