ON THE ENERGETICS OF THE PHOTOSYNTHESES IN GREEN SULFUR BACTERIA

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INTRODUCTION

The peculiar physiological characteristics of the photosynthetic bacteria have led to the development of a general concept of photosynthesis (1–3) which is most simply expressed by the generalized equation

$$CO_2 + 2H_2A \xrightarrow{\text{light}} (CH_2O) + 2A + H_2O,$$

in which H_2A stands for an "electron donor," and (CH_2O) for cell material. The electron donor can be any one of a number of reduced sulfur compounds, simple organic substances, molecular hydrogen, or water, each of which is oxidized concomitantly with the reduction of carbon dioxide to cell material. A thermodynamic consideration of the different types of photosyntheses shows that the minimum amount of radiant energy theoretically needed for the reduction of a unit quantity of CO_2 to cell material would depend upon the chemical potential between the reduced and the oxidized state of the electron donor. A certain negative potential of the electron donor implies that a corresponding amount of chemical energy is released during its oxidation, and it has been suggested that this chemical energy might be used for the reduction of CO_2 to cell material (4).

It has been pointed out, however, that this thermodynamic argument need not be strictly applicable. The mechanism of photosynthesis can be formulated as involving a primary photochemical reaction, similar in all cases, and consisting in the photochemical decomposition of H_2O under the influence of an excited pigment-enzyme complex. Such a reaction would yield a reducing and an oxidizing component, with the former capable of furnishing all the

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energy necessary for the reduction of CO_2 to cell material in subsequent dark reactions. Meanwhile, the oxidizing component is continuously reduced with the aid of the electron donor, except in the case of green plant photosynthesis in which the reduction is normally accomplished through the liberation of molecular oxygen. This concept implies that the utilization of radiant energy alone could be responsible for CO_2 assimilation, and that the excess chemical energy furnished by the oxidation of an externally supplied electron donor would be wasted for the formation of cell material from CO_2 (3, 5, 6).

A decision between these alternative hypotheses could be reached by measuring the quantum numbers for CO_2 assimilation by photosynthetic processes accomplished with electron donors of different chemical potentials. If the chemical energy of an electron donor were utilized in the synthesis of cell material, one should expect to find the lowest light quantum numbers in photosyntheses involving electron donors of the most negative potentials. If, however, the chemical energy were not so utilized, then the same quantum number should characterize all types of photosyntheses, irrespective of the chemical potential of the donor.

A study of the bacterial photosyntheses is of special advantage in connection with this problem because the photosynthetic bacteria can utilize various electron donors of widely different chemical potentials. Our knowledge concerning the energetics of the bacterial photosyntheses has been reviewed by van Niel (3), who concluded: "The present information concerning the energetics of bacterial photosyntheses leaves much to be desired. It seems that even for reactions which thermodynamically require little if any energy a considerable input of radiant energy is still necessary. But whether any regularities exist in the relation between the numbers of quanta and the energetic requirements of the reaction cannot yet be decided." Since the above-mentioned review appeared, Wassink *et al.* (7) have published data for photosynthesis of the purple sulfur bacterium *Chromatium*, indicating that just as much light energy is required for photosynthesis with an electron donor of a high as with an electron donor of a lower negative potential.

Because the green sulfur bacteria had not hitherto been studied from the point of view of photosynthetic efficiency the present investigation was undertaken in order to fill this gap. Furthermore, an attempt was made to obtain information concerning the quantum number for CO_2 assimilation during photosynthesis in the presence of electron donors of different chemical potentials. The results have served as a basis for discussion of the participation of the electron donor in the mechanism of photosynthesis.

