

*PREBIOTIC PORPHYRIN GENESIS: PORPHYRINS FROM
ELECTRIC DISCHARGE IN METHANE, AMMONIA,
AND WATER VAPOR*

BY G. W. HODGSON* AND CYRIL PONNAMPERUMA

EXOBIOLGY DIVISION, NASA, AMES RESEARCH CENTER, MOFFETT FIELD, CALIFORNIA

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Considerable evidence is emerging to show the possibility of the prebiotic genesis of biological organic compounds from the primitive atmosphere. Notable among these compounds, following the synthesis of amino acids by Miller,¹ are dipeptides, polypeptides, proteinoids, adenine, guanine, pentose sugars, deoxyadenosine, adenosine 5'-triphosphate (ATP), nucleotides, and fatty acids, as recently summarized by Fox,² and Ponnampereuma and Gabel.³

A major class of compounds missing from the foregoing group is that of the porphyrins, although these were sought in the classical Miller-Urey experiments.¹ Compounds with absorption features consistent with those for metal complexes of porphyrins were reported by Miller but identification as porphyrins was rejected on the basis of lack of fluorescence.

Porphyrins are synthesized in the laboratory principally through condensation reactions involving pyrrole compounds. Early methods for the preparation of porphine (unsubstituted porphyrin) and tetraphenylporphine employ the condensation of pyrrole with formaldehyde and benzaldehyde, respectively.⁴ This synthesis, particularly the step involving benzaldehyde, has been further developed in a number of interesting ways. For example, Calvin, Ball, and Aro-noff⁵ used zinc salts to promote the condensation reaction. Similarly, Szutka *et al.*⁶ used zinc acetate, and Szutka (1963)⁷ carried out the reaction with gamma radiation. Later, the reaction of pyrrole with benzaldehyde was found to be little affected by visible and ultraviolet light, but promoted to some degree by dispersing the reactants in water.⁸ A similar approach was used recently for the reaction between pyrrole and formaldehyde. The latter is more likely to occur in primitive environments. In these experiments simulating geochemically plausible conditions, porphyrins have been synthesized in dilute aqueous systems,⁹ confirming similar experiments carried out by Krashnovskii and Umrikhina.¹⁰

In a condensation reaction, δ -aminolevulinic acid (ALA) has been used as starting material. This reaction is analogous to the biogenic pathway recently reviewed by Lascelles.¹¹ Thus ALA is condensed under alkaline conditions anaerobically to porphobilinogen, a porphyrin precursor.¹² ALA is also converted in a small yield directly to porphyrin pigments using ultraviolet irradiation.¹³

The data for the ready reaction of pyrrole and formaldehyde under mild geochemical conditions increase the probability of porphyrin genesis in primitive-atmosphere conditions, since formaldehyde is known to be formed,^{1, 14, 15} and preliminary evidence in our laboratory indicates that pyrrole may be formed under such conditions.

The objective of the present investigation was, therefore, to answer the question whether porphyrins could be generated from methane, ammonia, and water by the action of electric discharges. Several series of exploratory discharge experiments were carried out, and microanalyses for porphyrins showed that porphyrins are indeed formed under such conditions.

Experimental.—The basic experimental procedure was to operate an electric discharge in an atmosphere of methane, ammonia, and water vapor, and to analyze for trace porphyrin pigments in the reaction products in the manner commonly used for trace biogenic porphyrins in geochemical work.¹⁶ The reactor used for many of the experiments was the same kind as that regularly used in prebiotic synthesis work in this laboratory: a 10-liter twin-flask apparatus with four tungsten electrodes in a plane, approximately 3–5 mm apart, in the upper flask, and a small volume of water in the lower, heated flask. The discharge was developed by four Tesla coils (about 12 kv). The atmosphere was an equal mixture of methane (25 cm) and ammonia (25 cm); during the experiments the total pressure in the apparatus did not exceed 1 atmosphere. After about two hours of discharge light-colored products appeared, and black polymeric material began to accumulate in the lower flask as the reaction proceeded.

