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# REVERSIBLE PHOTOCHEMICAL REDUCTION AND OXIDATION OF BACTERIAL CHLOROPHYLL AND BACTERIAL PHEOPHYTIN

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# REVERSIBLE PHOTOCHEMICAL REDUCTION AND OXIDATION OF BACTERIAL CHLOROPHYLL AND BACTERIAL pheophytin- PHEOPHYTIN

A.A.Krasnovskiy and K.K.Voynovskaya

The photochemical redox transformations of chlorophyll [1, 2] and of some of its analogs and drvs. as well as the formation of active intermediate substances in reactions sensitised by chlorophyll have already been discussed in prior publications [3]. This paper describes redox transformations of bacterial chlorophyll (BCh) and bacterial pheophytin (BP) under the action of light from the near infrared region of the spectrum, a region where the principal absorption maxima of these pigments are situated.

Gray-purple bacteria were grown in a medium containing malic acid [4] and kept in a luminostat. The suspension of bacteria was filtered through diatomaceous earth. The pigments were then extracted with MeOH, transfered into petroleum ether, and separated chromatographically in a column charged with saccharose. The solvents were saturated with $H_2S$ prior to the [5] Elution and washing out of carotinoids was carried with 4 parts petroleum ether, 1 part ethyl ether.

BCh was removed from the blue zone with ethyl ether. The experiments were usually carried out with freshly isolated BCh. BP was obtained in the solid state after the ether soln. of BCh had been treated with 10% HCl [6], whereupon the acid was washed out, and the ether solution evaporated after drying.

The experimental method used was the same as in our work with chlorophyll. The photoreaction was carried out in special vacuum tubes which could be kept in the cell-holder of a Beckman spectrophotometer. Five ml of the pigment solution (with an extinction coefficient: E= 0.5-0.8 at the infrared maximum of absorption) containing the oxidizing or reducing agent under investigation were exposed to a vacuum produced by a oil pump. The solution was shaken agitated during the evacuation. In experiments with oxygen evacuation was not carried out. The absorption spectrum was measured - - then illumination in a thermostatic device at 10° was carried out by focusing the light of a 500 wt motion picture bulb through a condenser and passing this light through an RC-5 filter. After exposing the sample to light in this manner, the absorption spectrum was measured; occasionally it was impossible to accomplish this because of the rapid reverse reaction. When oxygen of the air was used for the photooxidation, the following reducing agents were added in order to bring about the reverse reaction: ascorbic acid, hydrogen sulfide, etc. Upon photoreduction the final regeneration of the pigment was carried out with oxygen of the air. Some data obtained in the experiments in question are listed in tables 1 and 2 and



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shown in figures 1 and 2. In the results cited, the quantity of reacting pigment was calculated on the basis on the change in the value of the coefficient of extinction at the infrared maximum.

Results of Experiments on Photooxidation.

BCh is very rapidly oxidized under the action of oxygen of the air. This takes place most rapidly in ethyl alcohol and proceeds somewhat more slowly in pyridine. Upon exposure to light, the alcohol solution yields a strong reaction for peroxides with ~~an iron~~ ferrous sulfocyanide solution [1]. The reverse regeneration of the pigment is achieved by adding ascorbic acid or $H_2S$. Malic, citric, pyruvic, and succinic acid as well as thiosinamine [allyl thiourea] proved to be ineffective. The intermediate oxidation product is fairly stable; after the solution has stood for 10 hours, one can regenerate the ~~major part of~~ main quantity of pigment by adding ascorbic acid. Some oxidants (p-quinone, sodium nitrite, sodium nitrate, hematin) proved were found to be ineffective. However, BCh is oxidized even in the dark by o-quinone (in a toluene solution) under formation of a product similar to chlorophyll which has an absorption maximum at 680 mμ. BP is more stable towards photooxidation. However, a reverse course of the reaction is observed even here after ascorbic acid had been added.

Results of Experiments on Photoreduction.

BCh is reduced under the action of ascorbic acid ~~and~~ or sodium sulfide (The experiments in this case were conducted in a pyridine solution containing 10% of water in order to improve the solubility of $Na_2S$). Just as in the case of chlorophyll, the reaction proceeds furthest in pyridine, while in ethyl alcohol there is no apparent reaction. The reaction is ~~easily~~ readily reversible in the dark ~~(i.e.~~ (within a few seconds) in the absence of air, so that determination of the spectrum of the intermediate product is difficult under the circumstances. BP is reduced more rapidly and to a greater extent than BCh, and the reverse reaction in this case is slower than with BCh. The intermediate product of the reduction, which is green, exhibits a maximum at 640 mμ. Malic, succinic, citric, and pyruvic acid as well as thiosinamine and sodium thiosulphate were tested as reducing agents and found inactive.

