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INITIATION OF THE POLYMERIZATION OF METHYL METHACRYLATE BY REDUCED FORMS OF CHLOROPHYLL AND HEMATOPORPHYRIN

In the reaction of reversible photoreduction of chlorophyll and its analogs (^1), one could expect the formation of free radicals—semiquinones—as a result of electron transfer from the donor molecule (AH) to the excited chlorophyll molecule (X):

$$X + AH \rightleftharpoons X^- + AH^+.$$

In order to detect the formation of free radicals in the photoreduction reaction, we studied the photosensitized polymerization of monomers, which proceeds vigorously in the pigment—electron donor—methyl methacrylate system under the action of red light absorbed by the pigment (^2). In these studies, data were obtained on the initiation of polymerization by pigments and peroxide radicals. By the method of measuring electron paramagnetic resonance, upon illumination of the pigment—ascorbic acid system, a signal was detected belonging to the radical of the electron donor—monodehydroascorbic acid (^3). Thus, in the photoreduction reaction, different types of free radicals are formed: those of the pigment, of the electron donor, and of the products of their interaction with oxygen.

In order to study the action of the radicals of the reduced pigment, the radical of the electron donor must be excluded from the system. This is possible in systems in which a reducing agent is used that, upon donating an electron, is not converted into a free radical and remains in another phase—for example, metallic zinc.

In previous work from our laboratory, the reduction of chlorophyll and its analogs with zinc according to Timiryazev (^4) was studied, and the formation was shown (at the optimum acidity for the given pigment) of reduced forms

similar in absorption spectrum to the photoreduced forms (ˆ5). We found (ˆ6) that, when zinc amalgam is used instead of zinc dust or shavings, it is possible to carry out the reduction of chlorophyll and its analogs in pyridine without addition of acid (in the dark), i.e., under conditions in which accumulation in solution of aprotonic reduced forms—anion-radicals (X)—could be expected.

Chlorophyll and other pigments were obtained by methods adopted in our laboratory. The preparation of the methyl methacrylate monomer for experiments was described by us earlier (ˆ2). Reagent-grade pyridine, distilled without preliminary drying, was used. Zinc amalgam was obtained (ˆ7) by dissolving 3 g of granulated chemically pure zinc in 100 g of mercury with addition of 3-5 ml of 0.1 N sulfuric acid. The mercury with zinc was heated to 100° in a water bath until the zinc dissolved. The amalgam obtained was cooled and thoroughly washed with distilled water to remove the acid.

The experiments were carried out in vacuum Thunberg tubes with a side arm, adapted for measurement on a spectrophotometer (Fig. 1). Into the side arm were poured 4 ml of a solution of the pigment in pyridine and 0.1-0.2 ml of zinc amalgam; the arm was closed with a cotton filter and the air was removed by evacuation with a fore-vacuum pump. The pigment was then reduced by vigorous shaking of the pyridine solution with the amalgam (without illumination) in the side arm for several minutes. After complete reduction of the pigment, as evidenced by the change in color, the solution was filtered (in vacuo) through the cotton filter into the lower part of the Thunberg tube, and the absorption spectrum was measured.

Under these conditions chlorophyll is reduced with formation of a form having an absorption maximum at about 520 mμ (Fig. 2), similar to the photoreduced ...

form. When pheophytin is allowed to interact with zinc amalgam, a zinc complex of pheophytin is first formed, which is then reduced. Hematoporphyrin is reduced by the amalgam with the formation of a yellow product with an absorption maximum at 460 mμ (Fig. 3). The spectrum obtained differs from the absorption spectrum of hematoporphyrin photoreduced by ascorbic acid at room temperature in pyridine, where the reduction product has absorption maxima in the region of 650-740 mμ ([8]). In acidic aqueous solutions the maximum of the photoreduced form of hematoporphyrin lies at 500 mμ ([9]). Photoreduction at −40° led to an increase in absorption (corresponding to the "primary" photoreduced form of hematoporphyrin) in the region of 450-480 mμ ([10]). Flash spectroscopy of hematoporphyrin revealed a product with a maximum in the region of 400-500 mμ ([11]). Addition of 2-3 drops of glacial acetic acid to the chlorophyll solution had no effect on the spectrum of chlorophyll reduced by the amalgam. Reduction of hematoporphyrin in the presence of acid led to the formation of a reduction product with an absorption maximum in the region of 670-680 mμ.