The reaction products in each experiment were recovered from the reactor by decantation and subsequent extraction of the reactor with water, methanol, and/or other organic solvents such as benzene and chloroform. Dilute hydrochloric acid was also commonly used to ensure extraction of porphyrin-free bases. The general procedure was to transfer the discharge products as completely as possible to chloroform or benzene for resolution by silica gel column chromatography using increasingly polar solvent mixtures. Extracts and fractionation products were examined for absorption features in the range of 385–420 $m\mu$, which would include the Soret absorption bands of porphyrin compounds. Such features were evident in most of the experiments as very weak signals above background. Most of the synthesis experiments produced pigments which subsequent examination indicated to be porphyrins. The analytical problems, however, were severe because (a) the porphyrin yields were extremely small and (b) the yields of other colored substances, largely dark-colored polymeric material, were abundant. Two general attempts were made to overcome the difficulties: one in which reaction conditions were varied in an attempt to favor the production of porphyrin material in relation to other colored substances; the other in which the total product yield was increased by operating the discharge reactors under flow conditions with continuous addition of reactants. This was done by (a) modifying the regular reactor to permit addition of reactants during the course of the experiment, and (b) replacing the regular reactor with a small continuous-flow unit in which the electrodes were mounted in a glass flow tube about 20 cm long and 30 mm in diameter, the products collecting on the inside walls of the reactor.

Eighteen discharge experiments were carried out. While none of the variations in experimental approach resulted in marked improvements, yields and separations appeared adequate for testing the identification of the pigments as porphyrins.

Identification.—Minor absorption features in the 385–420-m μ range do not prove the generation of porphyrins in the electric discharge experiments. The compounds responsible for such absorption must satisfy a number of criteria to establish their identification as porphyrins. These involve detailed absorption spectra, solvent partition, chromatographic behavior, metal complexing, and fluorescence spectra. Selected fractions of the experimental products were accordingly examined in such detail.

(a) *Absorption spectra:* Chromatographic fractions from the discharge experiments contained compounds with spectral features outside the range for porphyrin Soret bands, and some of these were probably aromatics and others were nitrogen heterocyclic compounds. Other similar compounds were evident in the Soret range and were set aside in the present study. A discharge experiment in which pyrrole was included produced porphyrins with strong Soret absorption at 409 m μ and four less intense non-Soret peaks at 505, 541, 587, and 655 m μ . These pigments are probably closely related to the porphyrins produced directly from pyrrole and formaldehyde under mild, simulated geochemically plausible conditions.⁹

The corresponding spectrum of the product of a regular discharge experiment (i.e., methane, ammonia, and water vapor) after separation and purification showed a set of peaks at 400, 505, 532, 572, and 630 m μ , similar to those of the pyrrole-augmented discharge experiment, although somewhat distorted and obscured by dense background absorption. The yield of these pigments in the general discharge reactions was less than a microgram, based on estimated band intensities above background using extinction coefficients for porphyrin Soret bands. This represented a yield of about 0.00001 per cent (carbon basis), corresponding to yields of 0.002–0.00001 per cent for the pyrrole-formaldehyde experiments.⁹

(b) *Solvent partition:* Porphyrin pigments in free-base form are extractable from organic solvents with dilute aqueous acid solution due to the two protonated nitrogen atoms. Decrease of pH returns the pigments to the organic layer. The porphyrins in the pyrrole-augmented experiment, for example, behaved in this manner. The apparent porphyrins of the other experiments were readily extracted from chloroform with 6 *N* hydrochloric acid and returned by buffering with sodium acetate.

Acid extractability is a direct method of resolving porphyrin mixtures, and this was used in several experiments with extracting solutions ranging in pH from that of distilled water to 6 *N* hydrochloric acid. The bulk of the indicated porphyrin compounds could be extracted with HCl ranging from 0.01 to 3 *N* in concentration.

Porphyrins which are highly carboxylated have enhanced water solubility and are extracted with water or alkaline solutions. Such solubility was evident for a portion of the porphyrinlike pigments.

