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REVERSIBLE PHOTOREDUCTION OF CHLOROPHYLL *a* TO A COMPOUND, Ch_aH_2610, WITH AN ABSORPTION MAXIMUM AT 610 nm

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SUMMARY

1. A new reaction of chlorophyll *a* photoreduction was found. The reaction proceeded in non-polar solvents in the presence of rather strong primary amines (benzylamine, allylamine, ethanolamine).

2. The product of this reaction, named Ch_aH_2610 was photooxidized to chlorophyll *a* by quinones, 2,6-dichlorophenolindophenol or phenosafranine. Ch_aH_2610 was identified as dihydrochlorophyll *a*. The structure of the investigated compound was discussed; it was concluded that Ch_aH_2610 is 5,6-dihydrochlorophyll *a*.

3. The photoreduction of some chlorophyll derivatives was investigated.

INTRODUCTION

The investigations of chlorophyll photoreduction are of interest because this reaction is probably involved in the primary photochemical step of photosynthesis.

The direction of photoreduction depends on the conditions. As was found by KRASNOVSKY[1], chlorophyll *a* in the presence of pyridine and ascorbic acid was photoreduced to a compound with absorption maximum 525 nm. Similar effect was observed in photosynthetic tissues by COLEMAN AND RABINOWITCH[2] and WITT AND MORAW[3]. Two other photoreduction reactions were found by BROYDE AND BRODY[4] and SEELY[5]. After illuminating the concentrated solution of chlorophyll *a* and phenylhydrazine in ethanol the presence of a compound with band 640 nm was detected[4]. SEELY[5] found that in the presence of 1,4-diazabicyclo(2,2,2)octane chlorophyll *a* in the pyridine–ethanol solution was photoreduced to hypochlorophyll with absorption maximum 632 nm.

All these reactions took place in polar solvents, in the presence of either weak primary or tertiary amines. The investigation of photoreduction in non-polar solvents, in the presence of stronger primary amines led to discovering of a new reaction[6]. Properties and structure of the product of this reaction are discussed in this paper.

MATERIALS AND METHODS

Chlorophyll *a* was prepared from spinach by the method of ZSCHEILE AND COMAR[7], ethyl chlorophyllide *a* and pyrochlorophyll *a* by the method of PENNINGTON *et al.*[8]. Ethyl mesochlorophyllide *a* was prepared by H_2–Pt reduction in acetone[9]. 9-Deoxy-9-hydroxychlorophyll *a* was obtained by the method of HOLT[10]. Magnesium isochlorin-e_4-6-carboxypiperidide phytyl methyl ester was prepared by the reaction of chlorophyll *a* with piperidine.

All Mg^{2+}-free derivatives were prepared by treatment of methanol solutions of a suitable pigment with 2/3 part (v/v) of 2 M HCl.

All the pigments were purified by chromatography and controlled by their spectra and thin-layer chromatography on "Kieselgel G nach Stahl" with the mixture of petrol ether–ethyl ether–methanol (18:4:3, v/v/v) as eluent.

Experiments were performed with solutions containing approx. 10^{-5} M pigment, $3 \cdot 10^{-3}$ M phenylhydrazine and 10% (v/v) amine. The solution was flushed with N_2, purified over heated Cu. With light from 500 W projector bulb through a red filter (RG2, Schott, Jena, passing $\lambda > 620$ nm) conversion of chlorophyll *a* to Ch_aH_2610 was complete within 60 min. The course of reaction was controlled by measuring the spectra either in special holder with VSU, Zeiss spectrophotometer, or with recording SP800 Unicam spectrophotometer.

For reoxidation the pigment investigated was dissolved in benzene or ethyl ether and pyridine (2%, v/v) and suitable oxidant (5 mg/5 ml) was added. The solution was flushed with N_2 and irradiated with light through an orange filter (OG3, Schott, Jena, passing $\lambda > 570$ nm).

