

PHOTOCHEMICAL GENERATION OF PORPHYRIN. ESR AND ABSORPTION SPECTROSCOPIC STUDIES

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Irradiation by a high-pressure mercury arc of a mixture containing pyrrole, benzaldehyde, and Fe(III) in the medium of glacial acetic acid results in the formation of Fe(III)-*meso*-tetraphenylporphyrin and some amount of free *meso*-tetraphenylporphyrin. The products were identified by means of ESR and absorption spectra. When irradiated for long periods of time, the products undergo photochemical degradation. Since the photochemical incorporation of Fe(III) into the free *meso*-tetraphenylporphyrin ligand yields a complex with a structure different from that in the previous case, it may be concluded that Fe(III) acts as a template in laboratory synthesis from the above starting substances.

Porphyrins and metalloporphyrins are, in general, photochemically active compounds. Recently, their photochemical redox reactions and photosensitizing properties have been at the centre of interest¹. Less attention has been devoted to the influence of light on porphyrin synthesis, although the fact that porphyrins can be formed by an abiotic route² suggests that such studies would be useful.

Meso-tetraphenylporphyrin (TPP) is readily obtained by the reaction of pyrrole with benzaldehyde³. It has been observed that the course of the reaction is affected by sunlight⁴. The principle of photochemical reaction in the synthesis of porphyrin has been used by Beck and Rábai⁵, who prepared sulfonated TPP by exposure of an aqueous solution of pyrrole and 3,5-disulfo-benzaldehyde to visible light. A study of the reaction mechanism has shown that the reaction is photosensitized by the product. Both the thermal synthesis⁶ of TPP and the above photochemical reaction are catalyzed^{5,6} by Fe(III), but the mechanism of its catalytic action is unknown. In both cases free porphyrin is produced, and no metalloporphyrin formation has been reported.

In the present work we studied the photochemical reaction of pyrrole with benzaldehyde in glacial acetic acid in the presence of ferric chloride, with the aim to establish whether metalloporphyrin is formed directly and to clarify the role of ferric ions in the photochemical synthesis. The product identification relied on the fact that Fe(III)-porphyrin complexes give a characteristic ESR spectrum, the so-called heme iron spectrum.

EXPERIMENTAL

Materials

The photochemical synthesis was carried out using pyrrole (pro synthesis, Merck, F.R.G.) and benzaldehyde (analytical grade, Lachema, Czechoslovakia) purified by vacuum distillation and an additional procedure⁷. The other chemicals were of analytical grade (Lachema, Czechoslovakia). TPP (Aldrich, Europe) and Fe(III)-*meso*-TPP.Cl (Strem, Ventron, U.S.A.) were used as standards for comparing the ESR and absorption spectra.

Instruments

ESR spectra were measured on an ERS-220 instrument (Academy of Sciences, Berlin, G.D.R.) working in the X-band with a magnetic modulation of 100 kHz. The magnetic field was measured with a Radiopan ¹H NMR magnetometer (Poland), and the microwave frequency was measured using a C3-54 frequency counter (U.S.S.R.). The calibration was made using α, α' -diphenyl- β -picrylhydrazyl (DPPH), $g = 2.0037 \pm 0.0002$, and Mn(II)/ZnS (Academy of Sciences of the G.D.R.), $g = 2.0024 \pm 0.003$, $A = -0.0064 \text{ cm}^{-1}$. Absorption spectra were taken on a UNICAM SP-800B (Great Britain) and SPECORD UV-VIS (Zeiss, Jena) spectrophotometers, using cells with thicknesses of 5 and 10 mm. Irradiation was obtained from an HBO-200 high-pressure mercury arc (Osram F.R.G.).

Procedure

Pyrrole and benzaldehyde were injected with a microsyringe into the starting fresh solution of FeCl₃ in glacial acetic acid. The initial concentrations of the components were [pyrrole] = 0.1 mol dm⁻³, [benzaldehyde] = 0.1 mol dm⁻³, and [FeCl₃] = (1 to 10) · 10⁻³ mol dm⁻³. In comparative experiments, the reaction mixture without the addition of FeCl₃ was irradiated. The solutions placed in quartz or glass cells of 5 or 10 mm thickness open to the air were irradiated by a high-pressure mercury arc. Samples of 5 to 50 μ l volume were taken from the cells with a microsyringe for ESR measurements. For spectrophotometry, the samples were diluted with chloroform by a factor of 80 to 200. In long-term experiments, irradiation was always interrupted after about 7 h, and the samples were kept overnight in the dark at +5°C. The times listed in Table II represent the overall periods of irradiation. Check experiments indicated that the interruption of irradiation and the storage under the given conditions had no effect on either the ESR or absorption spectra.

