August, 1988] © *1988 The Chemical Society of Japan* NOTES *Bull. Chem. Soc. Jpn., 61, 2969—2970 (1988)* 2969

Pheophytinization of Chlorophyll *a* and Chlorophyll *a'* in Aqueous Acetone

Hitoshi Mazaki and Tadashi Watanabe*
Institute of Industrial Science, University of Tokyo, Roppongi, Minato-ku, Tokyo 106

(Received November 20, 1987)

Synopsis. Chlorophyll (Chl) *a* and its C-10 epimer, Chl *a'*, are compared for pheophytinization (demetallation) kinetics in aqueous acetone containing HCl. For each pigment the pheophytinization rate is of second order with respect to proton, and under identical conditions Chl *a'* is pheophytinized by roughly 50% faster than Chl *a*, due probably to weaker Mg-N bonds in Chl *a'*.

Our recent pigment composition analyses on higher plant chloroplasts, photosystem (PS) I and II particles, and several cyanobacteria showed for the first time that one molecule of Chl *a'*, or the C-10 epimer[1] of Chl *a*, is closely associated with the PS I reaction center (P700).[2,3] This has prompted us to carry out detailed physicochemical characterization of Chl *a'* against Chl *a*. In this Note we compare Chls *a* and *a'* for the kinetics of pheophytinization (replacement of the central Mg^{2+} ion with two protons), which would reflect the Mg-N bond strength in the chlorin macrocycle.

A few works have been reported on the pheophytinization of Chl derivatives. Following earlier observations by Mackinney and Joslyn,[4] Schanderl et al. compared Chl *a* and *b* pheophytinization rate constants in aqueous acetone at a single HCl concentration of 10^{-4} M ($M=mol\,dm^{-3}$).[5] A work of Berezin et al.[6] suggested indirectly that the Chl *a* pheophytinization rate is of second order with respect to proton in *t*-butyl alcohol containing trichloroacetic acid. The pheophytinization kinetics of Chl *a* in monomolecular films[7] and in heated green leaves[8] have been investigated. However, quantitative comparison between Chls *a* and *a'* with regard to pheophytinization kinetics has not been reported in the literature.

Experimental

Chls *a* and *a'*, with epimeric purity higher than 98%, were prepared by means of preparative scale high-performance liquid chromatography (HPLC) as described elsewhere.[9] Chl *a* or Chl *a'* was dissolved in an acetone/water (3/1, v/v) mixed solvent, and the solution was introduced in a temperature-controlled glass cell placed in a JASCO UVIDEC-650 spectrophotometer. The absorbance change due to the progress of pheophytinization was measured as a function of time at 430 nm [absorption peak for Chl *a*(*a'*)] or 409 nm [absorption peak for pheophytin (Pheo) *a*(*a'*)]. The concentration[10] of Chl *a*(*a'*) in aqueous acetone was 1.0×10^{-6} M, and that of HCl was varied in a range from 3×10^{-4} to 2×10^{-3} M. Thus the pheophytinization reaction

$$\mathrm{Chl}\ a(a') + n\mathrm{H}^+ \xrightarrow{k(k')} \mathrm{Pheo}\ a(a') + \mathrm{Mg}^{2+} \qquad (1)$$

can be regarded as pseudo-first-order with respect to Chl *a*(*a'*) concentration, with *k* and *k'* being the apparent rate constants for Chls *a* and *a'*, respectively. The *k* and *k'* values were obtained by fitting the time courses of Chl *a*(*a'*) concentration to the following kinetic formula:

$$\ln([\mathrm{Chl}]_t/[\mathrm{Chl}]_0) = -k(k')t \qquad (2)$$

where $[\mathrm{Chl}]_0$ and $[\mathrm{Chl}]_t$ are the concentrations of Chl *a*(*a'*) at the onset of measurement and at time *t*, respectively.

Results and Discussion

Figure 1 illustrates the temporal evolution of the relative concentration of Chl *a*(*a'*), observed at 430 nm, for different H^+ concentrations at 25 °C. The apparent rate constants are calculated from the slopes of the lines and Eq. 2. Under identical conditions, the *k* and *k'* values thus obtained were practically the same as those obtained by observing absorbance increase at 409 nm.