RESULTS

The photoreduction reaction

The anaerobic solutions of chlorophyll *a* in heptane, benzene or toluene with addition of rather strong primary amines (benzylamine, ethanolamine, allylamine) and phenylhydrazine were used. After irradiation of the solution with red light the decrease of main band and increase of the band near 610 nm was observed (Fig. 1). One can see that the disappearance of chlorophyll *a* is followed by formation of two products: a main product of photochemical reaction with band 610 nm, called

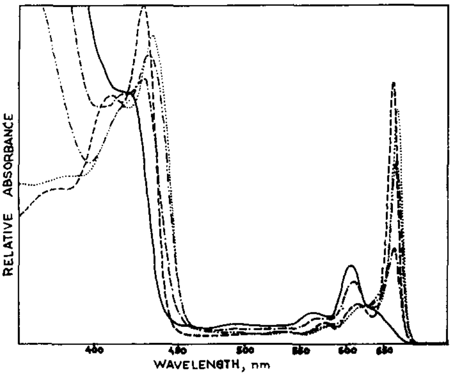

Fig. 1. Reduction of chlorophyll *a* to Ch_aH_2610 in red light (RG2 Schott, Jena). Composition of solution: 10% (v/v) benzylamine; 90% benzene; $1{,}7 \cdot 10^{-5}$ M chlorophyll *a*; $3.0 \cdot 10^{-3}$ M phenylhydrazine. Spectra recorded before illumination (······), and after illumination for 15 min (–··–··–), 45 min (–·–·–·) and 60 min (———). The spectrum of pure chlorophyll *a* (-----) ($1.7 \cdot 10^{-5}$ M in ethyl ether) is also given.

Ch_aH_2610, accompanied by a small amount of a compound with absorption maximum 642 nm, which is a product of dark aminolysis of chlorophyll *a*. Photoreduction was efficient in non-polar solvents only: addition of ethanol (10 %, v/v) or water (benzene saturated with water) inhibited this reaction. Oxygen had also an inhibitory effect. In the presence of strong amines (*n*-butylamine, dibutylamine) only small quantities of Ch_aH_2610 were formed. On the other hand, the photoreduction in the presence of pyridine proceeded in the direction of 525-nm product, which treated with benzylamine in darkness isomerized to Ch_aH_2610. Reversal isomerization of Ch_aH_2610 to 525-nm product was not found.

The properties of Ch_aH_2610

Pure compound Ch_aH_2610 was obtained by vacuum evaporation of solvent, addition of petrol ether, extraction of amine with water and column chromatography on sugar with a 1:1 mixture of ethyl ether and petrol ether as eluent. The spectrum of pure compound is shown in Fig. 2. Ethyl ether solution was strongly fluorescent. In the air Ch_aH_2610 was slowly oxidized. In the absence of air, in benzene or ethyl ether solution it was photochemically (orange light) oxidized to chlorophyll *a* by benzoquinone, menadione, 2,6-dichlorophenolindophenol or phenosafranine (Fig. 3). The reaction was accelerated by the addition of pyridine (2 %, v/v). The final product of oxidation was identified as chlorophyll *a* by thin-layer chromatography, spectrum and ability to repeated photoreduction. The SEELY's method[5] of oxidation with phenosafranine for determination of the amount of extra hydrogen atoms in $ChaH_2610$ showed that the compound investigated was dihydrochlorophyll *a*.

The further experiments were performed to study the reactivity of functional groups and to get information where the two extra hydrogen atoms were located.

Acetone solution of Ch_aH_2610 was reduced to its mesoderivative with hydrogen in the presence of platinum black; this was accompanied by shift of main band from 610 to 607 nm. Menadione oxidized this product to mesochlorophyll *a*. No reduction of Ch_aH_2610 in methanol or ethyl ether solution with $NaBH_4$ was found: after treatment with $NaBH_4$, Ch_aH_2610 transformed to ethyl ether showed unchanged spectrum

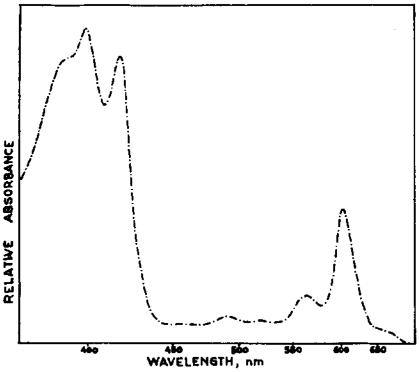

Fig. 2. The spectrum of Ch_aH_2610 in ethyl ether. Maxima: 602, 558, 518, 491, 417 and 399 nm.

