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Kinetics and Mechanism of the Hydrolysis of Chlorophyll *a* in Ternary Solvent Microemulsion Media

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Previous kinetic studies of the hydrolysis of chlorophyll *a* and other pheophytinate complexes have resulted in rate laws with no straightforward dependence on hydrogen ion concentration and have provided little mechanistic information. Use of detergentless microemulsion media consisting of toluene, water, and 2-propanol allows dissolution of lipophilic chlorophyll *a* and hydrophilic $HClO_4$ and provides a reasonably large surface area for interactions of these species. Over a wide range of values of the pseudo-first-order rate constant, from 1 to 10^{-2} s^{-1}, the reaction is first order in chlorophyll *a* and second order in $[H^+]$. For χ(toluene) = 0.411, $\chi(H_2O)$ = 0.180, and χ(2-propanol) = 0.408, at 25.0 °C and I = 0.10 M, k = 4.7×10^3 M^{-2} s^{-1}. As the composition of the medium is changed, the reaction rate shows a marked dependence on the concentration of toluene, being proportional to the 2.5 power of toluene concentration.

Introduction

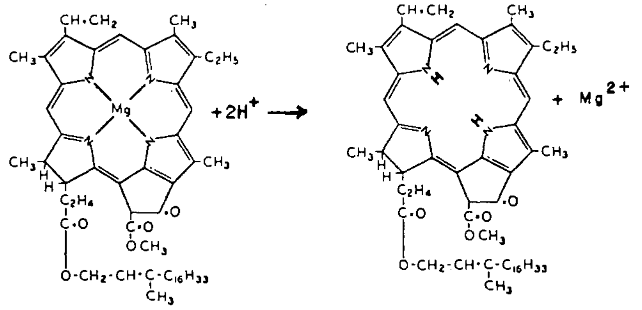
When plant cells are ruptured, chlorophylls are hydrolyzed to the magnesium-free pheophytins. The chlorophyll/pheophytin ratio has been used as a measure of the viability of aquatic flora.[2] It would be of interest to obtain mechanistic information about the hydrolysis reaction under a variety of conditions. Since chlorophylls are highly hydrophobic, it seems reasonable to assume that hydrolysis could take place at an aqueous–nonaqueous interphase. Another system in which the kinetics and mechanism of chlorophyll hydrolysis are of interest involves the transformations of the chlorophylls (and hemes and hemins) to the nickel and vanadyl porphyrins found in petroleum. Of the several mechanisms that can be envisioned for these conversions, the most reasonable scheme that has been presented involves a sequence of reactions in which the chlorophylls (and hemes and hemins) from plant and animal matter are hydrolyzed; the free-base chlorins (and porphyrins) then undergo competitive complexation reactions with metal ions available in the ground water and sedimentary rock with subsequent hydrolysis and competition sequences until the eventual products are the complexes that are most stable toward hydrolysis.[3] The pheophytins and metal pheophytinates, including the chlorophylls, and the most abundant natural porphyrins and metalloporphyrins are all highly lipophilic. However, the hydronium ion and common natural forms of metal ions are hydrophilic with low solubilities in organic solvents. Therefore, it also seems reasonable that the hydrolysis and complexation reactions involved in the transmetalation sequence may have taken place at aqueous–nonaqueous boundaries.

We have chosen to study the hydrolysis of chlorophyll *a* in a nonionic, detergentless microemulsion medium consisting of a hydrocarbon, water, and a somewhat polar organic solvent as a surfactant because the medium not only provides the convenience of high solubility of the chlorophyll *a* and acid but also may mimic the environment of natural hydrolysis reactions better than homogeneous solutions do. Herein we present kinetics results and a mechanistic interpretation of the acid-catalyzed hydrolysis of chlorophyll *a* in the microemulsion medium of toluene, water, and 2-propanol.

