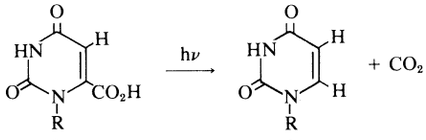
Chemical Evolution from Hydrogen Cyanide: Photochemical Decarboxylation of Orotic Acid and Orotate Derivatives

Abstract. *Irradiation of solutions at* p*H 7 to* p*H 8.5 of orotic acid, orotidine, and orotidine 5′-phosphate with light at 254 nanometers yields the corresponding uracil derivative via the singlet excited state. This reaction completes a plausible prebiotic synthesis of uracil and its derivatives starting from HCN as the only carbon source.*

Hydrogen cyanide was a likely starting material for the synthesis of biomolecules on the primitive earth (*1*). It condenses in dilute, mildly alkaline solutions to yield HCN oligomers, which in turn undergo hydrolytic decomposition at *p*H 8 to 9 to give an assortment of purines, pyrimidines, and amino acids (*1, 2*). The synthesis of the three major classes of nitrogen-containing molecules from HCN is an especially convincing model for their primitive earth syntheses because these three different types of biomolecules would have all been formed from the same starting material under identical reaction conditions (*2*).

Orotic acid, one of the pyrimidines formed by hydrolysis of the HCN oligomers, is a precursor, as the nucleotide derivative, to the RNA building blocks uridine 5′-phosphate and cytidine 5′-phosphate in contemporary biological systems (*3*). We now report the novel photochemical decarboxylation of orotic acid and its derivatives under plausible primitive earth conditions. Our results are not only applicable to the study of chemical evolution but also constitute a novel contribution to studies on the photochemical transformation of nucleic acid derivatives (*4*).



1 **2**

A R = H
B R = 1′-ribosyl
C R = 1′-ribosyl 5′-phosphate

Irradiation at 254 nm of orotic acid (**1A**), orotidine (**1B**), and orotidine 5′-phosphate (**1C**) results in the efficient formation of the corresponding uracil derivatives (*2*) (Table 1). The quantum yields for the decarboxylation of $6.4 \times 10^{-4}M$ solutions of orotic acid and orotidine are 1.6×10^{-4} and 1.7×10^{-2}, respectively (*5*). The more efficient decarboxylation of orotidine is a consequence of the less efficient photodimerization pathway (*6*). The greater yield of uracil from more dilute solutions of orotic acid (Table 1) is also consistent with the conclusion that bimolecular photoreactions limit the yield of the uracil derivatives.

The photochemical decarboxylation of carboxylic acids is a rare reaction and to our knowledge there have only been two other reports of the photodecarboxylation of a carboxyl group directly attached to an aromatic ring. One is the gas phase decarboxylation of benzoic acid which proceeds at temperatures higher than 110°C from a vibrationally excited ground state (*7*). The second is the photodecarboxylation of nicotinic acid derivatives which, in the case of nicotinic acid, gives only trace amounts of the decarboxylation product (*8*).

The decarboxylation of orotic acid proceeds from the singlet excited state as shown by the absence of uracil (**2A**) formation when benzophenone or acetone are used as sensitizers and by the observation that the uracil yield was not decreased by the addition of Fe(II), a paramagnetic cation which facilitates the decay of the triplet excited state (*9*). The decarboxylation reaction differs from the dimerization of orotic acid which proceeds from the triplet excited state (*4, 10*).

The decarboxylation of orotic acid promoted by Fe(III) and Cu(II) was also observed (*11*). A 7 to 8 percent yield of uracil was obtained when $6.4 \times 10^{-4}M$ orotic acid was irradiated for 6 hours in the presence of equimolar quantities of either of these metal ions. A 1 percent uracil yield was obtained in the absence of these metal ions. Prolonged irradiation (48 hours) in the presence of Fe(III) resulted in a 14 percent yield of uracil. Irradiation of an equimolar mixture of orotic acid and isopropanol ($6.4 \times 10^{-4}M$) together with $1.3 \times 10^{-3}M$ Fe(III) for 24 hours gave a 20 percent yield of uracil. These results are consistent with the oxidative decarboxylation reaction pathway proposed previously for the photoconversion of 2-pyridine carboxylic acid to pyridine and 2-hydroxypyridine which is promoted by metal ions (*11*).