The rate constants from Fig. 1 are plotted in Fig. 2 against $-\log[H^+]$. The slope of -2.0 common for both Chl *a* and Chl *a'* indicates that the rate is indeed of second order with respect to proton ($n=2$ in Eq. 1). The pheophytinization rate of Chl *a'* is 1.4-fold higher than that of Chl *a* at a given $-\log[H^+]$ value; this is in contrast to a qualitative observation by Hynninen[11] that Chl *a'* was pheophytinized much more rapidly than Chl *a*. The higher tendency of Chl *a'* to be pheophytinized is due probably to its weaker Mg-N coordination bonds in the chlorin ring. A recent ^{13}C NMR

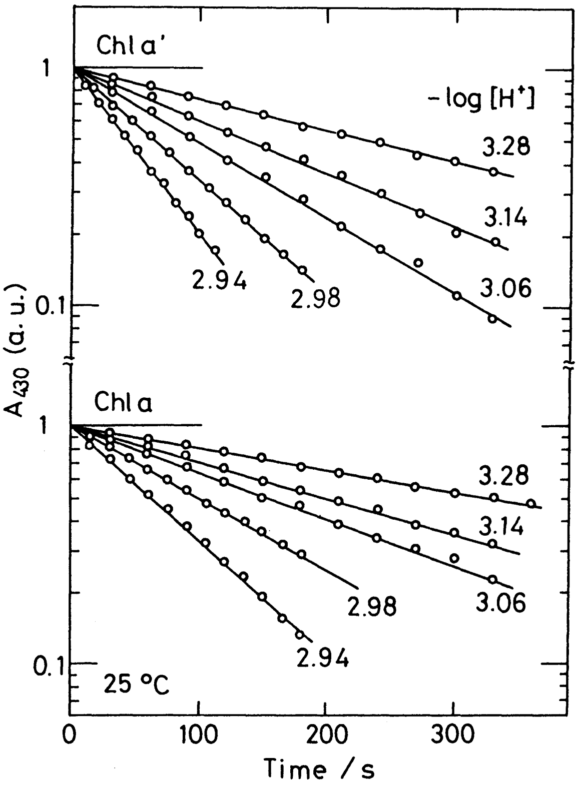

Fig. 1. Kinetic plots for the pheophytinization of Chls *a* and *a'* in aqueous acetone at different H^+ concentrations.

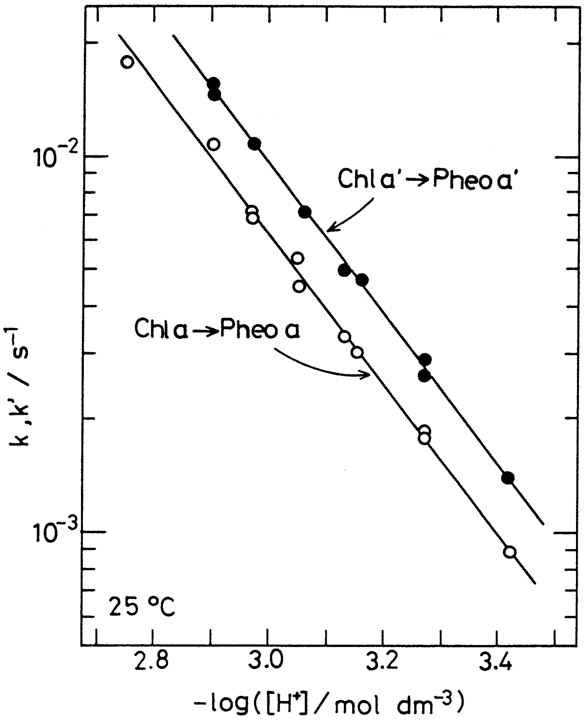

Fig. 2. Dependence of the pheophytinization rate constant k for Chl a (○) and k' for Chl a' (●) on H^+ concentration.