The products were identified by comparing their ESR and absorption spectra with those of the Fe(III)TPP and TPP standards dissolved in chloroform and in a chloroform-glacial acetic acid mixture (1 : 1, v/v). The formation of Fe(III)TPP can be detected from ESR spectra. The low concentrations of Fe(III)TPP and TPP produced were determined by measuring the solution absorbances at $\lambda = 416 \text{ nm}$ (Soret band) without discriminating between Fe(III)TPP and TPP. The resolution was achieved by measuring the solution absorbances at $\lambda = 685 \text{ nm}$ for Fe(III)TPP and at $\lambda = 548 \text{ nm}$ for TPP.

RESULTS

ESR Spectra

Solutions of pyrrole and benzaldehyde in glacial acetic acid at 77 K are ESR silent. An FeCl_3 solution ($1 \cdot 10^{-3} \text{ mol dm}^{-3}$) in glacial acetic acid at 77 K gives a signal (a singlet with $g = 4.34$) characteristic of Fe(III) in a tetrahedral environment. A mixture of FeCl_3 ($1 \cdot 10^{-3} \text{ mol dm}^{-3}$) in glacial acetic acid, pyrrole ($0.1 \text{ mol} \cdot \text{dm}^{-3}$) and benzaldehyde (0.1 mol dm^{-3}), when frozen 2 min after mixing, gives a weak signal of Fe(III) ($g = 4.34$) and another weak isotropic signal (a singlet with $g = 2.0031$) due to a free electron (radical).

Irradiation of the above pyrrole–benzaldehyde– FeCl_3 mixture results in changes in the ESR spectra (Fig. 1). First, the initial signal of Fe(III) with $g = 4.34$ (“free iron”) disappears, and a singlet with $g = 2.0331$ appears. An extreme amplification of the spectra revealed that this signal is the middle component (g_y) of the Krammer doublet due to the so-called low-spin heme Fe(III) complex, i.e. an Fe(III) complex with the porphyrin ligand. The other components of the Krammer doublet are $g_x \doteq 2.00$ and $g_z = 2.0570$. At the same time, there is an increase in the intensity of a narrow signal of the free radical (singlet, $g = 2.0031$, signal breadth $\Delta H_{pp} = 1.3 \text{ mT}$). Further irradiation removes the signal of low-spin heme Fe(III) and leads to the appearance of a new signal with $g_{\perp} = 6.01$, typical of high-spin heme⁸ complexes of Fe(III). The component of the heme Fe(III) signal with $g_{\parallel} \doteq 2.00$ is superimposed on the radical signal ($g_{\text{iso}} = 2.0031$). After continued irradiation, the signal of the high-spin heme Fe(III) is weakened in intensity and the Fe(III) signal

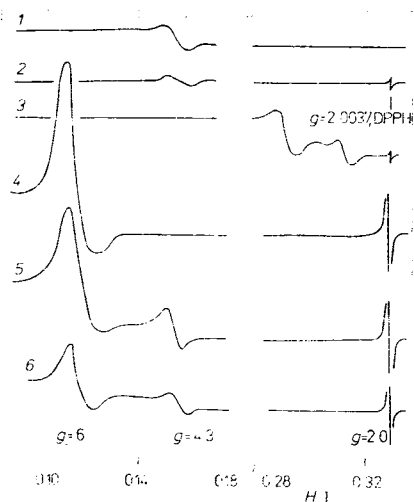


FIG. 1

ESR spectra of the reaction systems 1 FeCl_3 ($1.00 \cdot 10^{-3} \text{ mol dm}^{-3}$) in glacial acetic acid, 2 mixture of pyrrole (0.1 mol dm^{-3}), benzaldehyde (0.1 mol dm^{-3}) and FeCl_3 ($1.00 \cdot 10^{-3} \text{ mol dm}^{-3}$) in glacial acetic acid, unirradiated sample, 2 min after mixing; irradiation time (at room temperature): 3 2 h, 4 14 h, 5 22 h, 6 60 h. 77 K; microwave power, 20 mW; magnetic modulation, 100 kHz; modulation amplitude, 1.0 mT; chart speed, 74.6 mT min^{-1} ; response time, 0.1 s

with $g = 4.34$ reappears, indicating photochemical degradation of the heme complex.