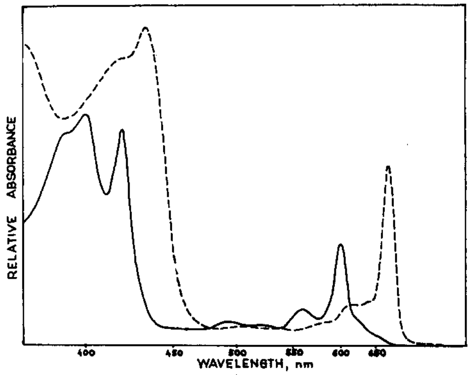

Fig. 3. Photooxidation of Ch_aH_2610 to chlorophyll *a*. ———, Ch_aH_2610 with menadione (5 mg/5 ml) in ethyl ether; -----, after transfer to benzene, addition of pyridine (2 %, v/v) and illumination for 20 min with orange light (OG_3 Schott, Jena).

and was oxidized with menadione to chlorophyll *a* and not to 9-deoxo-9-hydroxychlorophyll *a*. There was also no reaction between Ch_aH_2610 and piperidine.

The lack of reactivity of the 9-keto group could result from strong enolization of this group. Therefore the possibility of esterification or etherification and phase test were studied. It was found that Ch_aH_2610 did not react with acetic acid anhydride (15 %, v/v of anhydride in pyridine, 6 h in room temperature), or with diazomethane (ether solution, 12 h in room temperature). On the other hand, treatment of pyridine solution of Ch_aH_2610 with methanol solution of magnesium methoxide, under nitrogen, was accompanied by decrease of main bands 612 and 419 nm and increase of the broad band 510–470 nm. Addition of acetic acid regenerated the initial spectrum of Ch_aH_2610. Methanol solution of KOH (10 % KOH) caused irreversible change in Ch_aH_2610: the spectrum of intermediate showed maxima 651, 605, 565, 522 and 424 nm and resembled that of Mg^{2+}-unstable chlorin dimethyl ester[11].

It was possible to remove Mg atom from Ch_aH_2610 without oxidation. Methanol solution of Ch_aH_2610 treated with 2 M HCl changed its colour into green. After neutralization with pyridine the pigment, transformed to ethyl ether, showed spectrum with bands 626 and 416 nm. This compound was photooxidized with menadione in the presence of pyridine to pheophytin *a* (Fig. 4).

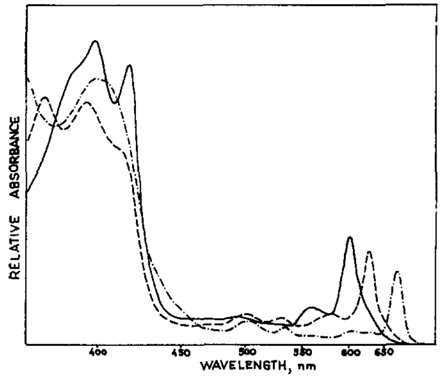

Fig. 4. Preparation of Mg-free derivative of Ch_aH_2610 and oxidation to pheophytin *a*. ———, initial solution of Ch_aH_2610 in ethyl ether; -----, after treatment of methanol solution of Ch_aH_2610 with 2 M HCl and transfer to ethyl ether; –·–·–, after addition of menadione and illumination for 10 min through orange filter (OG3 Schott, Jena).

The photoreduction of chlorophyll derivatives

Among compounds studied only ethylchlorophyllide *a* and pyrochlorophyll *a* were reversibly photoreduced to derivatives of Ch_aH_2610.

Pheophytin *a* underwent photoreduction to a compound with broad band 525 nm that did not isomerize to a product with band 626 nm. The photoreduction of 9-deoxo-9-hydroxypheophytin *a* resulted in the formation of a product with absorption maxima 552 and 492 nm. These two products were oxidized by air in darkness to initial substances.

Benzene solution of 9-deoxo-9-hydroxychlorophyll *a* with addition of benzylamine and phenylhydrazine was photobleached on irradiation. Mesochlorophyll *a* underwent irreversible photoreduction to a compound with band 410 nm, that was oxidized neither to the initial substance nor to the product of reduction of Ch_aH_2610

with hydrogen and platinum black. No photoreduction of magnesium-isochlorin-e_4-6-carboxypiperidide phytyl methyl ester was found in the presence of benzylamine.

All the described reactions are shown in Fig. 5.