The photochemical formation of uracil from orotic acid is a plausible extension of our pathway for the synthesis of pyrimidines from HCN (*2*). This extension requires only the use of ultraviolet light, one of the most abundant sources of energy on the primitive earth (*12*). In addition, the efficiency of such a photolysis reaction is not diminished by very low concentrations of starting materials. In this particular case, the efficiency increases as the concentration of orotic acid decreases because the rate of competitive biomolecular photoprocesses is diminished. This is an advantage for a prebiotic synthesis since this probably occurred at low concentrations of biomolecules on the primitive earth. A further advantage of this prebiotic synthesis is that there is not just one specific route from orotic acid to uracil, but rather an ensemble of orotic acid derivatives (**1A, B, C**) could have been converted to the corresponding uracil derivative (**2A, B, C**). This allows for the synthesis of the corresponding pyrimidine nucleotide both before and after decarboxylation and increases the likelihood that nucleotides would have formed by this route on the primitive earth. The photodecarboxylation of orotic acid promoted by metal ions is yet another prebiotic pathway to uracil and its derivatives. The Fe(III) or Cu(II) (or both) salts required for such a synthesis were likely to have been prevalent on the earth's surface (*12, 13*). In addition, these cations form 1:1 complexes with orotic acid, and therefore may have been bound to it on the primitive earth so that efficient decarboxylation could also have occurred by this pathway.

Table 1. The photochemical decarboxylation of orotic acid and its derivatives.*

Compound	Concentration (*M*)	Irradiation time (hr)	Product	Yield (%)
Orotic acid (**1A**)	6.4×10^{-4}	48	Uracil (**2A**)	4.9
Orotic acid (**1A**)	6.5×10^{-5}	6	Uracil (**2A**)	12.7
Orotidine (**1B**)	6.4×10^{-4}	3	Uridine (**2B**)	45
Orotidine 5′-phosphate (**1C**)	6.4×10^{-4}	4	Uridine 5′-phosphate (**2C**)	23

*Photolyses were performed in aqueous solutions adjusted to *p*H 8.5 with NaOH. The *p*H after irradiation was 7 to 8. Rayonet photochemical reactor equipped with 254-nm lamps was used for the irradiations. The products were purified by paper chromatography (Whatman 3MM paper; 1-propanol and concentrated NH_4OH, 3:1; 1-butanol, acetic acid, water, 12:3:5; ethyl acetate, formic acid, water, 7:2:1) or, in the case of nucleotides, by paper electrophoresis (Whatman 3MM paper, acetate buffer, *p*H 4.5). The structures and yields were established by the ultraviolet spectra of the eluted sample measured in acidic, basic, and neutral media. Uridine was also identified by the gas chromatographic retention index of its trimethylsilyl derivative. Uridine and uridine 5′-phosphate were further identified by acid hydrolysis to uracil.

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The close similarity between the proposed prebiotic pathway for the synthesis of uracil derivatives from orotic acid and the contemporary biotic pathway for the conversion of orotidine 5′-phosphate to uridine 5′-phosphate (*3*) provides further support to our proposed prebiotic synthesis. There would have been no major discontinuities in the chemical reactions involved in the prebiotic processes and the biotic processes that developed later. The transition from prebiology to biology would have been a smooth one; the only requirement would have been the evolution of enzymes to facilitate the prebiological chemical processes (*14, 15*). Such enzymes would have been essential if the orotate derivative were assimilated by a biological system living in a crevice shielded from ultraviolet radiation or when sufficient oxygen developed in the primitive atmosphere so that ozone was formed and effectively "turned off" the solar ultraviolet light required for photodecarboxylation.

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Aggregation Effects on Oxygen Binding of Sickle Cell Hemoglobin

Abstract. *Deoxygenation of concentrated solutions (0.33 gram per milliliter) of sickle cell hemoglobin show (i) a "crisis point" where the oxygen binding curve is unusually steep (Hill coefficient of 5 to 6), and (ii) a simultaneous increase in light scattering. Nearly identical oxygen binding curves are obtained upon oxygenation and deoxygenation of these solutions. The influence of aggregation is to shift the curve toward higher pressures.*