The data obtained permit the following conclusions:

1) BCh is rapidly photooxidized by air oxygen under formation of a peroxidic compound which does not show the characteristic spectrum of porphyrins; most likely is an interaction of oxygen with a lasting ~~biradical~~ excited state of the pigment (which assumes the form of a biradical) in accordance with A. N. Terenin's theory [3], a process which is accompanied by

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opening of the cyclically conjugated system of double bonds of the pigment molecule. A reverse reduction of the peroxide in question leads to formation of the initial molecule of the pigment.

2) Photoreduction of BCh and BP also leads to a drop in the infrared maximum of absorption. The course of the reaction of these pigments indicates that the reduction does not consist proceed at individual double bonds (all of them are hydrogenated in BCh), but involves opening of the ring under formation of an active intermediate product which is a ~~using formation of a~~ stable free radical of the semi-quinone type.

3) BCh is more easily oxidized and is reduced with greater difficulty than BP; the same relationship was observed by V. B. Yevstignayev and V. A Gavrilova [9] at our laboratory in the case of ordinary [non-bacterial] chlorophyll and pheophytins.

4) BCh is reversibly photooxidized with much greater facility than chlorophyll, but ~~is reduced with~~ its reduction is somewhat more difficult. The reversible reduction of both pheophytins is rapid and complete.

5) The reversible reactions of BCh and pheophytins which we discovered and investigated in this instance now explain the chemistry of reactions photosensitized by these compounds. When the photooxidation of organic substances with oxygen of the air is sensitized, the following possibilities are present (just as in the case of chlorophyll), depending on the nature of the medium: primary formation of the peroxide of the pigment or its photoreduction by the compound being oxidized. Subsequent reactions of the peroxide of the pigment with the oxidation substrate or reaction of oxygen with the photoreduced form of the pigments may then be expected to take place.

6) The results obtained confirm the conclusion that the pigments in question participate in the chemical process of bacterial photosynthesis. The photoreduced form of the pigment, which is formed in the reaction with hydrogen sulfide, transfers its hydrogen (proton and electron) to the intermediate enzyme system directing the reactions of carbon dioxide reduction that take place in the dark.

In accordance with this one may assume that Athiorhodaceae may utilize in photosynthesis more the more active compounds which are formed in dark phase transformations of the initial hydrogen donors, i. e. substances that are incapable of a direct photoreaction with the pigment.

Thanks are due to Academician A. N. Terenin for valuable advice in connection with this work.



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END.

[Two tables and two figures follow]



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Table 1. Reversible ~~Oxidation~~ Photooxidation of BCh and BP With Oxygen of the Air.

Conditions of the Experiment: Illumination was carried out in air through the light filter RG-5 - in the case of BCh solutions for 30 sec, in the case of BP solutions for 3 min. Regeneration was brought about by introduced adding the reducing agent immediately after exposure to light of the solution to light.

| Solvent. | Reducing agent | Quantity of pigment expressed in % of the initial product | | | |
|---|---|---|---|---|---|
| | | BCh | | BP | |
| | | photoreaction | reverse reaction | photoreaction | reverse reaction |
| Ethyl alcohol | Ascorbic acid | 9.1 | 7[illegible].8 | 80.7 | 59.8 |
| " " | $H_2S$ | 7.1 | 68.[illegible] | - | - |
| Acetone | Ascorbic acid | 11.2 | [illegible] | - | - |
| Acetone | $H_2S$ | 11.5 | [illegible] | - | - |
| Pyridine | Ascorbic acid | 20.0 | 2[illegible] | 87.0 | 78.7 |
| Toluene | $H_2S$ | 13.[illegible] | 3[illegible].5 | - | - |

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Table 2. Reversible Photoreduction of BCh and BP.