Experimental Section

Chlorophyll *a* was extracted from fresh spinach leaves and chromatographed on sucrose with use of standard procedures.[4] Nonaqueous solvents were dried and distilled by published procedures.[5] Water was doubly distilled, with the second distillation from alkaline permanganate solution. Acid concentrations of solutions made by dilution of doubly vacuum-distilled $HClO_4$ (G. F. Smith) were determined by titration. The Li_2CO_3 used to maintain ionic strength was prepared from recrystallized $LiCO_3$ and was dried to constant weight before use. The composition of each microemulsion was determined by weighing the solvent components. The acid concentrations quoted are based upon the volume of the entire microemulsion medium. The reactions were all run under pseudo-first-order conditions with chlorophyll *a* concentrations between 10^{-4} and 10^{-5} M.

Scheme I



The progress of each reaction was followed spectrophotometrically with either a Varian 635D conventional spectrophotometer or a Durrum-Gibson Model 110D stopped-flow spectrophotometer.

Results and Discussion

The reaction for the hydrolysis of chlorophyll *a* is illustrated in Scheme I. Although the reaction stoichiometrically involves two hydronium ions, the rate law, of course, need not be expected to be second order in acid concentration. For the analogous hydrolyses of zinc porphyrins, for example, the rate law at low acid concentrations is, in fact, third order even though the reaction also involves two hydronium ions.[6] Previous kinetics studies of the hydrolysis of chlorophyll *a* and other metal complexes of pheophytin *a* reported by Berezin and co-workers have involved a variety of media, but commonly, mixtures consisting of ethanol, glacial acetic acid, and, in some cases, sulfuric acid were used.[7] The rate laws found often have a complex dependence on acid concentration.[7a] The rate law for the hydrolysis of chlorophyll *a* in ethanol/glacial

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(1) (a) NIH-MBS program participant. (b) ACS SEED program participant cosponsored by Ciba-Geigy Corp.

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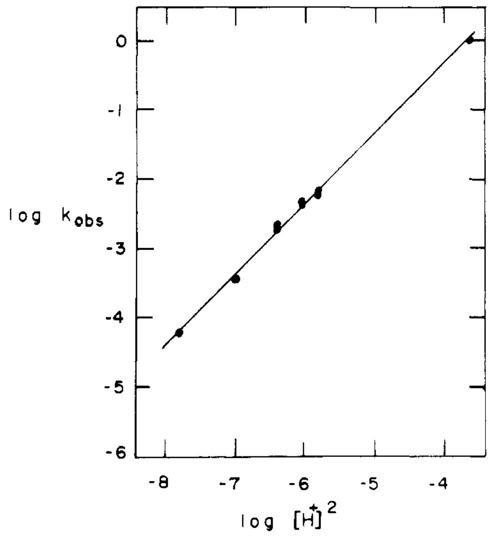
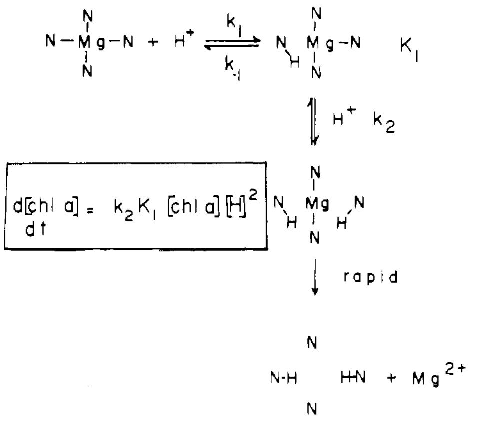


Figure 1. Rates of hydrolysis of chlorphyll *a* at 25 °C in a microemulsion medium consisting of toluene (χ = 0.411), water (χ = 0.180), and 2-propanol (χ = 0.409). The ionic strength was maintained at 0.10 M with $LiClO_4$.

