Sheer[1] on 1,2-butadiene. The indirect mechanism, on the other hand, fits their results quite well. In their work three of the major four carbon products were *trans*- and *cis*-2-butene and 2-butyne. It may be noted from eq 5 and 6 that for internal addition no 2-butyne is formed, which is contrary to their results. Hence it is possible that both internal and external addition take place, but it cannot be exclusively internal addition. Klein and Scheer claim that nonterminal addition does not occur.

An additional mechanism has been suggested[24] which would account for the formation of 2-butene and 2-butyne. After the initial addition reaction indicated in eq 1 the following addition and abstraction reactions take place.

$$H + H_3C\dot{C}{=}CHCH_3 \longrightarrow H_3CCH{=}CHCH_3 \quad (8)$$

and

$$H + H_3C\dot{C}{=}CHCH_3 \longrightarrow H_2 + H_3CC{\equiv}CCH_3 \quad (9)$$

The esr spectrum would be generated by reaction 7. Such a mechanism would require the immediate reaction of the $H_3C\dot{C}{=}CHCH_3$ radical with another hydrogen atom since this radical is not observed by esr. It would also mean that the reaction is not very specific as the hydrogen atom both adds and abstracts from both the parent molecule and the radical.

(24) The authors are indebted to Dr. Milton D. Scheer for this suggestion.

In the case of propyne an exactly analogous series of reactions can be postulated. The hydrogen addition takes place to form $CH_3\dot{C}{=}CH_2$. This radical abstracts from the parent compound, propyne, to yield the allenyl radical and either propylene or propyne and hydrogen. The same results are found from terminal or internal addition. Similar analyses can be applied to 1-butyne and 1,4-cyclohexadiene. However, for these three compounds we do not have any data on the resultant products when the compound is warmed to room temperature. Consequently, the esr spectra could be explained by the direct abstraction of H atoms from the initial compound or by the indirect method.

Conclusions

In the case of the addition reactions involving ethylene, 1,3-butadiene, and benzene, a simple addition of hydrogen atoms to a double bond appears to be sufficient to explain the results.

In the case of propyne, 1-butyne, 1,2-butadiene, and 1,4-cyclohexadiene the direct abstraction mechanism is enough to explain the esr spectra. However, for 1,2-butadiene the direct mechanism does not explain the results of Klein and Scheer, unless further abstraction and addition reactions take place between the radical and the hydrogen atom. The indirect mechanism does account for both phenomena. If either of these mechanisms is the correct one it indicates that the radical formed by direct addition, $CH_3\dot{C}{=}CHCH_3$, is very reactive and has a short lifetime at 77°K.

Fast Reaction Kinetics of Porphyrin Dimerization in Aqueous Solution[1]

Radha R. Das, Robert F. Pasternack, and Robert A. Plane

Contribution from the Department of Chemistry, Cornell University, Ithaca, New York 14850. *Received August 22, 1969*

Abstract: Temperature jump relaxation measurements were made on aqueous solutions and ethanol-water mixtures of the water-soluble porphyrin prepared by addition of four molecules of ethylenediamine to protoporphyrin IX. Measured relaxation times are of the order of magnitude of milliseconds and decrease slightly with pH in the range 3–7. Below pH 3, two different relaxation processes with different dependence on porphyrin concentration become apparent. From analysis of the kinetic data, confirmed by spectrophotometric and fluorometric studies, the phenomenon giving rise to the relaxation is found to be dimerization. At pH 6.0, 28°, in H_2O, the rate constant of dimerization is $7.8 \times 10^7\ M^{-1}\ sec^{-1}$. The reverse rate constant is 110 sec^{-1}. At lower pH, where the predominant porphyrin species contains three protons at its center, dimerization also occurs but at somewhat slower rate ($3.5 \times 10^7\ M^{-1}\ sec^{-1}$). In this case the rate increases with concentration (but not identity) of added salt, as for reactions between similarly charged ions. At still lower pH, where the predominant porphyrin species contains four protons at its center, simple dimerization apparently does not occur. Data are also given for dimerization equilibrium constants and rate constants for ethanol–water mixtures. All experiments were performed at several temperatures so that activation energies could be determined. These are found to be relatively small in all cases, indicating little energy barrier to dimerization. The fact that rates are considerably slower than diffusion controlled must indicate an unfavorable activation entropy resulting from necessary changes in water structure.