Conditions of the Experiment: Illumination was carried out through [illegible] light [illegible] in vacuum in the pre[illegible] to light [illegible] Twenty mg of ascorbic acid or 5 mg of $Na_2S$ were used. In the case of BP, exposure to light [illegible] 30 cc when alcohol was used as a solvent; for 0.5-1 min when pyridine was used as a solvent. In the [illegible] solutions in pyridine, exposure to light was continued for 3.5 min. Regeneration was brought about by [illegible] oxygen 5-10 min after exposure to light. The reverse reaction leading to regeneration proceeded in the absence of oxygen as well.

| Solvent. | Reducing agent. | Quantity of pigment expressed in % of the initial product | | | |
|---|---|---|---|---|---|
| | | BP | | BCh | |
| | | Photoreaction | Reverse reaction | Photoreaction | Reverse reaction |
| Alcohol | Ascorbic acid | 63.1 | 82.8 | 100 | 100 |
| | $Na_2S$ | 82.0 | 90.4 | 93.3 | [illegible] |
| Pyridine + 10% water | Ascorbic acid | 18.4 | 52.1 | 28.9 | [illegible] |
| | $Na_2S$ | 39.9 | 80.0 | 57.5 | 90.7 |

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Table 2. Reversible Photoreduction of BCh and BF.

Conditions of the Experiment: Illumination was carried out through the light filter RG-5 in vacuum in the presence of the reducing agent. Twenty mg of ascorbic acid or 5 mg of $Na_2S$ were used. In the case of BF, exposure to light was continued for 30 sec when alcohol was used as a solvent; for c.5-1 min when pyridine was used as a solvent. In the case of BCh solutions in pyridine, exposure to light was continued for 3.5 min. Regeneration was brought about by introducing oxygen 5-10 min after exposure to light. The reverse reaction leading to regeneration proceeded in the absence of oxygen as well.

| Solvent. | Reducing agent. | Quantity of pigment expressed in % of the initial product | | | |
|---|---|---|---|---|---|
| | | BF | | BCh | |
| | | Photoreaction | Reverse reaction | Photoreaction | Reverse reaction |
| Alcohol | Ascorbic acid | 63.1 | 82.8 | [illegible] | [illegible] |
| | $Na_2S$ | 82.0 | 90.4 | 93.3 | [illegible] |
| Pyridine + [illegible] | Ascorbic acid | 18.4 | 52.1 | [illegible] | [illegible] |
| | $Na_2S$ | 39.9 | 80.0 | 57.5 | [illegible] |

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Рис. 1. Обратимое фотоокисление кислородом воздуха бактериохлорофилла (БХ) в спирте с последующей регенерацией аскорбиновой кислотой. 1 – раствор БХ до облучения; 2 – после облучения 1 мин; 3 – после введения аскорбиновой кислоты на 26 мин после облучения и стояния в темноте в течение 1 часа.

Figure 1. Reversible photooxidation with oxygen of the air of bacterial chlorophyll (BCh) dissolved in alcohol followed by ~~with subsequent~~ regeneration with the aid ~~by means~~ of ascorbic acid. 1 - BCh solution before exposure to light; 2 - the same after exposure to light for 1 min; 3 - the same upon ~~after~~ introduction of ascorbic acid 26 minutes after exposure to light and then allowing to stand in darkness for 1 hour.

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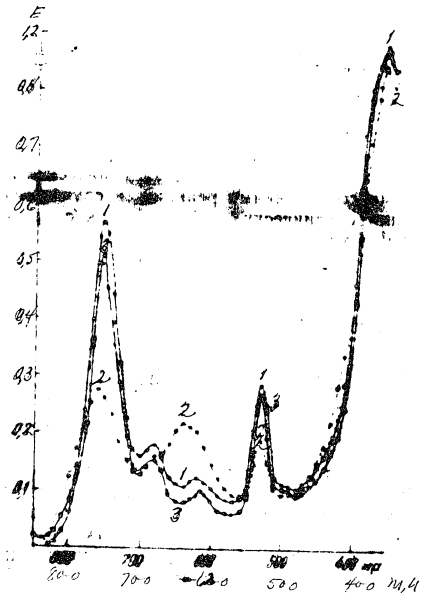

Рис. 2. Обратимое фотовосстановление раствора бактериофеофитина (БФ) в пиридине сернистым натрием. 1 — раствор БФ до облучения, 2 — промежуточный продукт реакции после 30 сек. освещения, 3 — конечный продукт (после впуска воздуха и стояния в темноте).

Figure 2. Reversible Photoreduction with ~~of~~ $Na_2S$ of Bacterial Pheophytin (BP) dissolved ~~in~~ in pyridine. 1 - BP solution before exposure to light; 2 - intermediate product of the reaction after 30 sec exposure to light; 3 - final product (after introduction of oxygen and standing in the dark).

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