acetic acid, for example, showed a nearly parabolic dependence on the square of glacial acetic acid concentration, which is equivalent to the fourth power of the solvated hydrogen ion concentration as calculated by Berezin.[7b] In one case, the hydrolysis of chlorophyll *a* in a *tert*-butyl alcohol/trichloroacetic acid mixture, a second-order dependence on the calculated effective solvated proton (H_{sol}^+) concentration was obtained (with use of indicators) but the range of $[H_{sol}^+]$ was very limited (1×10^{-3} to 3×10^{-3} M).[7c] In this medium the zinc pheophytinate complex showed a second-order dependence on $[H_{sol}^+]$ but the corresponding cadmium complex did not.[7c] Studies of metalloporphyrin hydrolysis have been performed in more tractable media and have often resulted in rate laws second order in acid concentration, and as mentioned above, third-order rate laws have been found in some cases when studies have been extended to low acid concentration.[8] The rate law found by Hambright and co-workers for the hydrolysis of zinc etioporphyrin[6] is rate = $k[ZnP][HCl]^3/(\rho + [HCl])$, which gives second-order behavior at high acid concentrations and third-order behavior at low acid concentrations.

The data for the hydrolysis of chlorophyll *a* in microemulsion media consisting of toluene (χ = 0.411), water (χ = 0.180), and 2-propanol (χ = 0.409) at 25.0 °C and 0.10 M ionic strength are shown in Figure 1. This microemulsion composition is nearly in the center of the microemulsion region of the toluene, water, 2-propanol pseudophase diagram.[9] Although the pseudophase diagram was obtained in the absence of added salt, studies of pseudophase diagrams of a closely related detergentless microemulsion system of hexane, water, and 2-propanol with and without $NaClO_4$ showed only slight alterations of the phase boundaries.[10] The best-fit line for the data in Figure 1 gives $K = 4.7 \times 10^3$ M^{-2} s^{-1}. As is evident from the figure, the second-order dependence on acid concentration extends over four orders of magnitude of rate with no evidence of third-order behavior. The simplest mechanism consistent with these results is shown in Scheme II. In this mechanism, the first protonation is reversible while a second protonation leads to hydrolysis. The rate constant for hydrolysis of chlorophyll *a* in a homogeneous solution consisting of water (χ = 0.100) and 2-propanol, in which the rate law is also second order in acid concentration, is 2.8×10^2 M^{-2} s^{-1}. The significant increase in rate in microemulsion media in which the water is confined to a droplet may be due to the ability of several water molecules in the vicinity of the magnesium atom to stabilize its release.

Scheme II



The detergentless, nonionic microemulsion medium employed in this study provides the advantages of facile dissolution of the hydrophobic chlorophyll *a* and hydrophilic $HClO_4$ and $NaClO_4$, a well-defined acid concentration, and a lack of charge effects at the interphase region due to charged surfactants. Hydrolysis reactions in such media may also be related to hydrolysis reactions that occurred as chlorophylls and hemins were converted to the nickel and vanadyl porphyrins found in petroleum.[3] The effect of changing this medium on the rate of hydrolysis of chlorophyll *a* has been reported.[3] Although changes in the concentrations of water on 2-propanol have no evident effect on the rate, an increase in toluene concentration causes the rate to increase. The rate constant is proportional to the toluene concentration to the power 2.5. In all cases, however, the rate law and presumably the mechanism remains the same. Hence, it appears to be worthwhile to attempt to study possible mechanistic differences in the hydrolysis reactions of related macrocyclic complexes in which there are no pyrrolic rings with saturated β–β bonds (magnesium porphyrins), those with two pyrrolic rings with saturated β–β bonds (bacteriochlorophylls), and the chlorophylls, which have one saturated β–β bond, using this microemulsion medium. These different types of magnesium complexes have been observed to have different hydrolysis rates, but no systematic kinetic study has been reported. In addition, it will be of interest to see if the rate laws for metalloporphyrins and metallopheophytinates with a variety of metal ions exhibit similar or different rate laws in microemulsion media.

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Registry No. Chlorophyll *a*, 479-61-8.

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