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Studies on Reactions of Imidazole with Two Iron(III) Porphyrin Dimers in Aqueous Solution

ALBERT N. THOMPSON and M. KRISHNAMURTHY

Department of Chemistry, Howard University, Washington, D.C. 20059, U.S.A.

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Kinetics of the reactions of imidazole with μ-oxo-bis(tetrakis(p-sulfophenyl)porphyrinatoiron(III)), [$(FeTPPS)_2O$], and μ-oxo-bis(tetrakis(p-trimethylammoniumphenyl)porphyrinatoiron(III)), [$(FeTAPP)_2O$], were investigated at 30 °C and 0.1 F ($NaNO_3$) ionic strength. The compound $(FeTPPS)_2O$ reacts at least forty times faster than $(FeTAPP)_2O$. From the pH dependence of this reaction, the reacting ligand species is inferred to be imidazolium ions. Rate laws and possible mechanisms of these reactions are discussed. Spectrophotometric titration was employed to determine the equilibrium constants of these reactions.

Introduction

The imidazole ring as a histidine moiety functions as a ligand toward transition metal ions in a variety of biologically important molecules such as hemoproteins [1]. Though there have been numerous investigations on the interaction of imidazole with iron porphyrins in nonaqueous solutions few studies have been reported in aqueous solutions [2–8]. The iron(III) porphyrins are known to exist as dimers in aqueous solution above a pH of six [9]. Recently Ostfeld and Colfax [10] investigated the break up of these iron porphyrin dimers into monomeric bis-imidazole complexes by imidazole and imidazolium ion in nonaqueous solutions. This report summarises our results on a similar reaction of imidazole with two water soluble porphyrins: sodium μ-oxo-bis-(tetrakis(*p*-sulfonatophenyl)porphyrinatoiron(III)), [$(FeTPPS)_2O$], and μ-oxo-bis(tetrakis(*p*-trimethylammoniumphenyl)porphyrinatoiron(III)) perchlorate, [$(FeTAPP)_2O$]*.

Experimental

Reagents

Imidazole was obtained from Sigma Chemical Co. Sodium nitrate, tris(hydroxymethyl)aminomethane (THAM) and other common chemicals such as sodium hydroxide were obtained from Fisher Scientific Co. The sodium salt of $(FeTPPS)_2O$ was synthesised by a literature method [11] and purified by Soxhlet extraction with methanol.

Synthesis of $(FeTAPP)_2O$

In a 250 ml beaker 1.0 g of H_2TAPP acetate synthesised by a literature method [12] was dissolved in 50 ml of water and heated to boil. Soon after the addition of the stoichiometric amount of ferrous sulfate to the boiling porphyrin solution, a color change is observed. A drop of this solution is diluted in a spectrophotometer cell and a drop of concentrated HCl is added to it. The visible spectrum of this solution is scanned from 700 nm to 500 nm. If the metalloporphyrin formation is incomplete as indicated by the presence of the diacid form of porphyrin in solution, additional ferrous sulfate is added and the heating continued. When the formation of the iron(III) porphyrin was complete as indicated by the spectral test, the solution was cooled, its pH was raised to about eight and the solution was filtered using a fine filter paper to remove the hydrated iron oxides. The clear filtrate was heated, and to the hot solution saturated sodium perchlorate solution was added. The crystalline product was filtered on a sintered glass funnel. *CAUTION: Though we did not encounter any explosions, perchlorate salts are known to be highly explosive; one should take adequate care in handling these compounds such as not to scrape the funnel to remove the sample etc. Anal.* Calcd for $C_{112}H_{120}N_{16}Cl_8O_{33}Fe_2 \cdot 4H_2O$: C 50.08%, H 4.77%, N 8.35%. Found: C 49.51%, H 4.57%, N 8.16%. The number of water molecules per mol of compound was chosen to fit the analyses. Elemental

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*The charges and counter ions of these porphyrin species are omitted for clarity in abbreviations. Neutral imidazole is abbreviated as Im and the imidazolium ion as HIm^+; to indicate the total concentration of any species subscript T is used.

analyses were performed by Schwarzkopf Microanalytical Lab. of New York.