The green sulfur bacterium *Chlorobium thiosulfatophilum* was used for the experiments. This organism can utilize various inorganic electron donors (8), and three of these, molecular hydrogen, thiosulfate, and tetrathionate, were selected as suitable for the purpose discussed above. The photosyntheses

carried out in the presence of these three donors are represented by the following equations:

$$CO_2 + 2H_2 \rightarrow (CH_2O) + H_2O; \quad \Delta F^\circ_{298} = +1{,}700 \text{ cal.}$$

$$CO_2 + 1/2\, S_2O_3^{--} + 3/2\, H_2O \rightarrow (CH_2O) + SO_4^{--} + H^+; \quad \Delta F^\circ_{298} = +29{,}150 \text{ cal.}$$

$$CO_2 + 2/7\, S_4O_6^{--} + 13/7\, H_2O \rightarrow (CH_2O) + 8/7\, SO_4^{--} + 12/7\, H^+; \quad \Delta F^\circ_{298} = +30{,}600 \text{ cal.}$$

It should be noted that the changes in free energy have been calculated from data at the standard state and signify first approximations.

EXPERIMENTAL

Determination of the maximum efficiency of photosynthesis involves (1) determination of the number of light quanta absorbed by the organism during a certain time and (2) determination of the number of molecules of CO_2 assimilated during the same period, under conditions in which the light intensity limits the assimilation rate, and with the organisms in a state of optimal photosynthetic activity. The light intensity should be in the range in which its relation to the assimilation rate is linear.

Preparation of Bacterial Suspensions.—*C. thiosulfatophilum* was grown in liquid cultures in synthetic medium as previously described (8), with 0.15 per cent $Na_2S_2O_3$ as electron donor. Bacteria were harvested by centrifugation of cultures still growing exponentially; usually cultures incubated for 22 to 28 hours after inoculation were employed. The sedimented bacteria were suspended in O_2-free synthetic medium without NH_4Cl and electron donor, and the suspension was aerated with O_2-free N_2 to maintain anaerobiosis.

Measurement of Assimilation.—All determinations were made by the customary manometric methods. Reaction vessels with two side arms but without center well were used. The sides of the vessels were covered with aluminum foil. This caused light transmitted by the suspension to be reflected back into the vessel, thus insuring a fairly uniform illumination of the bacteria.

The bacterial suspension was introduced into the main compartment. When thiosulfate or tetrathionate was supplied as electron donor, the substrates were initially placed in one side arm, the other receiving a solution of 10 per cent citric acid; the gas phase for such experiments consisted of O_2-free N_2 with 5 per cent CO_2. For measurements of CO_2 assimilation with H_2 as electron donor one side arm was provided with 2 N H_2SO_4, and a mixture of O_2-free H_2 with 2 per cent CO_2 served as the gas phase.

After equilibration in the water bath in darkness the assimilation was started by tipping in the substrate and turning on the light. In experiments with thiosulfate and tetrathionate as electron donors the amount of CO_2 assimilated was determined as the sum of the CO_2 changes in the liquid and the gas phase. In experiments with H_2 as

an electron donor total CO_2 assimilated was found either by gas analysis or by computation on the basis of total gas uptake, using an experimentally determined conversion factor.

In determinations of the quantum yield the first 10 minute period of assimilation was as routine eliminated from the calculations. This was necessary because it was found that a lag period of several minutes exists before the maximum assimilation rate is reached. Measurements were therefore started 10 minutes after the light had been turned on, and continued for a period of 30 to 40 minutes. Special attention was paid to the requirement that the photosynthetic rate be constant over this time interval. In experiments with H_2 this presented no difficulty, since the rate was found to be constant over a considerably longer period. When thiosulfate or tetrathionate was used, however, a decrease in the rate of CO_2 assimilation with time was frequently encountered; this could be avoided by using these substances only after repeated recrystallization from water-alcohol mixtures just before the experiment was begun. Aliquots of the same bacterial suspension were used for estimating the quantum yields with the three different electron donors; thus, three fully comparable experiments were carried out under closely similar conditions, one after the other in rapid sequence.

Light Intensity–Rate Curve.—It has been shown by French (9) and by Wassink *et al.* (7, 10) that the relationship between light intensity and rate of CO_2 assimilation by suspensions of purple bacteria in the presence of H_2 can be represented by a sigmoid curve. This renders the determination of maximum quantum yields precarious because the yield is proportional to the tangent of the intensity–rate curve. Similar experiments with *C. thiosulfatophilum* revealed that also in this case the relationship is expressed by a sigmoid curve, and that, below a certain intensity, the S shape is more pronounced in denser bacterial suspensions.