In earlier work,[2-4] we have studied the insertion of metals into porphyrins. Such studies indicate that the insertion reaction is not simple. In order to gain further insight into the steps of the reaction, temperature jump relaxation measurements were made on porphyrin

(1) Supported by a grant from the National Institutes of Health, U. S. Public Health Service.

(2) S. L. Baum and R. A. Plane, *J. Amer. Chem. Soc.*, 88, 910 (1966).

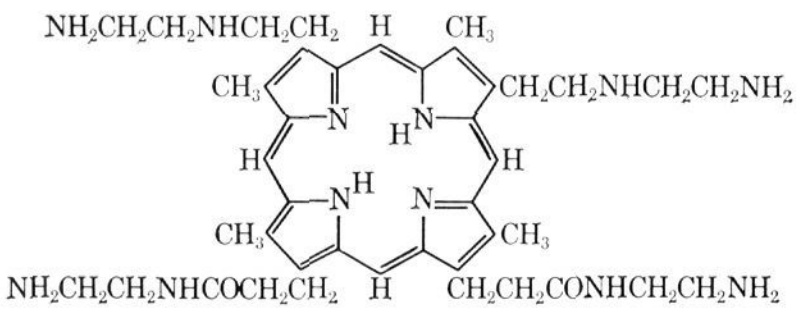
(3) R. Snellgrove and R. A. Plane, *ibid.*, 90, 3185 (1968).

(4) T. P. Stein and R. A. Plane, *ibid.*, 91, 607 (1969).

solutions in the absence of metal ions. Since Joule heating is the technique used for causing the temperature jump, a porphyrin soluble in polar solvents is necessary. The present study was performed using the water-soluble porphyrin formed by addition of four ethylenediamine molecules to protoporphyrin IX.[4] Preliminary experiments using deuteroporphyrin dimethyl ester disulfonate and tetraphenylporphine sulfonate indicate that the phenomena reported here are not limited to the ethylenediamine derivative porphyrin. In order to interpret and confirm present findings, spectrophotometric and fluorometric measurements were made on the porphyrin solutions.

Experimental Section

The water-soluble porphyrin was prepared by the method of Stein and Plane,[4] but the separation procedure was modified in that the porphyrin was eluted from a Dowex 50W-X2 column with 6 *M* HCl. Visible absorption spectra, nmr spectra, and chemical analysis of the product (containing 8 HCl molecules per porphyrin) were all consistent with the structure shown.



The temperature jump apparatus used to measure the rates of dimerization has been described in considerable detail elsewhere.[5] All the chemicals used were reagent grade. The solutions were made up in conductivity water, and the ionic strength of the solutions were adjusted in most experiments to 0.1 by addition of KNO_3. The solutions were brought to the desired pH by the dropwise addition of NaOH or HNO_3. Preliminary experiments showed considerable change in the optical density of the porphyrin solutions with changing temperature. The kinetics were followed by the direct observation of changes in absorbance of the Soret band at 390 and 450 nm. (Characteristic of porphyrins, the Soret band is the most intense absorption, by more than an order of magnitude.) The temperature after the jump was 28 ± 1°. Each relaxation time, evaluated from the slope of the plots of the logarithm of the amplitude *vs.* time, represents an average of at least three photographic determinations. The relative error for these determinations is ±10%. Results at both wavelengths gave identical relaxation times.

Most of the spectral measurements were made on a Bausch and Lomb 505 spectrophotometer and occasionally on a Cary 14. The fluorescence measurements were done on a Cary 15 spectrometer with additional accessories for fluorescence emission.

Results

Temperature jump experiments were performed on porphyrin solutions (without added or incorporated metal ion) at various porphyrin concentrations over a range of pH values. The optical density change at 390 nm was found to be accurately exponential so that single relaxation times could be easily determined. A typical result is shown in Figure 1. That the relaxation process is reproducible and not due to porphyrin decomposition on rapid heating was shown by repeating the experiment 20 times on the same solution (at 4-min intervals). It was found that not only were the 20 relaxation times identical within experimental error, but also the porphyrin absorption spectrum was unchanged at the end of the series.