(c) *Chromatography:* Porphyrin compounds encountered in geochemical analyses are commonly separated through column chromatography on silica gel or alumina. The elution order with increasing polarity of solvents is polynuclear aromatics, nickel porphyrins, vanadyl porphyrins, free bases, and carboxylated

porphyrins.¹⁶ In the electric discharge products, the polycyclic aromatics were eluted from silica gel (Baker-analyzed reagent, unactivated) by hexane and hexane-chloroform mixtures. Traces of some 408-m μ pigments were eluted by chloroform and chloroform-methanol mixtures. These may have been metal porphyrin mixtures with the metal coming from erosion of the electrode and glass surfaces. They were followed by the bulk of the porphyrinlike pigments in chloroform-methanol (99/1 or 98/2 v/v). The corresponding position with more polar alumina (Alco F1, deactivated with methanol) was chloroform-methanol (95/5 v/v) for the free porphyrinlike pigments.

(d) *Gel permeation*: Gel permeation of porphyrins is useful in establishing molecular sizes. Much of the gel permeation work has involved polystyrene gels (e.g., Waters, 40 Å) with the results reported as elution volume (% of bed volume) or elution ratio (fraction of bed volume). Synthetic porphyrins are readily eluted with benzene, or benzene-methanol (90/10 v/v) to suppress adsorption, e.g., etioporphyrin I at 0.67.¹⁷ Major petroleum and related porphyrin metal complexes show similar elution with only slightly smaller ratios of 0.61–0.63,¹⁷ indicating somewhat larger molecular sizes. Many of the electric-discharge porphyrinlike pigments have similar values, i.e., in the range of 0.64–0.70, as does one of the classes of porphyrin pigments from the pyrrole-formaldehyde experiments, at 0.63.

Minor amounts of electric-discharge pigments in the present study appear to be significantly larger in molecular size with elution values approaching 0.4, and it is interesting to relate this to similar observations for terrestrial geochemical porphyrins which are attributed to molecular complexing of porphyrins with large but undefined moieties of several thousand or more molecular weight.^{9, 18, 19}

At the same time, other pigments from the current experiments exhibited rather larger elution ratios, e.g., 0.80, indicating smaller molecular sizes very similar to those exhibited by one group of porphyrins from the pyrrole-formaldehyde reactions, perhaps closely related to unsubstituted porphine.

(e) *Metal complexing*: Porphyrin-free bases readily form metal complexes with cations through reaction in glacial acetic acid. In this manner, the free porphyrin of the pyrrole-augmented experiment formed copper complexes, and a parallel reaction with nickel acetate for a regular discharge product showed the apparent formation of a nickel complex as indicated by its acid insolubility and loss of fluorescence excitation. The greater yield of porphyrinlike pigments made it possible to demonstrate clearly the difference between porphyrin complexes with paramagnetic ions and those with diamagnetic ions. Contact with cupric acetate in glacial acetic acid at 118°C for one hour gave a copper complex accompanied by marked suppression of what was evidently Soret excitation, as noted below, while similar contact with diamagnetic zinc ion produced no change.

The product of the electric discharge experiments carried out by Miller¹ contained pigments regarded by Hodgson and Baker⁹ as likely to have been porphyrin metal complexes. Although the bulk of the evidence for porphyrins in the present study indicates porphyrin-free bases, there is some minor evidence

for metal complexes in some of the experiments. For example, the "Soret" concentrate from several experiments in the gel permeation fraction 0.52 showed weak but real absorption at almost 402 $m\mu$ while exhibiting no fluorescence excitation response for free porphyrin bases.

(f) *Fluorescence spectra*: Excitation of free porphyrin bases produces fluorescence emission in the red, commonly in the 550–700- $m\mu$ range.^{20–22} Primitive-atmosphere discharge products with absorption features at about 400 $m\mu$ show emission at three principal points in the indicated range, viz. about 635, 660, and 690.

