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KINETIC AND THERMODYNAMIC PARAMETERS FOR THE PHEOPHYTIN *a*/*a'* EPIMERIZATION IN ORGANIC SOLVENTS (1)

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The epimerization rate constants and equilibrium compositions for the pheophytin *a*/*a'* pair have been determined for the first time in ten organic solvents. The rate constant varies by five orders-of-magnitude with the nature of solvent. A free energy diagram for the epimer pair is constructed from temperature dependencies of the two parameters in N,N-dimethylformamide.

Several lines of recent researches(2-7) evidence that pheophytin (Pheo) *a*, the demetallated chlorophyll (Chl) *a* which has long been believed absent *in vivo*,(8,9) does exist in chloroplasts at a molar ratio of Pheo *a*/Chl *a* ≃ (1 - 2)/100 and plays a key role in photosystem (PS) II reaction center. We have confirmed further that Pheo *a'*, the C10 epimer(10) of Pheo *a*, is not contained in green leaves.(5) A comparison of such a biased epimer constitution *in vivo* with kinetic and thermodynamic features of the Pheo *a*/*a'* epimerization *in vitro* would provide an insight into the state of existence of functional pigments in photosynthetic apparatus. These features, however, have not been quantified to date. In a previous work we developed an experimental procedure for studying the epimerization of Chl derivatives.(11) The procedure is applied here to determine the epimerization rate constants and equilibrium compositions for the Pheo *a*/*a'* pair in organic solvents.

Pheo *a* (epimeric purity > 98%) and Pheo *a'* (90-97%) were prepared as in Ref. 12, except that the latter was obtained here by treating the former with triethylamine. Each pigment was dissolved at a concentration of 3 mM (1 M = 1 mol dm^{-3}) in reagent grade N,N-dimethylformamide (DMF), pyridine, ethanol/chloroform (50/50), methanol/chloroform (50/50), ethyl ether, acetone, 2-propanol, chloroform, tetrahydrofuran (THF), or benzene, and the solution was left standing in darkness at 10 °C under N_2 atmosphere. The temporal evolution of the Pheo *a*/*a'* molar ratio was measured by the high-performance liquid chromatography (HPLC) specified in Ref. 11. The forward and backward rate constants for the epimerization process:

$$\text{Pheo } a \underset{k'}{\overset{k}{\rightleftarrows}} \text{Pheo } a' \qquad (1)$$

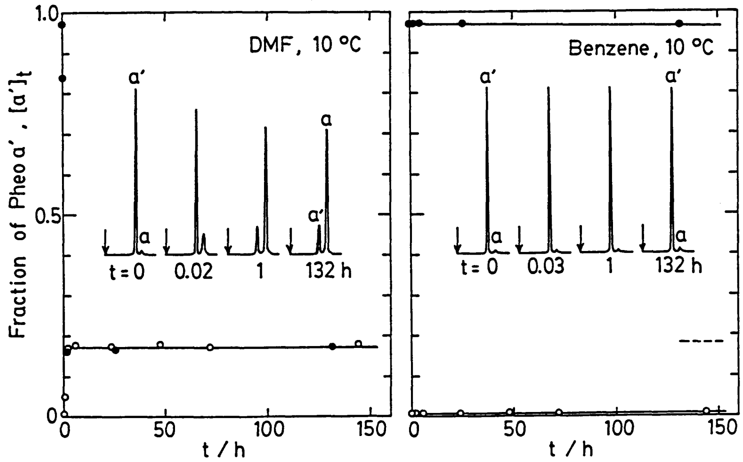

Fig. 1. Temporal change in the mole fraction of Pheo *a'* ($[a']_t$) starting either from 99.3% Pheo *a* (○) or from 97% Pheo *a'* (●) in DMF (the fastest case) and benzene (the slowest case) at 10 °C. The insets show typical HPLC charts with 97% Pheo *a'* as the starting composition. See Fig. 2 for the $[a']_t$ *vs.* t profiles in DMF at a shorter time scale.

were then calculated by fitting the $[a']_t$ (mole fraction of Pheo *a* at time t) *vs.* t profiles to a theoretical curve given by Eq. 2 in Ref. 11. In DMF and pyridine, the measurements were conducted also at other temperatures.