The disappearance of the Fe(III) signal at the start of the reaction is due partly to the thermal and photochemical reduction of Fe(III) by benzaldehyde⁹ to Fe(II), and partly to the formation of low-spin Fe(III) complex, which passes into the high-spin form on further irradiation. Owing to the exposure of the reaction mixture to the air during irradiation, Fe(II) is reoxidized to Fe(III).

Table I compares the g -factors of ESR spectra of the standard, i.e. commercial metalloporphyrin Fe(III)TPP in chloroform and a 1 : 1 (v/v) chloroform-acetic acid mixture, with the g -factors of Fe(III) found in the spectrum of the reaction mixture. It is seen that the photochemical reaction gives rise to the high-spin heme Fe(III) complex. Chloroform was used because of a limited solubility of Fe(III)TPP in glacial acetic acid. The small differences in the g -factors are due to solvation effects. Since benzaldehyde was the starting component, there is no doubt that the metalloporphyrin formed was Fe(III)-*meso*-tetraphenylporphyrin. The same complex is also produced by the thermal ("dark") reaction, but at a substantially lower rate. The ESR spectrum is observable on standing for 60 h in the dark.

The concentrations of the products and intermediates of the photochemical reaction were obtained by using the standards DPPH ($g = 2.0037$, $N = 2.66 \cdot 10^{14}$ spins) and Mn(II)/ZnS ($g = 2.0024$, $N = 2.20 \cdot 10^{14}$ spins for a sextet line). The results are summarized in Table II. At the optimum after 14 h irradiation, 60.3% of iron is incorporated in the metalloporphyrin complex. Side reactions give rise to solid polymeric products^{10,11}, whose amount increases with increasing iron concentration. At $[\text{Fe(III)}] = 1.00 \cdot 10^{-2} \text{ mol dm}^{-3}$, the whole volume of the sample solidifies on 1 h irradiation. When the concentrations of pyrrole and benzaldehyde were lowered to a level corresponding to Fe : pyrrole ratios of 1 : 10 to 1 : 1, no metalloporphyrin formation was observed. Optimum conditions favouring the formation of metalloporphyrin occurred at a Fe(III) : benzaldehyde : pyrrole ratio of 1 : 100 : 100.

Absorption Spectra

Unlike the ESR spectra, absorption spectra allow both the possible reaction products, metalloporphyrin and free porphyrin, to be identified. A disadvantage is a background spectrum that arises from absorption by the polymeric products. Absorption spectra were therefore used to find out whether the photochemical reaction in the absence of ferric salts gives rise to free porphyrin and whether free porphyrin is produced along with metalloporphyrin in the presence of ferric salts. The wavelengths of the absorption bands and the molar absorption coefficients for solutions of the Fe(III)TPP and TPP standards in chloroform are listed in Table III. The intense Soret band at 416 nm, common to metalloporphyrin and free porphyrin, indicates the presence

of the product at a concentration as low as 10^{-7} mol dm $^{-3}$. The reaction components pyrrole and benzaldehyde do not show absorption at wavelengths above 400 nm.

On mixing pyrrole and benzaldehyde in the absence of iron two absorption bands, at 442 nm and 474 nm, appear which are due to as yet unidentified intermediates,

TABLE I

Comparison of ESR spectra of the Fe(III)-*meso*-tetraphenylporphyrin standard and the photochemically generated complex. 77 K; microwave power, 20 mW; magnetic modulation, 100 kHz

| Spectral parameters | Fe(III)TPP.Cl standard | | Photochemically generated complex glacial CH ₃ COOH |
|---------------------|------------------------|---|---|
| | CHCl ₃ | CHCl ₃ : CH ₃ COOH 1 : 1 (v/v) | |
| | High-spin | | |
| g_{\perp} | 6.113 | 6.160 | 6.01 |
| g_{\parallel} | 2.00 | 2.00 | (2.00) |
| | Low-spin | | |
| g_x | 1.9806 | 2.0037 | 2.00 |
| g_y | 2.1310 | 2.1494 | 2.0331 |
| g_z | 2.4185 | 2.4191 | 2.0570 |