The experiments were performed by illuminating the vessels with white light from below. Neutral transmission filters, covering suitable points between 0 and 100 per cent transmission, were attached to the bottoms of individual vessels. The aluminum foil covering the sides of the vessels prevented illumination of the bacteria with light other than that coming in from the bottom.

Fig. 1 shows the results of two typical experiments, comparable except for the density of the suspensions used; in Experiment B the density was about 4 times as great as in Experiment A. These and other similar experiments proved that it was of advantage to carry out the quantum yield experiments with relatively thin bacterial suspensions, and at incident light intensities as close as possible to the maximum. In that way it was possible to minimize the error introduced by neglecting the S shape. In the actual quantum yield experiments from 4 to 7 mg. (wet weight) of bacteria were used per vessel, corresponding to a density of about 1.3 to 2.3 mg. bacteria per ml. suspension. An additional advantage of using thin bacterial suspensions was that no gas changes could be detected as long as the bacteria were kept in the dark.

The light intensity–rate curve for photosynthesis with thiosulfate and tetrathionate as electron donors also showed an S shape, though much less pronounced than with H_2. It could only be demonstrated when dense bacterial suspensions were used. With about 5 mg. of bacteria (wet weight) per vessel the relation was expressed by a per-

fectly straight line starting at the origin up to the point at which the light intensity no longer is the limiting factor.

Light Source and Optical System.—Quantum yields were determined in infrared light at a wave length close to that corresponding to the absorption maximum (747 mμ) of the green bacteria chlorophyll.

The available monochromator did not produce a high enough light intensity at this wave length to cause photosynthesis to proceed at a rate close to saturation. A

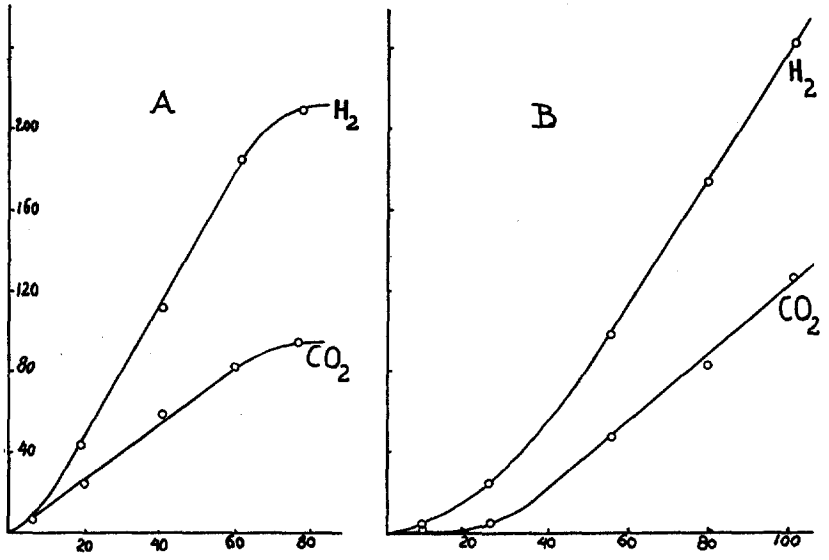

FIG. 1. Relation between incident light intensity and rate of assimilation for photosynthesis of *Chlorobium thiosulfatophilum* with H_2 as electron donor. Abscissae, light intensity in ergs per mm.^{2} per second; ordinates, H_2 and CO_2 absorbed in microliters per hour.

A, density of suspension 2.5 mg. bacteria per ml. suspension.

B, density of suspension 10.0 mg. bacteria per ml.

fairly monochromatic and sufficiently intense light beam was therefore isolated from white light by the use of filters. Light from an ordinary 500 W projection lamp, emitted through a circular opening and rendered parallel, was passed through an interference filter (Farrand Optical Co.) with a transmission peak at 732 mμ. The short-wave length tail of the emergent beam was partly cut off by a solution of commercial food dyes, the long-wave length tail by passage through a 5 cm. layer of water. It was established that not more than 1 per cent of the total energy transmitted by this combination represented radiation outside the region 700 to 780 mμ; the long-wave length absorption of intact *Chlorobium* chlorophyll protein falls entirely within this range.