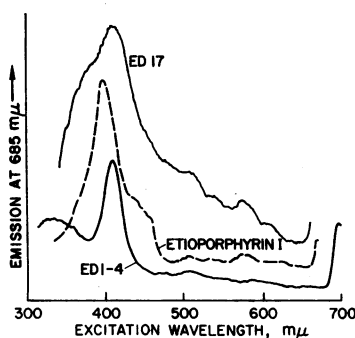


FIG. 1.—Fluorescence excitation spectra of discharge porphyrin pigments and etioporphyrin I, showing Soret and non-Soret emissions corresponding to characteristic porphyrin absorption bands.

Excitation spectra for the indicated porphyrin compounds yield strong spectral confirmation for the identification of the fluorescing compounds as porphyrins. Figure 1 shows excitation fluorescence spectra for products of the continuous flow reactor (ED 17) and the regular reactor (ED 1–4), along with that of synthetic etioporphyrin I for comparison. In all curves the Soret and non-Soret bands are clearly those of porphyrin compounds. In addition, the relationship between the intensity of the absorption bands and that of the corresponding excitation bands was found to be in accordance with that for authentic etioporphyrin I.

A further test of the identification of the discharge pigments as porphyrins was offered by the spectra of the pigments in acid solution.

On transfer to acid solution, free-base porphyrins show a change in intensity of the non-Soret bands from roughly equal intensity to a strong preference for a single band midway through the non-Soret region. This was evident in the discharge pigments, in which a 550 band became clearly dominant.

It is not important at this time to dwell on the other variation in the spectra which are probably largely due to variation in porphyrin structure, but rather to note that the fluorescence analyses strongly support the other analytical data and confirm the generation of porphyrin compounds in methane–ammonia–water discharge systems.

Experimental and Analytical Controls.—Extreme care was taken during both the discharge synthesis and the analytical procedures to avoid introducing trace porphyrin contaminants which might have arisen through the use of glassware and equipment involved in related studies on porphyrins and chlorophylls. Some of the comparative work with known porphyrins (e.g., for the gel permeation data) was done in a different laboratory using identical equipment; the remainder (e.g., for the etioporphyrin I data) was done in the present laboratory after the completion of the analyses of the discharge products. In addition, all solvents were examined for porphyrin residues and found to be free of such

pigments; similarly, blank analytical procedures showed no evidence of the introduction of porphyrin pigments in the course of the analyses.

Discussion.—The present study shows that microgram quantities of free-base porphyrins are generated under electric-discharge conditions in methane–ammonia–water systems. The mechanism of the reactions is not clear, although it is reasonable to infer that they involve both formaldehyde and pyrrole. Formaldehyde is known to be present and active in the synthesis of porphyrins. Pyrrole markedly augments the porphyrin yield and is known to be active in the formation of porphine. It is possible that pyrrole is formed through condensation of formaldehyde to sugars which give rise to mucic acid with which ammonia condenses to give the pyrrole ring.²³ Hydrogen cyanide is also abundant in the discharge system and is likely to be involved in the formation of pyrrole. The role of water in the mechanism is not clear. While Soret-absorbing materials appeared to form in the anhydrous experiments, their stability was low, particularly in aqueous media. Mechanism studies are presently underway.

The significance of the formation of porphyrins in the prebiotic context is fairly obvious since porphyrins, as pointed out by Pullman and Pullman,²⁴ are vital conjugated systems for many biological systems and may have been incorporated into such evolving systems because of their prebiotic availability. Two further points warrant notice at this time: one, that the porphyrins generated in the discharge systems exhibit a fairly ready solubility in water, evidently due to carboxylic side chains resulting from cyanide hydrolysis, and are accompanied by an abundance of surfactant products as well; and the other, that the porphyrins with major spectral absorption in the 400-m μ range apparently represent the first compounds with biological significance in chemical evolution to absorb at wavelengths in the visible spectrum, thereby making available significantly greater quantities of solar energy for further chemical and biological evolution. Thus, there appear to be significant new opportunities for homogeneous catalysis and energy transfer emerging from the evolution of porphyrin compounds.

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* Senior postdoctoral research associate of the National Academy of Sciences, Ames Research Center, National Aeronautics and Space Administration, Moffett Field, California; permanent position: head of petroleum research, Research Council of Alberta, Edmonton, Alberta, Canada.

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