(5) G. C. Kresheck, E. Hamori, G. Davenport, and H. A. Scheraga, *J. Amer. Chem. Soc.*, **88**, 246 (1966).

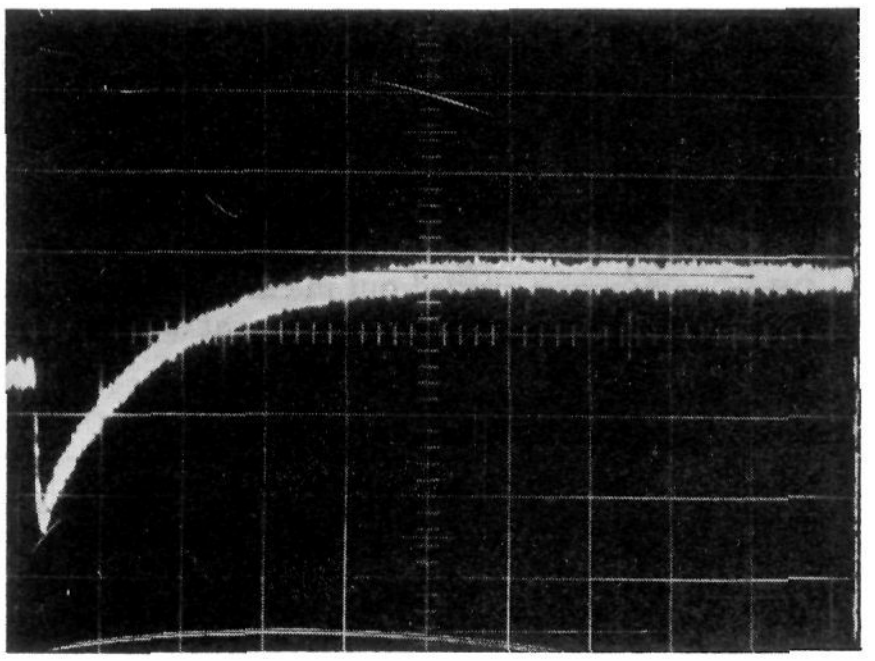


Figure 1. A typical relaxation spectrum of the porphyrin: $\Sigma P = 1.08 \times 10^{-4}$ *M*, pH 3.5, μ 0.1. The abscissa scale is 500 μsec per major division and the vertical scale is in arbitrary units of absorbancy. The relaxation effect corresponds to an increase in absorbance with time.

The results of one series of such studies at a temperature of 28° and an ionic strength of 0.1 *M* KNO_3 is given in Table I. As the measured relaxation times show, there is but a small increase in rate as the acid concentration is lowered by two orders of magnitude. On the other hand, increasing the total porphyrin concentration increases the rate by a factor proportional to the square root of porphyrin concentration. Both of these generalizations were borne out in other series of experiments, covering wider concentration ranges. So long as the pH is kept within range 3–7, there is little increase in rate with pH; in one series performed at 7 pH values between 3.1 and 7.0 the rate is increased by but 50% throughout the entire range.

Table I. Temperature Jump Relaxation Times at 28° in Aqueous 0.1 *M* KNO_3

[Total porphyrin] $\times 10^5$ *M*	τ, msec pH 4.0	pH 4.85	pH 6.0
1.2	1.4	1.3	1.0
2.4	0.98	0.80	0.75
3.6	0.82	0.75	0.65
4.8	0.70	0.50	0.50

To test the effect of salt concentration on the relaxation rate, experiments were performed as a function of ionic strength, both at pH 4.2 and 6.0. The results are listed in Table II. It is seen that at the lower pH there

Table II. Effect of Ionic Strength on Relaxation Time at 25°

pH	[KNO_3], *M*	[Porphyrin] $\times 10^5$, *M*	τ, msec
4.2	0.1	3.8	0.70
4.2	0.2	3.8	0.60
4.2	0.3	3.8	0.52
4.2	0.5	3.8	0.50
6.0	0.1	4.9	0.62
6.0	0.2	4.9	0.62
6.0	0.3	4.9	0.54
6.0	0.4	4.9	0.57
6.0	0.5	4.9	0.55

is a rate increase with increasing salt concentration while at higher pH there is little if any effect. To see whether salt effects might be specific, experiments were run at pH 3.2 and 6.0 with $LiNO_3$ and $NaClO_4$ substituted for KNO_3. All results of added salt on both absorption and relaxation spectra were identical within experimental error (±5%).