The light beam, impinging on a mirror attached to the manometer support of the

apparatus, was reflected through a window in the water bath, and illuminated the bottom of the reaction vessel. In this manner the beam remained focussed on the bottom of the vessel at all times during the experiment. Care was taken to adjust the positions of light source and lens so that the parallel light beam had a cross-section of nearly uniform intensity. A small correction factor, separately determined, permitted the computation of the average light intensity for the entire cross-section when the intensity was measured in the center of the beam. The light intensity could be regulated by a variable resistance.

Incident light intensity was measured with an Eppley linear type thermopile with Zn-black coating and quartz window. The thermopile was standardized against a standard carbon filament lamp before each experiment, and a factor applied to correct for that part of the infrared radiation from the glass bulb of the standard lamp which was absorbed by the quartz window of the thermopile. The thermopile was placed in a colorless, transparent plastic box in the water bath in a position corresponding to that of the bottom of the Warburg vessel, and the proper correction factor introduced for the extra reflection from the plastic-air interphase.

Measurement of Absorption.—Only a fraction of the incident light was absorbed by the thin bacterial suspensions used for the experiments. It was thus necessary to determine the per cent absorption. This was done in a large Ulbricht integrating sphere; the construction was similar to that used by Kok (11) and Rieke (12) for a similar purpose. The aluminum foil remained on the vessels during the measurements.

The Ulbricht sphere was placed in the extension of the parallel light beam, and per cent absorption was measured immediately before or immediately after a quantum yield experiment. The absorption values were kept within 40 to 60 per cent of the total emission of the beam described above with a transmission peak at 732 mμ.

The use of the Ulbricht sphere for the determination of light absorption has the great advantage that the measurements are not subject to correction for scattering. It has been shown by the investigations of Kok (11) and Rieke (12) that this method gives quite reliable results.

From the experimental values of the amount of CO_2 assimilated, incident light intensity, per cent absorption, vessel bottom area, and time of exposure, it was possible to calculate the *quantum yield* as the number of molecules of CO_2 assimilated per light quantum absorbed by the bacteria, or the *quantum number* as the number of light quanta used to convert 1 molecule of CO_2 into cell material.

RESULTS

In Table I are summarized the values of the quantum numbers as determined in several series of experiments. Figures on the same horizontal line represent quantum numbers calculated from experiments with bacteria from the same culture; these values are directly comparable. The average quantum numbers for all simultaneous experiments with hydrogen and thiosulfate as electron donors are 9.8 for hydrogen, and 10.1 for thiosulfate. Simultaneous runs with hydrogen and tetrathionate as electron donors yielded the average

values of 9.1 for hydrogen, and 9.3 for tetrathionate; for thiosulfate and tetrathionate the corresponding average values were 9.5 and 9.2 respectively. The differences between the individual average values are best accounted for by the slight variations in photosynthetic activity of bacteria from one culture to another.

The sigmoid shape of the light intensity–rate curve has been disregarded in the calculation of the quantum numbers listed in Table I. In the case of photosynthesis with thiosulfate and tetrathionate this is entirely justified because, as mentioned earlier, the densities of the bacterial suspensions used in the experiments were such that at low light intensities not the slightest indication of a non-linear relationship was observed. The maximum inaccu-

TABLE I

Minimum Number of Light Quanta Used to Convert 1 Molecule of CO_2 to Cell Material by Chlorobium thiosulfatophilum with Molecular Hydrogen, Thiosulfate, and Tetrathionate, Respectively, as Electron Donors

Experiment No.	Electron donor		
	H_2	$Na_2S_2O_3$	$Na_2S_4O_6$
1	8.7	10.0	9.0
2	9.0	9.6	9.4
3	9.3	8.9	8.9
4	8.2	9.4	9.3
5	10.0	10.1	—
6	11.8	11.0	—
7	10.4	10.4	—
8	11.2	11.4	—
9	10.3	—	9.7
10	7.8	—	—

racy introduced by following this procedure for the computation of the quantum numbers of photosynthesis in the presence of H_2 has been estimated as not more than 0.5 unit; the corresponding quantum numbers in Table I might therefore be too high by this amount. It will be evident that the application of even this maximum correction yields values for quantum numbers of photosynthesis with the three different electron donors that agree within the limits of experimental accuracy. It is therefore concluded that the quantum yield of photosynthesis in *C. thiosulfatophilum* is independent of the nature of the electron donor, at least in the three cases studied.