Instrumentation

A Beckman model CIII Acta uv/visible spectrophotometer with thermostatted cell compartment was used for titration studies and for kinetic studies of reactions with half-lives greater than ten seconds. A Durrum stopped-flow spectrophotometer was used to study the kinetics of reactions with half-lives less than ten seconds. Transmittance *versus* time curves from the stopped-flow runs were displayed on an oscilloscope and photographed with a camera attachment. All pH measurements were made with a Radiometer model PHM64 pH meter.

Techniques

The spectrophotometric titrations and kinetics of imidazole substitution reactions of $(FeTPPS)_2O$ and $(FeTAPP)_2O$ were investigated at 30.0 ± 0.2 °C and an ionic strength of 0.1 F($NaNO_3$ and NaOH). Wherever the pH's of these solutions were maintained constant, 0.1 F THAM buffer was employed for that purpose.

The spectrophotometric titrations were carried out by scanning the spectra from 450 to 380 nm with each addition of the ligand. The pH was maintained constant at 9.1 with THAM buffer. As the reactions of $(FeTPPS)_2O$ were very fast the spectra were recorded soon after (about 5 min) each addition of the ligand to 500 ml of the porphyrin solution. These additions did not alter the total volume significantly since the ligand is solid. For $(FeTAPP)_2O$, as the reactions were slower, aliquots of porphyrin solution with varying ligand concentrations were made and stored in the constant temperature bath overnight. The next day the spectra were recorded. Since the spectrum of the final product was the same as that of the bis(imidazole)iron(III) porphyrin obtained at low pH's, the following stoichiometry was assumed for the titration reaction:

$$2H^+ + 2n(Im) + (FeTAPP)_2O \rightleftharpoons 2FeTAPP(Im)_n + H_2O$$

For such an equilibrium process, the following relationship can be easily derived:

$$\log\left[\frac{(A_0 - A_X)^2}{(A_X - A_\infty)}\right] = \log\left[\frac{K(A_0 - A_\infty)}{2C_T}\right] 2n \log(Im)$$

where n is the number of molecules of ligand reacting with monomeric iron(III) porphyrin, K is the equilibrium constant, C_T is the total concentration of the iron porphyrin dimer, A_0 is the absorbance of the dimer before the addition of the ligand, A_X is the absorbance of the solution when specific amounts of the ligand are coordinated and A_∞ is the absorbance of the coordinated metalloporphyrin at the end of the reaction. The equilibrium constants of these reactions were obtained from a plot of log $[(A_0 - A_X)^2/(A_X - A_\infty)]$ *versus* log(Im) yielding a linear plot with a slope of 2n and an intercept of log $[K(A_0 - A_\infty)/2C_T]$.

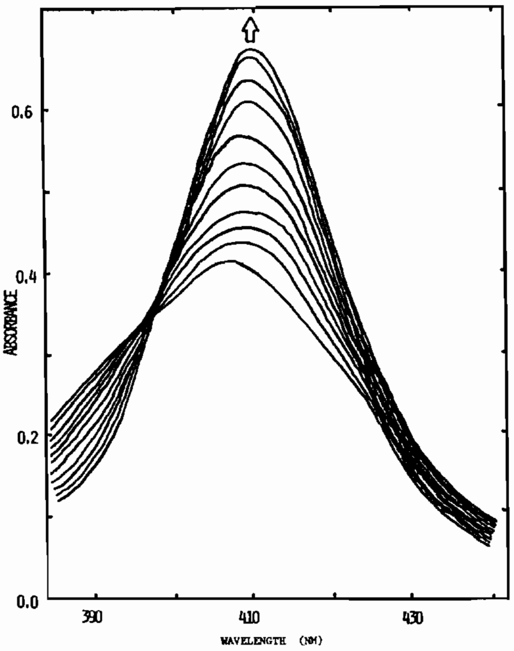

Fig. 1. Changes in the spectrum of $(FeTAPP)_2O$ with the addition of different amounts of imidazole at pH 9.1 (30 °C and 0.1 F $NaNO_3$).

The kinetics of the reactions of the dimers with imidazole were monitored at 415 nm. The rate constants, k_{obs}, were obtained from the slope of $\ln(A_t - A_\infty)$ *versus* time plots, where A_t and A_∞ are absorbances of the solution at time t and at the end of the reaction. These plots were linear over at least three half-lives. Duplicate runs agreed within 5% error.