The pigment forms reduced by zinc amalgam are extremely unstable. In vac-

Figure 1

Figure 1: Figure 1

Figure 2

Figure 2: Figure 2

uum the reduced form of chlorophyll reverts to the original chlorophyll in 5-6 min. When air is admitted, instantaneous regeneration of chlorophyll occurs. Hematoporphyrin reduced (in the absence of acid) in vacuum is fairly stable, but after air is admitted it changes into the original porphyrin practically instantaneously. The hematoporphyrin reduction product obtained in the presence of acid, in the reverse reaction, first passes into the "460 mμ" form and then into the initial form. The reverse reaction in vacuum proceeds slowly; with air it is very rapid.

Fig. 1. Vacuum tube for carrying out experiments on the reduction of pigments with zinc amalgam and on initiation of polymerization by the reduced forms: **1** —pigment solution in pyridine in the side arm; at the bottom of the side arm — zinc amalgam; **2** —cotton filter; **3** —methyl methacrylate

Fig. 2. Reversible reduction of chlorophyll *a* by zinc amalgam in pyridine: **1** —spectrum of chlorophyll *a* in pyridine (initial); **2** —after reduction with zinc amalgam; **3** —after the reverse reaction

The instability and high reactivity of the pigment forms reduced by zinc amalgam distinguish them from the photoreduced forms, which are stable in vacuum and react slowly with oxygen. Therefore, despite the spectral similarity of both forms, they probably have different natures. Indeed, we showed that the "red" photoreduced form of chlorophyll in the presence of an excess of ascorbic acid did not initiate polymerization of methyl methacrylate ([2]) and, in accordance with the experiments of Weissman and Livingston ([12]), did not give an EPR signal ([3]). Evidently, the "red" photoreduced form is not a free radical but, most likely, a valence-saturated product.

In the reaction of the pigment with zinc amalgam we expected the formation of radical reduced forms. To detect free radicals

we studied the initiation of chain polymerization of methyl methacrylate by porphins reduced with zinc amalgam. The experiments were carried out with chlorophyll and hematoporphyrin in Thunberg vacuum tubes with a side arm. The pigment in pyridine solution at a concentration of $5 \cdot 10^{-5}$ M was reduced in the side arm with zinc amalgam by the method described above (in vacuum, in the dark). Then the solution of the reduced pigment was filtered into the lower end of the tube, into which methyl methacrylate (3 ml) had previously been poured. The final ratio of the volumes of pyridine and methyl methacrylate was

Fig. 3. Reversible reduction of hematoporphyrin by zinc amalgam in pyri-

Figure 3

Figure 3: Figure 3

dine. **a** –spectral changes of hematoporphyrin in the absence of acid: 1 –initial spectrum, 2 –after reduction, 3 –after the reverse reaction; **b** –spectrum of hematoporphyrin reduced in the presence of acetic acid (0.04 ml per 4 ml of solution)

1 : 3. After mixing methyl methacrylate with the pyridine solution of the reduced pigment, the test solution was kept in vacuum in the dark at room temperature for one hour to allow chain polymerization to develop. Then the tube was opened, the solution was poured into an excess of methyl alcohol (1 : 10), and the occurrence of polymerization was judged from the amount and character of the polymer precipitate. The experiments were carried out in several variants; the results of the experiments are given in Table 1.

From the data in Table 1 it is evident that the most active polymerization is observed when a solution of pigment reduced with zinc amalgam is added to methyl methacrylate in vacuum. The greatest effect was obtained in the experiment with chlorophyll reduced in the presence of 2–3 drops of acetic acid. In this case a large flocculent precipitate of polymer is formed in methyl alcohol. Shaking a non-evacuated pigment solution with zinc amalgam, followed by dark storage with methyl methacrylate in vacuum, did not lead to polymerization. This indicates that the radicals initiating polymerization are formed as a result of reduction of the pigment in vacuum. In the next variant of the experiment, the solution of reduced pigment was kept in vacuum for 5–10 min before being filtered into methyl methacrylate. During this time the reduced chlorophyll had almost completely regenerated. As can be seen from the data of Table 1, in this case polymerization was also completely absent. In an analogous experiment with hematoporphyrin, the reduced form of which is more stable in va-