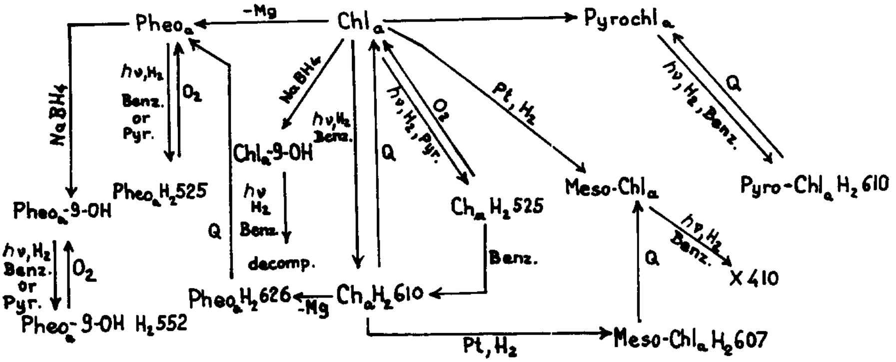

Fig. 5. Schema of photoreduction reactions in the presence of benzylamine. Chl$_a$-9-OH, 9-deoxo-9-hydroxychlorophyll *a*; Pheo$_a$-9-OH, 9-deoxo-9-hydroxypheophytin *a*; Chl$_a$H$_2$525, product of chlorophyll *a* photoreduction with maximum 525 nm; *hv*, H$_2$, reaction of photoreduction; Benz., benzylamine; Pyr., pyridine; Q, photooxidation with quinone.

DISCUSSION

On the basis of presented material it is evident that Ch$_a$H$_2$610 is dihydrochlorophyll *a*, similarly to SEELY's hypochlorophyll and pink product of KRASNOVSKY. These three compounds must differ in the position of two extra hydrogen atoms only. According to SEELY, KRASNOVSKY's product is β,δ-dihydrochlorophyll *a*: the breaking of the system of conjugated bonds caused strong shift of absorption band from 660 to 525 nm. The spectra of two other photoreduction products are very alike: the similarity to the spectra of chlorins argues for reduction of two adjacent pyrrole rings on β-positions[12–14], and sharpness of the bands in visible part of the spectra argues for retention of planarity and closed conjugation in the ring. Therefore only two structures are possible: 1,2-dihydrochlorophyll *a* and 5,6-dihydrochlorophyll *a*. SEELY considered his hypochlorophyll *a* is probably 1,2-dihydrochlorophyll *a*. It means Ch$_a$H$_2$610 should be a 5,6-dihydro derivative. Some results support this conclusion. Photoreduction of chlorophyll *a* toward hypochlorophyll proceeded simultaneously with photoreduction of the vinyl group to ethyl group, what suggest the neighbourhood of these two reactive sites. On the other hand the photoreduction of chlorophyll *a* to Ch$_a$H$_2$610 and reduction of chlorophyll *a* to mesochlorophyll *a* are independent processes. The reduction of Ch$_a$H$_2$610 to its mesoderivative caused the change of spectrum suggesting the conjugation of vinyl group with porphyrin ring. These are arguments for accepting the structure of 5,6-dihydrochlorophyll *a* for Ch$_a$H$_2$610.

It is not clear why the carbonyl group at C_9 is not reactive both in hypochlorophyll and Ch$_a$H$_2$610. The positive phase test for Ch$_a$H$_2$610 suggest that the β-keto-ester grouping is unchanged. On the other hand there is lack of reactivity of $C_9{=}O$ group either as keto group (lack of reduction with NaBH$_4$ and of aminolysis with piperidine) or as enol group (negative results of esterification and etherification experiments).

The reduction to Ch_aH_2610 requires electron attracting groups (vinyl and carbonyl) at the 2- and 6-positions, and the central Mg atom. It is not clear, whether these groups play a role in relocation of hydrogen during the base-catalized isomerization[5], or in formation of special complexes with amines[15] sensitive to photoreduction to the desired product.

There are some arguments in favour of biological significance of the chlorophyll photoreduction to Ch_aH_2610: (a) this reaction is directed by ethanolamine that was found in the leaves of the higher plants[16]; (b) this reaction is reversible; (c) this reaction proceeds in non-polar media, therefore it is possible that this reaction would undergo in hydrophobic part of photosynthetic apparatus.

The further experimental material is necessary for proving this hypothesis.

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