Results

Spectrophotometric titration studies of $(FeTAPP)_2O$ and $(FeTPPS)_2O$ reacting with imidazole were carried out to determine the stoichiometry and the equilibrium constant for the reaction. In both titration studies the pH of the solution was maintained constant at pH 9.1 utilising 0.01F THAM as the buffer and the ionic strength was maintained constant at 0.1 F with $NaNO_3$. A typical set of spectra obtained during the titration of $(FeTAPP)_2O$ as a function of total imidazole concentration is presented in Figure 1. A typical plot of the changes

TABLE I. Kinetic Data for the Reaction of Imidazole with $(FeTPPS)_2O$ and $(FeTAPP)_2O$ at Constant pH.[a]

$(FeTPPS)_2O$		$(FeTAPP)_2O$	
10^2 $[Im]_T$, M	10^2 k_{obs} sec^{-1}	10^2 $[Im]_T$, M	10^3 k_{obs} sec^{-1}
2.2	12.5	6.0	4.39
4.5	28.6	12.0	6.96
9.0	63.7	14.0	7.89
12.0	93.5	16.0	9.09
15.0	104.3	18.0	9.79
31.5	198.0	20.0	11.20
46.5	293.8	22.0	11.80
61.5	306.0	24.0	14.10
76.5	321.0	42.0	21.00
90.0	335.0	62.0	32.60
		82.0	47.00
		102.0	57.50

[a] pH = 9.1; μ = 0.1 M $NaNO_3$; temperature = 30 °C; wavelength = 415 nm.

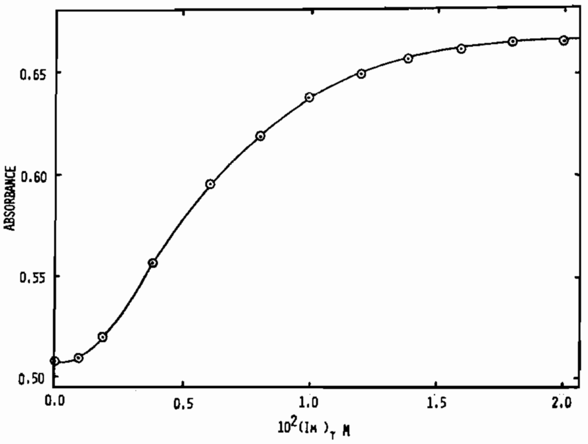

Fig. 2. Changes in the absorbance of $(FeTAPP)_2O$ *versus* $(Im)_T$ at 415 nm at pH 9.1.

in the absorbances at 415 nm *versus* (Im) is presented for $(FeTPPS)_2O$ in Figure 2.

A plot of log $[(A_o - A_X)^2/(A_X - A_\infty)]$ *versus* log(Im) at 415 nm for the reaction of $(FeTPPS)_2O$ with imidazole yielded a straight line. The slope, 2n, and intercept $\log[K(A_o - A_\infty)/2C_T]$ were both calculated using a linear least squares computer program. The equilibrium constant, log K, obtained by proper substitution of the values for the initial concentration of the dimer and its initial absorbance and final absorbance of the solution into the equation for intercept, is 21.0 ± 0.01*. From the slope the value of n, the number of molecules of imidazole coordinating per molecule of metalloporphyrin, was found to be 1.94 ± 0.05 implying that the product is the bis(imidazole)iron porphyrin complex. An analysis of titration curves obtained in completely aqueous solution yielded similar results as those obtained in a 50% methanol–water solution with respect to the slopes and intercept**.

Analysis similar to the one described above for $(FeTPPS)_2O$ was utilised to obtain the equilibrium constant K and n, the number of molecules of imidazole reacting with $(FeTAPP)_2O$. The equilibrium constant, log K, was found to be 15.56 ± 0.07. The value of n obtained was 1.76 ± 0.07, again indicating the product to be a bisimidazole complex.