TABLE II

Dependences of the concentration ($\cdot 10^4$ mol dm $^{-3}$) of Fe(III)TPP and some intermediates on time. Microwave power, 20 mW; magnetic modulation, 100 kHz; [FeCl₃]₀ = $1.00 \cdot 10^{-3}$ mol . dm $^{-3}$; [pyrrole]₀ = [benzaldehyde]₀ = 0.1 mol dm $^{-3}$

| Irradiation time, h | [Fe(III)] tetrahedral $g = 4.34$ | [FeTPP] HS ^a $g_{\perp} = 6.01$ | [FeTPP] LS ^b $g = 2.0331$ |
|---------------------|--|--|--|
| 2 | 0 | 0 | 0.59 |
| 3.5 | 0 | 0 | 0.01 |
| 10 | 0 | 0 | 0.08 |
| 14 | 0 | 6.3 | 0 |
| 22 | 1.20 | 4.10 | 0 |
| 60 | 0.71 | 2.03 | 0 |

^a High-spin; ^b low-spin.

probably pyrrole-benzaldehyde condensates. About 60 min irradiation gives rise to a Soret band, which under these conditions denotes unambiguously the presence of the TPP free ligand. Figure 2 shows the spectrum of the irradiated reaction mixture and a reference spectrum for the dark reaction. The height of the Soret band at 416 nm after 3 h irradiation corresponds to a TPP free ligand concentration of $6.30 \cdot 10^{-6} \text{ mol dm}^{-3}$. For the thermal reaction, a weak Soret band was observed after 22 h.

Figure 3 shows a plot of the absorbance at 416 nm (Soret band) as a function of time for the reaction in the presence of iron. Here, curve 1 relates to the reaction mixture irradiated by a mercury arc, curve 2 to the mixture exposed to scattered daylight, and curve 3 to the mixture standing in the dark. Once again, as in experiments followed by means of ESR spectra, the product is formed much more rapidly

TABLE III

Absorption spectra of Fe(III)TPP.Cl and TPP standards in chloroform

| Absorption band | Fe(III)TPP.Cl | | TPP | |
|-----------------|------------------------------|---|------------------------------|---|
| | λ_{max} nm | $\epsilon \cdot 10^{-3}$ $\text{dm}^3 \text{ mol}^{-1} \cdot \text{cm}^{-1}$ | λ_{max} nm | $\epsilon \cdot 10^{-3}$ $\text{dm}^3 \text{ mol}^{-1} \cdot \text{cm}^{-1}$ |
| Soret band | 416 | 122.314 | 416 | 577.731 |
| IV | 509 | 15.896 | 514 | 23.775 |
| III | 575 | 4.585 | 548 | 12.575 |
| II | 654 | 3.265 | 587 | 7.310 |
| I | 685 | 3.461 | 644 | 5.466 |

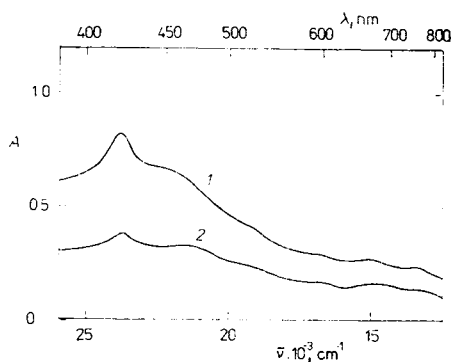


FIG. 2

Absorption spectra of *meso*-tetraphenylporphyrin generated in the absence of Fe. 1 photochemical reaction (13 h irradiation), 2 thermal reaction. Initial concentrations: pyrrole 0.1 mol dm^{-3} , benzaldehyde $0.1 \text{ mol} \cdot \text{dm}^{-3}$ in glacial acetic acid

by the photochemical reaction, but subsequently undergoes photochemical degradation. The absorption at 416 nm may contain a contribution from both the Fe(III)TPP metalloporphyrin and the TPP free porphyrin. Evidence for the formation of Fe(III)TPP has been provided by ESR spectra. The presence of free TPP is indicated by a band at 548 nm, which does not overlap with any other band due to Fe(III)TPP. Owing to a low intensity of this band and because of the background spectrum, it was only possible to obtain approximate values of the concentrations and the ratio of free porphyrin to metalloporphyrin; after 14 h irradiation, the photochemical reaction yields 20 to 30% of TPP and 80 to 70% of Fe(III)TPP. The concentrations of both components are of the order of 10^{-4} mol dm $^{-3}$.