DISCUSSION

In spite of the fact that the quantum yield determinations were carried out with bacteria in a physiologically active state, and grown under presumably optimal conditions, it would be rash to claim that the values re-

ported in Table I represent maximum obtainable efficiencies. It seems, however, permissible to compare them with those reported for photosynthesis by green plants and by various types of purple bacteria.

It then appears that the number of quanta involved in the assimilation of 1 molecule of CO_2 by photosynthesizing green sulfur bacteria (average about 9) is practically identical with that most frequently encountered in all other photosynthetic processes (10 ± 2). Apart from emphasizing the close similarity of the photosynthetic mechanisms operating in these different organisms, this result, and particularly the finding that the quantum number for green bacteria photosynthesis is independent of the nature of the electron donor, has important implications in connection with the problem raised in the introduction.

TABLE II

Minimum Quantum Requirements and Observed Quantum Numbers of Photosynthesis in Chlorobium thiosulfatophilum

Electron donor	ΔF^0_{298} in calories per mole of CO_2	No. of quanta needed for assimilation of 1 mole of CO_2		Thermodynamic efficiency
		Theoretical minimum	Observed	
				per cent
Hydrogen	1,700	0.044	9	0.5
Thiosulfate	29,150	0.75	9	8.3
Tetrathionate	30,160	0.77	9	8.5

At a wave length of approximately 730 mμ, corresponding to the maximum transmission of the filter combination used in our experiments, the amount of energy per mole quantum is equivalent to about 39,000 calories. Using this figure, and the values for energy changes involved in the three types of green bacteria photosyntheses investigated, the minimum theoretical quantum numbers for these processes can be calculated. They are listed in Table II, column 3. The experimentally determined values, shown in column 4, are very much greater; hence the thermodynamic efficiencies of these photosyntheses (column 5) are very low indeed. For green plant photosynthesis, which does not require the presence of a reducing substance, the efficiency is conceded to be at least 25 per cent. This comparison makes the green bacteria photosyntheses look rather wasteful from a thermodynamic point of view.

The most striking fact is, however, that the efficiency is least for photosynthesis in the presence of H_2, the system with the highest chemical potential. This furnishes a strong argument in favor of the contention that the extra chemical energy released by the oxidation of the electron donor is not utilized for the assimilation of CO_2, and that the latter is coupled only with

the absorption of light quanta. The same conclusion is reached by comparing the quantum numbers of CO_2 assimilation by green bacteria in the presence of thiosulfate and tetrathionate with those determined for green plant photosynthesis.

It thus appears that in all photosynthetic processes so far studied the same number of quanta is utilized for the assimilation of a molecule of CO_2. This obviously suggests that the primary photochemical reaction in all these processes is the same. The studies with isolated chloroplasts in particular have left no doubt that this reaction must be interpreted as a photolysis of water. By inference this would also be the case in the bacterial photosyntheses, a conclusion that has also been reached on the basis of other lines of reasoning (3, 5).

A few remarks may be added concerning previously reported quantum numbers, especially for photosynthesis in the presence of H_2. From his experiments with the non-sulfur purple bacterium, *Streptococcus varians* (now *Rhodopseudomonas capsulatus*), French (9, 13) has inferred that CO_2 assimilation by this organism can be accomplished with 5 quanta per molecule when H_2 is used as electron donor. The calculations leading to this result are, however, subject to doubt because the curve representing the relationship between light intensity and assimilatory rate shows a pronouncedly sigmoid shape. Rabinowitch (14, p. 1127) has calculated from French's data a lower limit of the quantum efficiency of 0.11, corresponding to a quantum number of 9. Experiments with the purple sulfur bacterium, *Chromatium*, (10) yielded values of 8.5 to 16, and 8.5 to 13.5 quanta, respectively, for photosynthesis with H_2 and thiosulfate as electron donors. A conservative evaluation of these figures reveals that they agree reasonably well with our own results obtained with green sulfur bacteria.