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*During the write up of this report, Fleischer's article [8] appeared in the literature. They have also determined the equilibrium constant for the reaction of imidazole with $(FeTPPS)_2O$, though their description of the equilibrium is slightly different from ours. If one multiplies our equilibrium constant, K, by $(K_w)^2$, one should arrive at an equilibrium constant for the reaction as defined by Fleischer and Fine. Though these workers determined the equilibrium constant at 25 °C, no mention of ionic strength could be found in the article. Nevertheless our value of K computed as above, 1.0×10^{-7}, determined at 30 °C and 0.1 F $NaNO_3$, agrees reasonably well with 2.8×10^{-7} reported in [8].

**Though these iron(III) porphyrin dimers are treated as μ-oxo dimers in solution by earlier workers, we have evidence to believe that the dimers exist in two forms in solution depending upon various factors such as their concentration, ionic strength, presence of detergents or non-aqueous solvents *etc.* R. F. Pasternack, P. A. Malek and J. D. Albert report (*A.C.S. 176th National Meeting Abstract,* paper 13 in Inorganic Chemistry) a fast first reaction in this $(FeTPPS)_2$-O–Im system. This fast reaction is most probably the interconversion of these dimers, which can be effected by the addition of an organic species such as methanol, pyridine, imidazole *etc.* Such interconversion in the reverse direction is effected by the addition of salts such as NaCl, $NaNO_3$, NaCN *etc.* Most probably the fast first reaction we reported earlier [14] is also this interconversion. Details of this dimer system will be published shortly in this Journal. We have taken adequate care in this investigation to have essentially one form of the dimer.

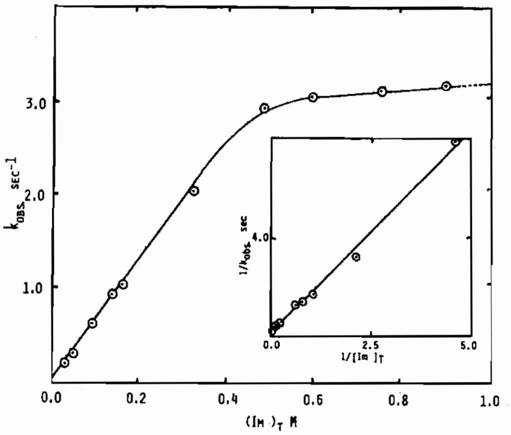


Fig. 3. Plot of pseudo-first-order rate constants, k_{obs}, *versus* $(Im)_T$ for the reaction of $(FeTPPS)_2O$ with imidazole at pH 9.1 (insert: Plot of $1/k_{obs}$ *versus* $1/(Im)_T$ at pH 9.1).

Kinetic studies of substitution reactions of $(FeTPPS)_2O$ with imidazole were investigated at a constant pH of 9.1 and ionic strength of 0.1 F $NaNO_3$ in a buffered solution, over a total imidazole concentration range of 0.22 F to 0.9 F. In this concentration range the formation of the bis(imidazole)-hemin complex is complete as indicated by the titration studies. The pseudo first-order rate constants, k_{obs}, levelled off to a constant value at higher ligand concentrations as shown in Fig. 3. The values of observed rate constants ranged from 0.125 sec^{-1} to 3.35 sec^{-1} and are presented in Table I. A plot of $1/k_{obs}$ *versus* 1/(Im) gives a linear relationship shown in Fig. 3. The values of pseudo first-order rate constants, k_{obs}, for the substitution reaction of $(FeTAPP)_2O$ obtained at a constant pH (9.1) and ionic strength (0.1 F $NaNO_3$) over a total imidazole concentration range of 0.061 F to 1.02 F are also presented in Table I. A plot of k_{obs} *versus* [imidazole] yields a straight line passing through the origin. On the average $(FeTPPS)_2O$ reacts about 45 times faster than $(FeTAPP)_2O$. Both these reactions obey the rate law:

$$\text{Rate} = k_{obs}(P) = \{k(Im)/[1 + k'(Im)]\}(P) \qquad (1)$$

Therefore:

$$k_{obs} = [k(Im)/[1 + k'(Im)] \qquad (2)$$

and on rearranging

$$1/k_{obs} = 1/k\,(Im) + k'/k \qquad (3)$$

If $k'(Im) <<<< 1$ in the case of the reaction of $(FeTAPP)_2O$ at pH 9.1, equation 2 resolves to $k_{obs} = k(Im)$.