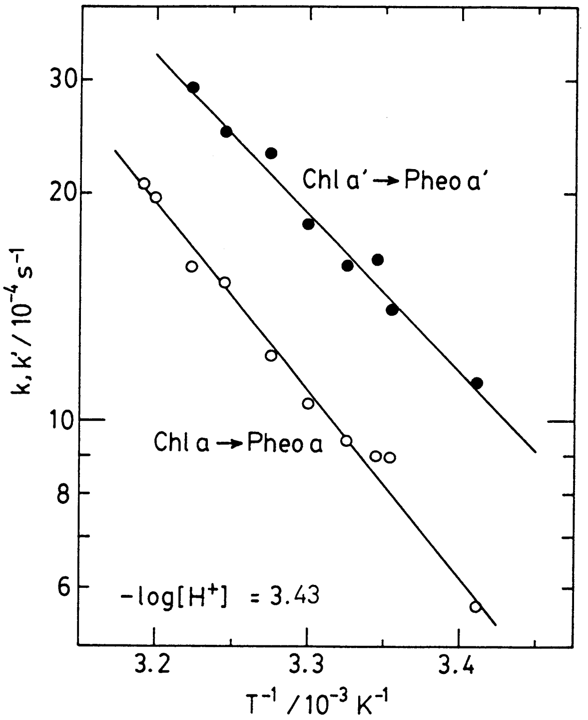

Fig. 3. Arrhenius plots over a temperature range 10—40 °C for the pheophytinization rate constants for Chl a (○) and Chl a' (●).

study on Chl derivatives[12] indicates that an enhanced steric hindrance between the bulky substituents (the C-7 phytyl chain and C-10 methoxycarbonyl group) in the Chl a' molecule induces a peripheral strain, especially in rings IV and V,[1] which in turn influences the conformation and stability of the whole macrocycle.

In aqueous acetone at 25 °C, though with a slightly different acetone/water volume ratio (4/1), Schanderl et al.[5] obtained a k value of 1.72×10^{-4} s^{-1} at $-\log[H^+]=4.0$. Extrapolation of our k vs. $-\log[H^+]$ plot in Fig. 2 to $-\log[H^+]=4.0$ yields $k=6.97\times10^{-5}\ s^{-1}$. The 2.5-fold difference seen between these two values might reflect a difference in the solvent composition.[13]

The temperature dependence of the pheophytinization rates was examined in a range from 10 °C to 40 °C at a $-\log[H^+]$ value of 3.43. The Arrhenius plots in Fig. 3 gives the activation energies of 47.4 and 40.4 kJ mol^{-1} for Chls a and a', respectively. The former value compares well with a literature value[5] of 43.5 kJ mol^{-1}.

Based on the results presented above, the pseudo-first-order rate constants $k(k')$ for the Chl $a(a')$ pheophytinization in aqueous acetone are expressed as follows:

$$k\ /s^{-1} = 1.30\times10^{12}\,[H^+]^2 \exp(-47400[J]/RT)$$
$$k'/s^{-1} = 1.20\times10^{11}\,[H^+]^2 \exp(-40400[J]/RT)$$

The authors are grateful to Mr. M. Nakazato, Nampo Pharmaceutical Co., Ltd., for his help in preparing Chl a and a' samples. This work has been supported partially by a Grant-in-Aid from the Ministry of Education, Science and Culture (No. 62602508).

References

1) See Ref. 2 for the carbon numbering and structure of Chl derivatives.

2) T. Watanabe, M. Nakazato, H. Mazaki, A. Hongu, M. Konno, S. Saitoh, and K. Honda, *Biochim. Biophys. Acta,* **807**, 110 (1985).

3) T. Watanabe, M. Kobayashi, M. Nakazato, I. Ikegami, and T. Hiyama, "Progress in Photosynthesis Research," ed by J. Biggins, Martinus Nijhoff, Dordrecht, The Netherlands (1987), Vol. 1, pp. 303—306.

4) G. Mackinney and M. Joslyn, *J. Am. Chem. Soc.,* **62**, 231 (1940); **63**, 2530 (1941).

5) S. H. Schanderl, C. O. Chichiester, and G. L. Marsh, *J. Org. Chem.,* **27**, 3865 (1962).

6) B. D. Berezin, A. N. Drobysheva, and L. P. Karamanova, *Russ. J. Phys. Chem.,* **50**, 1194 (1976).

7) M. Rosoff and C. Aron, *J. Phys. Chem.,* **69**, 21 (1965).

8) D. R. Haisman and M. W. Clarke, *J. Sci. Fd. Agric.,* **26**, 1111 (1975).

9) T. Watanabe, M. Nakazato, M. Konno, S. Saitoh, and K. Honda, *Chem. Lett.,* **1984**, 1411.

10) The volume reduction by mixing acetone with water was taken into account in calculating concentrations.

11) P. Hynninen, *Acta Chem. Scand.,* **27**, 1487 (1973).

12) S. Lötjönen and H. Hynninen, *Org. Magn. Reson.,* **21**, 757 (1983).

13) However, by closely tracing the measurement described in Ref. 5, we obtained a k value of 5.4×10^{-5} s^{-1} at 25 °C and $-\log[H^+]=4.0$.