Other studies were carried out in ethanol–water mixtures. Table III records the results at various pH

Table III. Relaxation Times in Ethanol-Water[a]

Vol % ethanol	pH	τ, msec
0	4.0	0.62
10	4.0	0.55
25	4.0	0.35
50	4.0	0.28
50	3.0	0.45
50	7.0	0.20

[a] 25°, 0.1 M KNO_3 and 4.4×10^{-5} M total porphyrin.

values as read with a glass electrode. Adding ethanol increases the rate and the effect of pH is again relatively small although there is a more marked retardation on lowering the pH from 4 to 3.

Measurements were made at temperatures other than 28°. Results are listed in Table IV.

Table IV. Relaxation Times at Various Temperatures in Aqueous 0.1 M KNO_3

T, °C	pH	[Porphyrin] $\times 10^5$, M	τ, msec
20	4.0	1.5	1.0
	4.0	3.0	0.95
	4.0	6.0	0.70
	4.0	9.0	0.60
28	4.0	1.5	0.90
	4.0	3.0	0.70
	4.0	6.0	0.50
	4.0	9.0	0.40
45	4.0	2.0	0.45
	4.0	4.0	0.30
	4.0	6.0	0.27
	4.0	8.0	0.24
25	6.0	2.4	0.87
	6.0	3.6	0.70
	6.0	4.8	0.62
28	6.0	1.2	1.00
	6.0	2.4	0.75
	6.0	3.6	0.65
	6.0	4.8	0.50
45	6.0	2.0	0.40
	6.0	4.0	0.28
	6.0	6.0	0.24
	8.0	8.0	0.20

Analysis of the data is complicated by the fact that within the pH range studied, three porphyrin species (ignoring protonation of the ethylenediamine groups, which should not cause spectral changes) may exist: PH_2, PH_3^+, PH_4^{2+}. Although the slight pH dependence of the data seems to clearly rule out formation of protonated species as the reaction responsible for the relaxation process, the various protonated species would be expected to undergo the relaxation process at different rates. Furthermore, the dependence of relaxation rate on the square root of total porphyrin concentration indicates that the process in question is a dimerization. Also consistent with the proposed dimerization is the observation that the fluorescence intensity at 610 nm of the porphyrin solutions (in the concentration range 1×10^{-3}–3×10^{-4} M) is proportional to the square root of total porphyrin concentration, and that it increases on addition of ethanol and on lowering the pH. These results can be understood by noting that in general porphyrin monomers fluoresce while porphyrin aggregates do not.[6] For these reasons, spectrophotometric studies of porphyrin solutions at various concentrations and at various pH were carried out in the Soret region. These are summarized in Table V in terms of a dimerization equilibrium K_D = [dimer]/[monomer]^2.

Table V. Spectrophotometric Determination of Porphyrin Dimerization, 0.1 M KNO_3

T, °C	pH	Solvent	$K_D \times 10^{-5}$, M^{-1}
25	6.0	Water	7.5
25	4.5	Water	4.0
25	2.5	Water	2.1
40	6.0	Water	3.1
40	3.0	Water	2.0
25	6.0	25% ethanol	1.0