Kinetic studies of the reaction of imidazole with $(FeTPPS)_2O$ and $(FeTAPP)_2O$ were also investigated as a function of varying pH values, keeping the total imidazole concentration constant at 0.615 F. These reactions were investigated in the pH range of 7.18 to 10.9 and 7.16 to 9.64 in the case of $(FeTPPS)_2O$ and $(FeTAPP)_2O$ respectively. Kinetic data for these reactions are presented in Table II. Thes reactions are accelerated at high imidazolium ion concentrations. At low imidazolium ion concentrations, the pseudo first-order rate constants, k_{obs}, levelled off to a constant value as shown in Figures 4 and 5. A plot of $1/(HIm^+)$ *versus* $1/k_{obs}$ shown in the insert of Figs. 4 and 5 were found to be linear. A linear least squares computer fit was utilised to obtain the slope $1/k$ and intercept k'/k. Both dimers in this study were found to obey the rate law presented earlier.

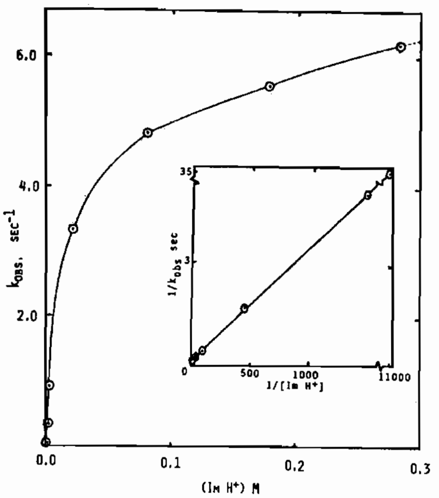


Fig. 4. Plot of k_{obs} *versus* HIm^+) for the reaction of $(FeTPPS)_2O$ with imidazole at various pH's; (insert: Plot of $1/k_{obs}$ *versus* $1/(HIm^+)$).

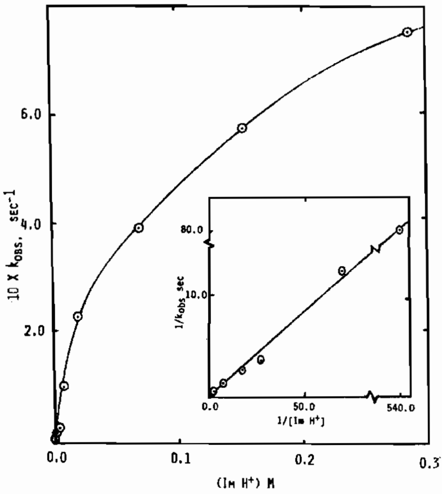


Fig. 5. Plot of k_{obs} *versus* (HIm^+) for the reaction of $(FeTAPP)_2O$ with imidazole at various pH's (insert: Plot of $1/k_{obs}$ *versus* $1/(HIm^+)$).

TABLE II. Kinetic Data for the Reaction of Imidazole with $(FeTPPS)_2O$ and $(FeTAPP)_2O$ at various pH's.[a]

$(FeTPPS)_2O$			$(FeTAPP)_2O$		
pH	10^4 [ImH$^+$], *M*	10^2 k_{obs} sec^{-1}	pH	10^4 [ImH$^+$], *M*	10^3 k_{obs} sec^{-1}
7.18	2860	614	7.16	2930	749
7.50	1800	558	7.62	1480	561
8.01	702	498	8.04	660	362
8.54	225	330	8.45	275	220
9.52	24.0	66.0	8.73	147	84.6
10.02	7.70	24.3	9.64	18.5	13.1
10.96	0.90	2.80	10.08	6.70	5.00
			10.85	1.10	1.20

[a] $[Im]_T = 0.615$ *M*; $\mu = 0.1$ *M* $NaNO_3$; temperature = 30 °C; wavelength = 415 nm.