Nearly 30 years ago it was recognized (*1*) that sickling of erythrocytes from patients with sickle cell anemia is due to gelation of deoxygenated sickle cell hemoglobin (HbS). The same tendency toward aggregation is manifested in the much lower solubility of deoxygenated HbS compared to oxygenated HbS (*2*). Oxygen binding curves are sensitive to such aggregation and crystallization effects, as has been shown in general by Wyman and co-workers (*3, 4*) and considered in detail for HbS by Minton (*5, 6*). The study of such curves provides the basic information for understanding the oxygen-linked aggregation process and the physiological consequences. Solutions of HbS at nongelling concentrations show binding curves similar to normal hemoglobin (*7*). However, experimental difficulties, such as slow diffusion, arise in the study of gelling solutions. These difficulties have been circumvented by use of natural erythrocytes, but the presence of 2,3-diphosphoglycerate and the distribution of intracellular HbS concentrations complicates interpretation for these materials (*8*). Even cells with controlled concentrations of HbS have given scattered P_{50} values (*9, 10*).

Here we report "reversible" binding measurements on highly concentrated HbS solutions where a "crisis point" is detected upon deoxygenation and extraordinarily high cooperative oxygen binding is observed. Conditions were chosen (*p*H 7.1, 0.15*M* phosphate buffer, 25°C, 0.15 to 0.33 g of HbS per milliliter) to coincide with studies (*11, 12*) on critical gelation and kinetics of the aggregation process. We used a purified sample of HbS obtained from a homozygous source (*13*). The sample contained at least 98 percent HbS, the remainder being fetal hemoglobin (HbF). No more than 2 percent methemoglobin could be detected spectrally at the beginning of an experiment and no more than 5 percent formed in the course of the run.

Oxygen saturation was determined by

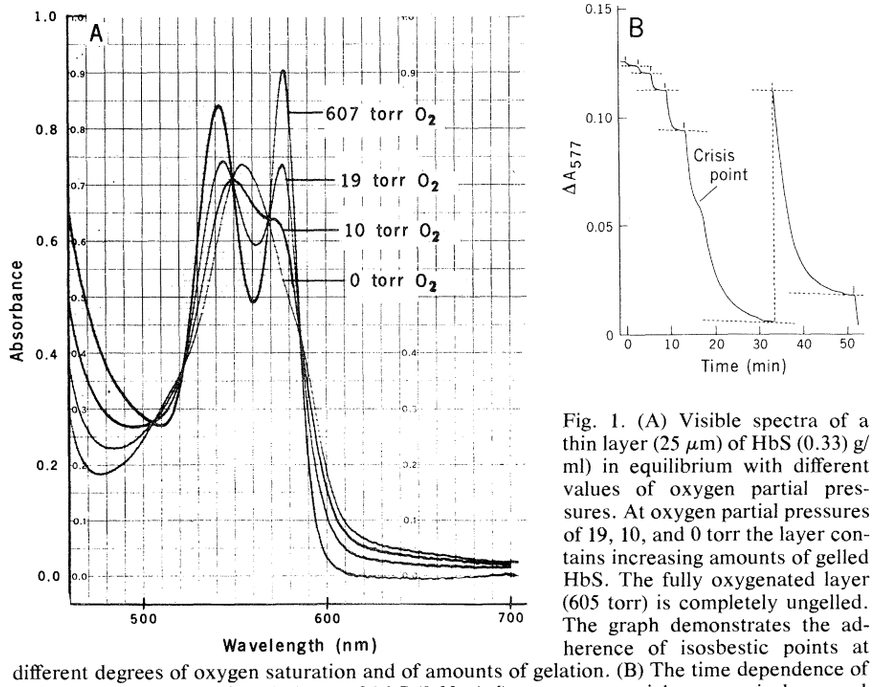

Fig. 1. (A) Visible spectra of a thin layer (25 μm) of HbS (0.33) g/ml) in equilibrium with different values of oxygen partial pressures. At oxygen partial pressures of 19, 10, and 0 torr the layer contains increasing amounts of gelled HbS. The fully oxygenated layer (605 torr) is completely ungelled. The graph demonstrates the adherence of isosbestic points at different degrees of oxygen saturation and of amounts of gelation. (B) The time dependence of absorbance at 577 nm of a thin layer of HbS (0.33 g/ml) as oxygen partial pressure is decreased in precise steps. The total absorbance at full oxygenation was 0.8. Maximum instrument sensitivity (0.1 absorbance, full scale) was used. The inflection point in the fifth step is defined as the crisis point. This point corresponds to a fraction of oxygen saturation, $\theta = 0.78$.

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