Table 1

Initiation of methyl methacrylate polymerization by products of the interaction of pigments with zinc amalgam
Pigment concentration $5 \cdot 10^{-5}$ M, 0.1 ml of zinc amalgam, 1 ml of pyridine and 3 ml of methyl methacrylate. Experiments at room temperature, dark exposure 1 h.
b.a. –without acid. a. –0.02 ml of glacial CH_3COOH

Experimental conditions	Chlorophyll b.a.	Chlorophyll a.	Hematoporphyrin b.a.	Hematoporphyrin a.	Without pigment b.a.	Without pigment a.
Reduction of the pigment in vacuum and addition to methyl methacrylate in vacuum	+++	+++	+++	+++	−	−
Shaking of the pigment solution with zinc amalgam in air, evacuation, addition to methyl methacrylate	+	+	+	+	−	−

Experimental conditions	Chlorophyll b.a.	Chlorophyll a.	Hematoporphyrin b.a.	Hematoporphyrin a.	Without pigment b.a.	Without pigment a.
Reduction in vacuum, reverse reaction in vacuum for 5 min, addition to methyl methacrylate	−	−	++	++	−	−
Reduction in vacuum, admission of air to the reduced form, evacuation, addition to methyl methacrylate	−	++	−	++	−	−

Note. +++ large flocculent precipitate of polymer in methyl alcohol. ++ homogeneous suspension of polymer in methyl alcohol, measured on a spectrophotometer. + traces of polymer in methyl alcohol. − complete absence of polymer in methyl alcohol.

···vacuum, polymerization took place. In another variant of the experiment, air was admitted to the solution of the reduced pigment, after which the pyridine solution of the regenerated pigment was quickly filtered into methyl methacrylate and again evacuated. After dark exposure, polymer was detected both with chlorophyll and with hematoporphyrin in the case when the pigments had been

reduced in the presence of acid. Evidently, the reaction of the hydrogen reduced form with oxygen leads to the formation of peroxide radicals that initiate polymerization. Shaking pyridine with zinc amalgam without pigment, in vacuum and in the presence of air (with acid and without acid), did not lead to the formation of products initiating polymerization of methyl methacrylate.

Thus, the results of the experiments show that, upon interaction of pigments with zinc amalgam in vacuum, in the dark, reduction products are formed that are similar in absorption spectrum to photoreduced pigments. In the presence of the reduction products, active polymerization of methyl methacrylate is observed. It is very probable that the pigments reduced by amalgam are ion-radicals, the formation of which may be expected upon electron transfer from zinc to the pigment molecule. The instability and high reactivity of pigments reduced by amalgam make it possible to suppose their chemical difference from the photoreduced forms of pigments, which do not initiate polymerization and are stable in vacuum and in air. The fact that reduced forms of pigments similar in their spectral properties may differ in their nature (be radicals or valence-saturated products) should be taken into account when analyzing the results of studies of chlorophyll photoreactions by methods of flash and differential spectroscopy.

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REFERENCES CITED

1. A. A. Krasnovskii, DAN, **60**, 421 (1948). Usp. khim., **29**, 736 (1960).

2. A. A. Krasnovskii, A. V. Umrikhina, DAN, **104**, 882 (1955); Biophysics, **3**, 547 (1958).

3. N. N. Bubnov, A. A. Krasnovskii, A. V. Umrikhina, V. F. Tsepalov, V. Ya. Shlyapintokh, Biophysics, **5**, 121 (1960).

4. L. M. Kosobutskaya, A. A. Krasnovskii, DAN, **74**, 103 (1950).

5. V. B. Evstigneev, V. A. Gavrilova, DAN, **108**, 507 (1956).

6. A. V. Umrikhina, Dissertation, Institute of Biochemistry named after A. N. Bach, Academy of Sciences of the USSR, 1960.

7. Yu. V. Karyakin, *Pure Chemical Reagents*, L., 1947, p. 582.

8. A. A. Krasnovskii, K. K. Voinovskaya, DAN, **96**, 1209 (1954).

9. A. A. Krasnovskii, A. V. Umrikhina, DAN, **122**, 1061 (1958).

10. V. B. Evstigneev, V. A. Gavrilova, DAN, **118**, 1146 (1957).

11. A. N. Terenin, Trans. Farad. Soc., Gen. Discussion, 1959.

12. H. Linschitz, S. I. Weissmann, Arch. Biochem. and Biophys., **67**, 491 (1957).

Note: Figure translations are in progress. See original paper for figures.