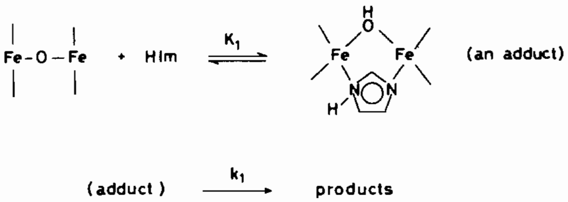
Discussion

Sadasivan *et al.* [13] investigated the ligand exchange and dimerization reactions of deuteroporphyrin-IX dimethylester iron(III) derivatives (deuterohemins). From interrelationships among conditions giving rise to monomeric and dimeric hemins, they proposed mechanisms by which ligand exchange occurs. Ligand exchange through doubly bridged intermediates is predicted to precede the collapse of the double bridged dimer. The ready conversion of the oxobridged dimer into monomeric hemins in the presence of aqueous salt solutions favors the formation of the doubly bridged dimeric intermediate. Hambrigt *et al.* [14] investigated the kinetics of cyanide addition to $(FeTPPS)_2O$. In a fast first step a cyanide ion binds the dimer forming a cyanoiron dimer. This step was also found to be pH-dependent. The cyano iron dimer dissociates into dicyano iron monomers by two possible pathways; one first order in (H^+) and the other proportional to $(H^+)(CN^-)^2$. In both the formation of cyano iron dimer as well as in the formation of dicyano iron monomer, the proposed mechanism involves HCN as the reacting species. But Stong and Hartzell [7] did not find any dissociation of the μ-oxo-iron dimer in their investigation of the reaction between imidazole and μ-oxo-bis(tetrakis(*p*-carboxyphenyl)porphyrinato iron(III). Instead these workers found simultaneous addition of two imidazole units to each dimer. In recent studies Ostfeld and Colfax [10] found that in dichloromethane–nitromethane solution μ-oxo-bis(tetraphenylporphyrin)iron(III) was cleaved by imidazole. Proposed mechanism involves a rapid preequilibrium in which the μ-oxo-dimer reacts to form an adduct with midazole. The splitting of the dimer is then proposed to occur by one of two kinetically indistinguishable routes. One where the hematin itself can be cleaved forming the products and the other where the imidazole adduct is cleaved leading to products. In either of these routes two simultaneously rate determining steps have been proposed: one independent and the other dependent on imidazolium ion concentration.

In this investigation, in the case of both peripherally negatively and positively charged porphyrins, the rates of imidazole substitution reactions are faster at low pH's and slower at high pH values. Considering the equilibrium:

$$(HIm^+) \rightleftharpoons Im + H^+ \quad pK_d = 7.12\ [15]$$

it is reasonable to conclude that imidazolium ion is the reactive species. If the imidazolium ion attacks the μ-oxo bridge of the dimer and in the process gets deprotonated, it can contribute to the formation of the bridged preequilibrium adduct proposed herein. This adduct can then possibly split into two monomers in the rate determining step. Such adducts have been proposed as possible intermediates earlier [13]. Electrostatic repulsion and structural considerations assign a high energy and consequently rule out from consideration any possible metal–imidazolium intermediate. The mechanism proposed for this reaction is as follows:



Then rate = $\{k_1K_1\,(HIm^+)/[1 + K_1(HIm^+)]\}(dimer)_T$

This rate law resembles the empirical rate law (equation 1) obtained earlier. This mechanism is similar to the one proposed by Ostfeld and Colfax [10] except for two basic differences: (1) the imidazolium ion rather than the neutral imidazole is the reactive species forming the preequilibrium adduct and (2) no path involving a second imidazole species is noted in the dissociation of the adduct.

The values of k_1 and K_1 computed for the reaction of $(FeTPPS)_2O$ are 7 ± 2 sec^{-1} and 47 ± 9 M^{-1} respectively. The value of corresponding k_1 and K_1 computed for the reaction of $(FeTAPP)_2O$ is 1.3 ± 0.1 sec^{-1} and 5.6 ± 0.5 M^{-1} respectively. Since the formation of the adducts results in an increase in the net positive charge, the reactions studied herein are more favored in the case of negatively charged porphyrin than for the positively charged porphyrin. The same trend is indicated by the equilibrium constants observed for $(FeTPPS)_2O$.

In conclusion, this study indicates that in the reaction of formation of bis(imidazole) complexes of hemin from hematin dimers (1) imidazolium ion is the reacting species independent of the peripheral charge of the porphyrin and (2) the rate determining step is the cleavage of the adducts formed between hematin dimers and imidazolium ions. It is most likely that the protonated ligand is the reactive species in cleaving the oxo dimers in general.

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