The most interesting case for comparison is, however, the so called "photoreduction," or CO_2 assimilation with concomitant H_2 oxidation, exhibited by some algae (15, 16), and studied by Rieke (12) from the point of view of its quantum efficiency. The significance of the comparison stems from the fact that the phenomenon of photoreduction is open to two alternative interpretations, neither of which can be ruled out on the basis of available evidence. The currently favored view appears to be that the assimilation of CO_2 with the simultaneous oxidation of H_2, by algae previously adapted to H_2 and illuminated in an O_2-free environment, represents a metabolic process essentially similar to that carried out by the photosynthetic bacteria. It is, however, conceivable that photoreduction is the net result of two different reactions, *viz.* (*a*) "normal" photosynthesis with evolution of O_2, and (*b*) oxidation of H_2 with the O_2 so produced. In the latter case H_2 oxidation would be dependent upon illumination, but photosynthesis should proceed also in the absence of H_2. The fact that at moderate light intensity O_2 is produced, even

when H_2 is present, might be considered as an argument in favor of the second alternative.

Gaffron (15) has shown that "adapted" cells of *Scenedesmus* can assimilate CO_2 also in darkness, while oxidizing H_2 with O_2. This assimilation, or "chemosynthesis," is both qualitatively and quantitatively comparable with that carried out by hydrogen bacteria. In both cases the amount of CO_2 assimilated may be equal to one-half of the amount of O_2 used (Doudodoroff; Schatz; personal communication). The following equation represents this energetically linked reaction system:

$$6H_2 + 2O_2 + CO_2 \rightarrow 5H_2O + (CH_2O).$$

Considering photoreduction as a "normal" photosynthesis with superimposed chemosynthesis, the actual CO_2 assimilation per absorbed quantum of radiant energy might here be greater by 50 per cent than in ordinary photosynthesis, as shown by the equations:

$$\begin{array}{ll} \text{Photosynthesis:} & 2[CO_2 + H_2O \rightarrow (CH_2O) + O_2] \\ \text{Chemosynthesis:} & 6H_2 + 2O_2 + CO_2 \rightarrow 5H_2O + (CH_2O) \\ \hline \text{Measured:} & 3CO_2 + 6H_2 \rightarrow 3(CH_2O) + 3H_2O. \end{array}$$

Assuming a quantum number of 8 to 10 for the photosynthetic reaction, the postulated sequence might then result in an "apparent" quantum number for photoreduction of 5.3 to 6.7.

Unfortunately, Rieke's results are not precise enough to decide whether the quantum number of photoreduction is lower than that of photosynthesis. The reason is that, as in other cases, the light intensity–rate curve is distinctly sigmoid. While various correction factors could be applied, none appears preferentially justifiable. As Rieke remarks: "By manipulating these corrections—in ways which at present seem to be entirely unjustified—the data might be reconciled with any value [for quantum efficiency] within the limits 0.090–0.160;" the corresponding quantum numbers would be 11 to 6.25, thus covering the range of special interest.

In view of the rather excellent agreement of the quantum numbers for the various types of green bacteria photosyntheses it seems permissible to conclude that in these processes a "chemosynthetic" CO_2 assimilation is not involved.

SUMMARY

The quantum efficiency of photosynthesis by the green sulfur bacterium, *Chlorobium thiosulfatophilum*, has been determined in systems in which thiosulfate, tetrathionate, and molecular hydrogen served as electron donors. It was found that about 10 ± 1 quanta are used for the assimilation of 1 molecule of CO_2, and that the quantum number is independent of the nature of the electron donor. These results are considered as support for the view that

also in the bacterial photosyntheses the primary photochemical reaction consists in the photolysis of H_2O, and that the chemical energy released during the oxidation of the electron donor is not utilized for CO_2 assimilation. Hence the photosynthetic processes of the green sulfur bacteria are thermodynamically less efficient than is green plant photosynthesis.

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