In all the ten solvents employed, no molecular alterations of Pheo *a*/*a'* other than epimerization were noted in HPLC charts up to 150 h, and the time course of epimerization was found to obey the theoretical formula.[11)] Two extreme examples for the temporal evolution of Pheo *a*/*a'* composition, together with representative HPLC charts, are illustrated in Fig. 1. An equilibrium state with $[a']_{eq} = 0.187$ is attained in about 1 h in DMF, while in benzene the epimerization is much slower. In DMF, and also in pyridine, the temporal evolution of the composition was followed in a shorter time range (cf. Fig. 2) to obtain the rate constants. Similar measurements were carried out in other solvents, and the results are summarized in Table 1. The value of $[a']_{eq}$ ranges from 0.13 to 0.20, indicating that the free energy difference between Pheo *a* and *a'* is 3.3-4.5 kJ mol^{-1}.

Table 1. Rate Constants and Equilibrium Compositions for the Pheo *a*/*a'* Epimerization at 10 °C [a)]

Solvent	k	k'	τ [b)]/h	$[a']_{eq}$ [c)]
DMF[d)]	22900	99900	0.226	0.187
Pyridine[d)]	2440	11800	1.96	0.172
Ethanol[e)]	5.7	38	640	0.13
Methanol[e)]	5.4	33	720	0.14
Ethyl ether	6.1	24	920	0.20
Acetone	3.9	22	1100	0.15
2-Propanol	2.5	11	2000	0.19
Chloroform	1.0	4.4	5000	0.18[f)]
THF	0.68	3.1	7200	0.18[f)]
Benzene	< 0.5	< 2	>10000	0.18[f)]

a) k and k' are in 10^{-8}s^{-1}. b) Epimerization time constant defined by $1/(k+k')$.[11)] c) Mole fraction of Pheo *a'* at equilibrium, corresponding to $k/(k+k')$.[11)] d) See Figs. 2 - 4 for the results at other temperatures. e) Mixed 50/50 (v/v) with chloroform. f) Assumed value.

The rapid epimerization in two N-containing solvents suggests that base catalysis is the underlying mechanism for process (1). In a preliminary experiment, addition of imidazole, a well-known base catalyst,[13)] to a concentration of 0.1 M indeed resulted in a roughly 10^4-fold enhancement of the epimerization rate constant in ethyl ether (details to be published). These results indicate that the use of basic solvents or coexistence of basic compounds should be carefully avoided in experiments for *in vitro* characterization of Chl derivatives, since the epimers behave quite differently in many physicochemical aspects.11)

The temperature dependencies of k, k', and $[a']_{eq}$ were measured in DMF and pyridine. Figure 2 shows the temporal evolution of Pheo a/a' composition in DMF at three temperatures. The measured points fit well to the theoretical curves, and the rate constants thus obtained in DMF and pyridine are plotted in Fig. 3 against reciprocal of temperature.

Finally the equilibrium Pheo a/a' compositions were measured in the two solvents as a function of temperature. In going from 5 to 50 ^{o}C the $[a']_{eq}$ value changed from 0.185 to 0.196 in DMF, and from 0.171 to 0.186 in pyridine. Figure 4 depicts that $\ln K$ ($K = [a']_{eq} / [a]_{eq}$) shifts linearly with reciprocal of temperature in each solvent.

The thermodynamic parameters calculated from the data in Figs. 3 and 4 are given in Table 2. Figure 5 is a schematic illustration of the free energy diagram constructed by using

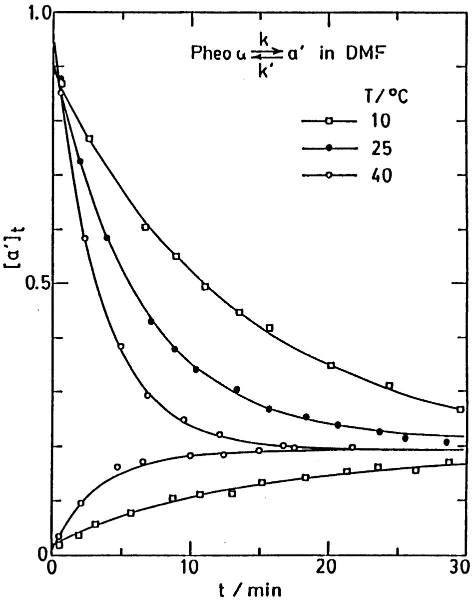

Fig. 2. Temporal evolution of the Pheo a/a' composition in DMF starting from 98-99% Pheo a or 90-95% Pheo a' at different temperatures. Curves are drawn according to the theoretical formula, Eq. 2 in Ref. 11.