It might be noted that the monomer and dimer have Soret bands at the same frequency but differ in optical density. The value of $K_D = 7.5 \times 10^5$ at pH 6 may be compared to that of 9.3×10^7 obtained for phthalocyaninetetrasulfonate.[7] The data show that the extent of dimerization decreases somewhat with decreased pH, increased temperature, and addition of ethanol. The decrease with lowered pH is likely due to protonation equilibria. The pK_a's of the porphyrin were determined by spectrophotometric titration (Figure 2). It was found that as the porphyrin solution was titrated with acid, below pH 6.5 the spectrum changed in that the Soret at 393 nm shifted to shorter wavelength (375 nm), the shift being maximum at pH ~3.5. This shift can reasonably be attributed to the protonation of one of the ring nitrogens. Below pH 3 a new Soret peak at 404 nm appeared, the intensity of which increases with decrease in pH and reaches a maximum at pH 1.5. Simultaneously the 375-nm peak decreases in intensity and broadens. The visible spectrum at pH 1.5 has two peaks, showing that at this pH the species existing in solution is the diprotonated one.[8] The total porphyrin concentration was of the order of 10^{-5} M for the studies in the Soret region and 10^{-4} M for the visible region. From measurement of the spectral changes,[9] the following equilibrium constant was determined in H_2O.

$$[H^+][PH_2]/[PH_3^+] = 1.0 \times 10^{-5}$$

Because its value was constant over the concentration range where monomer and dimer coexist, it follows

(6) J. N. Phillips, "Comprehensive Biochemistry," Vol. IX, M. Florkin and E. H. Stotz, Ed., Elsevier Publishing Co., New York, N. Y., 1963, p 48.
(7) H. K. Bernauer and S. Fallab, *Helv. Chim. Acta*, **45**, 2487 (1962).
(8) B. Dempsey, M. B. Lowe, and J. N. Phillips in "The Porphyrins," L. Lemberg and R. K. Morton, Ed., Pergamon Press, London, 1961, p 29.
(9) A. Neuberger and J. J. Scott, *Proc. Roy. Soc., Ser. A.*, **213**, 307 (1952).

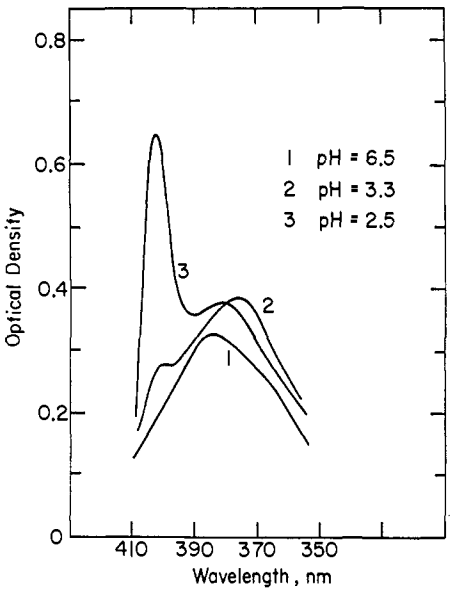


Figure 2. The Soret of the water-soluble porphyrin at different pH's: [porphyrin] = 0.8×10^{-5} *M*, KNO_3 = 0.1 *M*.

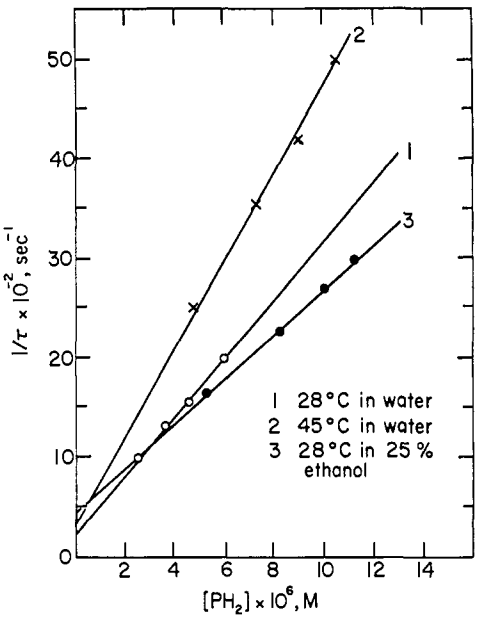


Figure 3. Plot of $1/\tau$ against concentration of free porphyrin, in water and in 25% ethanol.

that the protonation equilibrium is the same for porphyrin in the monomeric and dimeric form. It should be noted that the spectral changes ascribable to the monocation PH_3^+ are dependent on ionic environment. The value above is for 0.1 *M* salt concentration and it was found that the salt could be KNO_3, $NaNO_3$, $LiNO_3$, or $NaClO_4$ without affecting the results. Thus it seems unlikely that a specific ion pair complex such as previously postulated[10] could be responsible for the spectral change. At lower pH, a second protonation is apparent. The constant determined for it was

$$[H^+][PH_3^+]/[PH_4^{2+}] = 1.6 \times 10^{-3}$$

PH_4^{2+} apparently shows markedly less tendency toward dimerization.