Photochemical Incorporation of Fe(III) into the TPP Free Ligand

The results obtained indicate that the photochemical reaction in the presence of Fe(III) gives rise to free porphyrin in addition to metalloporphyrin. Under the given conditions, the latter may be formed by two routes:

a) a catalytic mechanism, in which ferric ions catalyze the formation of free porphyrin, and metalloporphyrin is produced by photochemically accelerated incorporation of the metal into the ligand;

b) a template mechanism, in which metalloporphyrin is formed by a direct reaction of ferric ions with the other reaction components, whose sterically favourable arrangement is governed by the coordination properties of the central ion.

In order to be able to decide between the two mechanisms, we studied separately the incorporation of Fe(III) into the free ligand under the same conditions as in the previous experiments. After 2 h irradiation, a 1 : 1 mixture of FeCl $_3$ and commercial *meso*-tetraphenylporphyrin gave the ESR spectrum displayed in Fig. 4. The spectrum contains two signals arising from the low-spin Fe(III) with $g_1 = 2.0621$, $\Delta H_{pp(1)} = 9.50$ mT, and $g_2 = 1.9838$, $\Delta H_{pp(2)} = 6.00$ mT. Superimposed on each signal are nine lines of a hyperfine structure with the splitting constant $a_N = 1.58$ mT. The

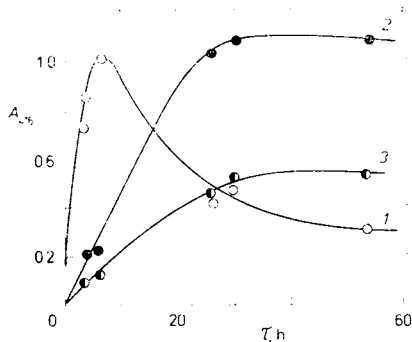


FIG. 3

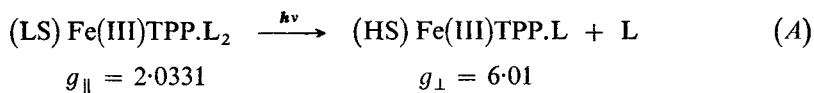
A plot of the absorbance at 416 nm (Fe(III)-TPP + TPP) against the irradiation time. 1 high-pressure mercury arc, 2 scattered daylight, 3 dark reaction. Initial concentrations: FeCl $_3$ $1.00 \cdot 10^{-3}$ mol dm $^{-3}$, pyrrole 0.1 mol dm $^{-3}$, benzaldehyde 0.1 mol dm $^{-3}$

ratios of signal intensities are 1 : 4 : 10 : 16 : 19 : 16 : 10 : 4 : 1. Accordingly, the hyperfine structure stems from an interaction of an unpaired electron on the low-spin Fe(III) with the four pyrrole nitrogens of the ligand ($^{14}\text{N}, I = 1$), thus from the formation of a metal ion complex with the ligand. After about 60 h irradiation, a weak signal with $g_{\perp} = 6.01$ due to the high-spin Fe(III)TPP appears. The spectrum with the hyperfine structure has been obtained for the photochemical reaction of FeCl_3 with the TPP free ligand, but not on a control left for the same time in the dark. Apparently, it arises from an intermediate of the incorporation, an Fe(III)-TPP complex of a structure different from that of the observed high-spin Fe(III)TPP, probably one with the central ion out of the ligand plane¹².

Since no such intermediate has been observed in the formation of metalloporphyrin from the starting components, it follows that the metalloporphyrin is produced by direct reaction with the template mechanism. The template effect of the central ion may operate during the first step of the condensation reaction of pyrrole and benzaldehyde, or in a further step of cyclization of dipyrromethane, a compound which has been considered as a possible reaction intermediate⁵.