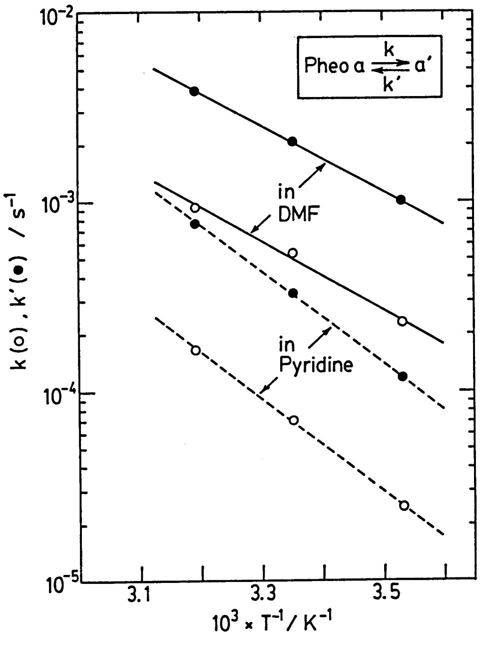

Fig. 3. Arrhenius plots of the Pheo a/a' epimerization rate constants in DMF and pyridine.

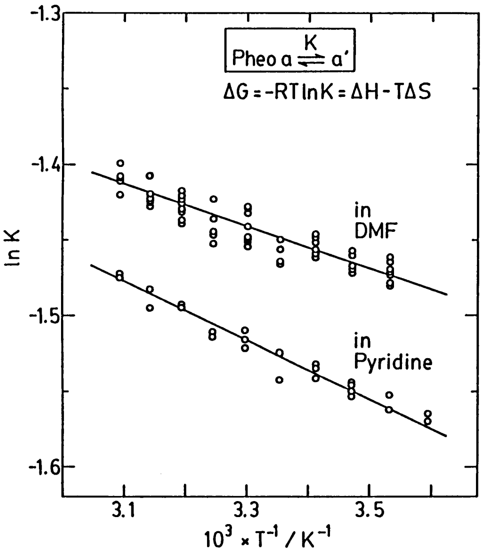

Fig. 4. Temperature dependence of the Pheo a/a' equilibrium constant K in DMF and pyridine. Measurements were repeated 2-8 times for each temperature.

Table 2. Thermodynamic Parameters for the Pheo *a*/*a'* Epimerization in Two Solvents[a)]

Solvent		Activation Parameters				Equilibrium Parameters			
		$E^{\ddagger}$	$\Delta H_{298}^{\ddagger}$	$\Delta S_{298}^{\ddagger}$	$\Delta G_{298}^{\ddagger}$	$\ln K_{298}$	ΔH_{298}	ΔS_{298}	ΔG_{298}
DMF	$a \rightarrow a'$	34.3	31.8	-202	92.1	-1.450	1.1	-8.2	3.6
	$a' \rightarrow a$	33.3	30.8	-193	88.3				
Pyridine	$a \rightarrow a'$	47.3	44.8	-175	96.7	-1.525	1.6	-7.3	3.8
	$a' \rightarrow a$	45.8	43.5	-167	92.9				

a) $E^{\ddagger}$, $\Delta H^{\ddagger}$, $\Delta G^{\ddagger}$, ΔH, and ΔG in kJ mol^{-1}; $\Delta S^{\ddagger}$ and ΔS in J K^{-1}mol^{-1}. $K = [a']_{eq}/[a]_{eq}$

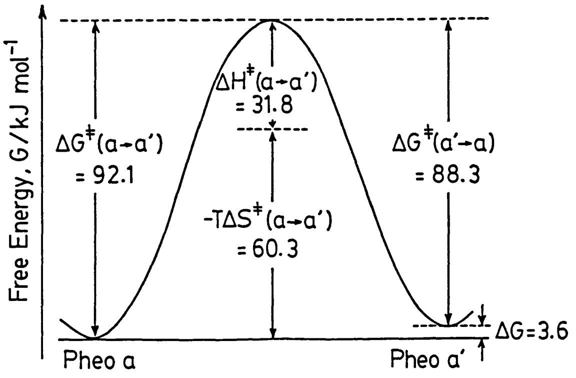

Fig. 5. Free energy diagram for the Pheo *a*/*a'* epimer pair in DMF at 25 °C. $\Delta G^{\ddagger}$, $\Delta H^{\ddagger}$, and $\Delta S^{\ddagger}$ are taken from the kinetic data (Fig. 3), and ΔG from the equilibrium data (Fig. 4).

these data. This is the first case where any energetic correlation between the epimer pair of Chl derivatives has been established. It is seen in Fig. 5 that the entropy term contributes by about 65% to the overall activation free energy for the Pheo *a*→ *a'* conversion in DMF. Similar investigations concerning the epimerization of Chl *a*/*a'* and other couples are currently in progress.

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