At pH values below 3, the relaxation behavior changes. In the pH range 2–3, two relaxation processes are apparent, the slower having a relaxation time of 9.0 msec at a total porphyrin concentration of 1.9×10^{-5} *M*, 4.0 msec at 3.8×10^{-5} *M*, and 2.3 msec at 7.6×10^{-5}. That the relaxation time is considerably longer and that it shows a first-order dependence on porphyrin concentration indicate this to be a different process from that at higher pH. In addition, there is a much faster relaxation process apparent in the pH region 1.5–3.0. For this faster process, relaxation times of 14 ± 2 μsec were found in a series of 11 experiments which ranged in porphyrin concentration from 2×10^{-5} to 1.4×10^{-4} *M*. No dependence of relaxation time on porphyrin concentration was observed. Nor was there any dependence on salt concentration which ranged from 0.1 to 0.5 *M*. The latter observation is important since it rules out the possibility that these very short relaxation times are merely heating times for the solution. In control experiments using phenolphthalein the heating time was found to be 8 μsec at 0.1 *M* salt, 4 μsec at 0.3 *M*, and too fast to measure at 0.5 *M*.

(10) E. B. Fleisher and J. H. Wang, *J. Amer. Chem. Soc.*, 82, 3498 (1960).

Discussion

The results measured are consistent with the relaxation process involved being a dimerization of the porphyrin. This is most easily seen at pH 6.0 where the reaction should be free from complications due to protonation. For the reaction

$$PH_2 + PH_2 \underset{k_{-22}}{\overset{k_{22}}{\rightleftharpoons}} P_2H_4$$

standard derivation for the relaxation-time expression[11] gives

$$1/\tau = 4k_{22}[PH_2] + k_{-22}$$

As was noted, experimentally, $1/\tau$ varies with the square root of total porphyrin concentration. This is indeed the expected result for porphyrin concentrations sufficiently large that most of the porphyrin is dimerized so that $[PH_2]$ is proportional to the square root of total porphyrin concentration. If the data at 28° are plotted as $1/\tau$ *vs.* square root of total porphyrin concentration, a straight line results with an intercept of 190 sec^{-1} which is the value for k_{-22}. From such plots it is possible to also obtain the value for k_{22}; however, a preferable procedure involves use of the independently measured equilibrium constant to determine the value of the monomer concentration, $[PH_2]$. Such a plot is given in Figure 3. The slope of the line for 28° and aqueous solution is 3.1×10^8 which gives a forward rate constant of $k_{22} = 7.8 \times 10^7$ M^{-1} sec^{-1}, and the intercept again gives $k_{-22} = 190$ sec^{-1}. The rate of the dimerization is very near to that found by Hammes and Hubbard[12] for the dimerization of acridine orange.

In analyzing rate data, it becomes obvious that there is less certainty in fixing slopes than intercepts. Hence, the forward rates are known better than the reverse rates. To obtain better values for reverse rate con-

(11) M. Eigen and L. DeMaeyer, "Technique of Organic Chemistry," Vol. VIII, Part II, 2nd ed, A. Weissberger, Ed., Interscience Publishers, New York, N. Y., 1963.

(12) G. G. Hammes and C. D. Hubbard, *J. Phys. Chem.*, 70, 1615 (1966).