DISCUSSION

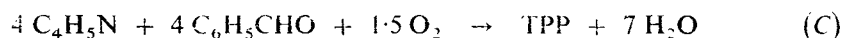
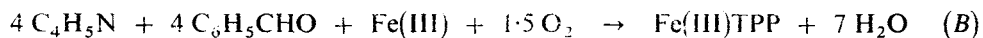
Since Fe(III) complexes with porphyrins give typical ESR spectra of the so-called heme iron, it was possible to demonstrate that the photochemical reaction of Fe(III) with pyrrole and benzaldehyde in glacial acetic acid yields Fe(III)-metalloporphyrin. The rate of the thermal reaction is negligible compared to that of the photochemical reaction. (The latter can be utilized for the synthesis of porphyrins in a stirred-tank or flow photoreactor with control over the product concentration to eliminate the subsequent photochemical decomposition.) The ESR spectra do not, however, provide any information about the free ligand and ferrous compounds, for these are ESR silent. At the start of the reaction, before irradiation, it was possible to observe a partial thermal reduction of Fe(III) by benzaldehyde, which on exposure to radiation was accompanied by photoreduction⁹. The reduction was attended by reoxidation of Fe(II) with oxygen from the air. The ESR spectrum indicates first the formation of a low-spin Fe(III) heme complex ($g = 2.0331$) with the coordination number 6, which during irradiation is converted into the final product, the high-spin Fe(III)-TPP heme complex. It is well-known that high-spin heme iron complexes are penta-coordinated¹³ and that radiation induces substitution reactions, especially aquation, of metal complexes. It is therefore reasonable to conclude that in our case the high-spin Fe(III)TPP is formed by photoinitiated dissociation of one of the axial ligands and reduction of the coordination number.



The decrease in the intensity of the Fe(III)TPP signal after long periods of irradiation may be caused partially by photoreduction of the central ion, and partially by photochemical degradation of the complex. Evidence for the photochemical degradation is provided by the appearance of a signal of "free", i.e. uncomplexed, Fe(III) with $g = 4.34$. Partial photoreduction of the central ion is indicated by the overall balance for Fe(III) according to Table II.

The photochemical generation of TPP free ligand in the absence of iron has been indicated by absorption spectra. After long periods of irradiation the product undergoes photochemical degradation. The TPP free ligand is also formed along with Fe(III)TPP in the presence of iron.

The formation of the products can be summarized as



Reactions involving attachment of the central ion to the porphyrin ligand, the so-called metal ion incorporations or porphyrin metallations, are generally very slow^{1,12}, and no data are available for the effect of radiation on their course. The ESR spectrum shown in Fig. 4 indicates that the ferric ion interacts with the four pyrrol nitrogens of the ligand, so that a complex is formed, but its structure differs from that of a heme complex. By analogy with products of thermal incorporation, the complex is probably of the sitting-atop type, with the central ion attached out of the ligand plane. Our experiments have shown that the formation of the complex by photochemical reaction occurs much more rapidly than the thermal generation and that the subsequent reorganization of the complex to the heme type is very slow. The experiments have also shown that incorporation of the central ion is not involved in the photochemical generation of metalloporphyrin and that, consequently, ferric ions exert a template effect in the formation of metalloporphyrin.

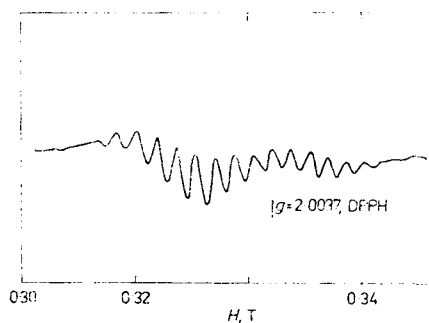


FIG. 4

ESR spectrum of the complex formed by photochemical reaction of Fe(III) with the *meso*-tetraphenylporphyrin free ligand. Irradiation time (at room temperature), 130 min. $[\text{FeCl}_3] = 2.50 \cdot 10^{-3} \text{ mol dm}^{-3}$, $[\textit{meso}$ -tetraphenylporphyrin] = $2.50 \cdot 10^{-3} \text{ mol} \cdot \text{dm}^{-3}$, 77 K; microwave power, 10 mW; magnetic modulation, 100 kHz

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