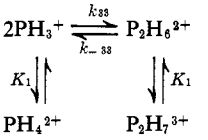
Table VI. Rate Constants and Activation Energies

Temp, °C	Solvent	pH	k_{22}, M^{-1} sec^{-1}	$\Delta E_{22}^{\ddagger}$, kcal mol^{-1}	k_{-22}, M^{-1} sec^{-1}	$\Delta E_{-22}^{\ddagger}$, kcal mol^{-1}
25	Water	6	7.0×10^7		93	
28	Water	6	7.8×10^7	4.1	110	12.0
45	Water	6	11.0×10^7		350	
20	Water	4	2.5×10^7		62	
28	Water	4	3.5×10^7	7.4	90	12.9
45	Water	4	7.0×10^7		350	
20	25% ethanol	6	4.5×10^7		370	
28	25% ethanol	6	5.5×10^7	5.5	540	10.1
45	25% ethanol	6	10.0×10^7		1450	

stants, an alternate method can be employed. In it the reverse rate constant is obtained by dividing the measured equilibrium constant into the forward rate constant. Values so obtained are summarized in Table VI together with derived activation energies.

The values of activation energy for dimerization are quite small indicating that the energy barrier for the reaction is about that for diffusion. The activation energy for diffusion of aromatic and cycloparaffin hydrocarbons in water has been measured as 4.6 kcal/mol,[13] which is also that for the self diffusion of H_2O.[14] The diffusion of the larger hydrocarbons is slower than that for the smaller ones but both exhibit the same activation energy.[13] Based on such findings, the present results would seem to indicate that the main energy barrier to dimer formation is that imposed by necessary structural changes in the aqueous solvent. That the reaction is less rapid than diffusion-controlled reactions of smaller species is reflected in an unfavorable activation entropy which may result from changes of water structure with some contribution from electrostatic repulsion.[15]

At lower pH, the relaxation times decrease slightly but the dependence on concentration remains the same. It would seem that a similar dimerization is occurring, but now it involves protonated species. This postulate is consistent with the finding that the reaction now shows a salt effect. Increased salt concentration increases the rate—as it should for the dimerization of similarly charged species. To handle the data quantitatively, and take into account the protonation equilibria for both monomer and dimer, becomes a formidable task. However, at pH 3.0, some simplification is possible since $[PH_2] \ll [PH_3^+]$ and PH_4^{2+} apparently does not dimerize. The vertical, protonation,



steps are assumed to be much more rapid than the dimerization and the equilibrium constants for the protonations of monomer and dimer are assumed to be equal. Even with these simplifications the expression for the relaxation rate is complicated.

$$1/\tau = 4k_{33}[PH_3^+] + 2\gamma k_{-33} = k_{33}(4[PH_3^+] + 2\gamma/K_{33})$$

$$\gamma = \frac{1}{2}\left(\frac{K_1 + [P_2H_6^{2+}] + [PH_3^+] + [H^+] - [P_2H_7^{3+}]}{K_1 + [P_2H_6^{2+}] + [PH_3^+] + [H^+] - [PH_4^{2+}]/2}\right)$$

Graphical evaluation leads to a value for $k_{33} = 2.2 \times 10^7$ M^{-1} sec^{-1}. From the measured dimerization constant at this pH, k_{-33} is found to be 280 sec^{-1}. Note that these rates are not very different for those at higher pH involving dimerization of the unprotonated porphyrin. However, it seems clear that as the porphyrin is converted to PH_4^{2+} dimerization does not occur as at higher pH. The causes for the two relaxation times at pH between 2 and 3 is not clear. However, it may well be a general phenomenon for porphyrins in that similar behavior was found for hematoporphyrin in this pH range, where it is water soluble.

Finally, the occurrence of dimerization of the water-soluble porphyrin could have important consequences in the kinetics of metal ion incorporation reactions. In the one case (Zn) studied for the present porphyrin, the kinetics were found to be first order in porphyrin.[4] This must mean that the metal ion is able to react directly with the dimer at rates quite comparable to those for reaction with the monomer. The dimerization of metalloporphyrins is currently being studied and will be the subject of a future publication.

(13) L. Bonoli and P. A. Witherspoon, *J. Phys, Chem.*, 72, 2532 (1968).
(14) J. H. Wang, *J. Amer. Chem. Soc.*, 73, 510 (1951).
(15) K. U. Linderstrom-Lang and J. A. Schellman in "The Enzymes," Vol. 1, 2nd ed. P. D. Boyer, H. Lardy, and K. Myrbäck, Ed., Academic Press, New York, N. Y., 1959, pp 445–510.