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Photochromic Behavior of Schiff Base–Metal Complexes. I. Photoinduced Hydrolysis of Pyridoxal 5′-Phosphate- and Salicylaldehyde-α-amino Acid–Zinc(II) Complexes

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Synopsis. Pyridoxal 5′-phosphate-α-amino acid–Zn(II) complexes and their salicylaldehyde analogues showed photochromic behavior in borate buffer at pH 9.5. A detailed comparison of UV spectral changes during the thermal back reaction with those for the complex formation indicates that the photoexcitation of the complexes induces the hydrolysis of the Schiff base ligands and that the back reaction corresponds to regeneration of the complexes.

Pyridoxal 5′-phosphate (PLP) is an essential cofactor for many amino acid transformations in biological systems. PLP or pyridoxal (PL) itself also non-enzymically catalyzes some of these reactions, which proceed *via* the Schiff bases with amino acids.[1] We have found that the photoexcitation of the PLP Schiff bases causes a half-reaction of decarboxylation-dependent transamination.[2] This type of decarboxylation has not been observed in thermal model systems for α-amino acids having an α-hydrogen. This difference between the photochemical and thermal reactions led us to investigate the photochemical behavior of the Schiff base-metal ion complexes which have been used much more frequently in the non-enzymic model systems.

The Zn(II) complex of PL–Val (Val=valine) Schiff base has been reported by Metzler *et al.*[3] to be exceedingly photolabile in aqueous solutions. We here report that the Zn(II) complexes of PLP–α-amino acid Schiff bases and their salicylaldehyde (SAL) analogues are photochemically hydrolyzed in an aqueous solution.

Experimental

PLP (Sigma), α-amino acids (Wako),[4] and zinc perchlorates (Kishida) were of reagent grade or better and were used without further purification. SAL (Wako) was distilled under reduced pressure and stored in the dark at about −20 °C. UV absorption spectra were taken with a Shimadzu UV 202 spectrophotometer. All kinetic runs were carried out spectrophotometrically in 0.05 mol dm^{-3} borate buffer (pH 9.5) at an ionic strength of 0.5 (KCl) with a Shimadzu MPS-5000 spectrophotometer. The cell compartments were thermostated (±0.1 °C) at desired temperatures.

Complex Formation and Rate Determination. α-Amino acid, zinc perchlorate, and PLP or SAL were simultaneously mixed. The Zn(II) complex solutions equilibrated within 30 min or less showed absorption maxima at 378 nm (0 in Fig. 1, ε 5620) for PLP–Ala (Ala=alanine), 375 nm (ε 5820) for PLP–AIB (AIB=α-aminoisobutyric acid), 351 nm (ε 5880) for SAL–Ala, and 350 nm (ε 5920) for SAL–AIB complexes. The molar absorption coefficients in parentheses were based on the initial concentration (1.00×10^{-4} mol dm^{-3}) of PLP or SAL, and were obtained under the conditions of the relative concentrations of three components given in the footnote of Table 1. These concentration conditions gave the greatest absorbances at the absorption maxima of the complexes. The molar absorption coefficient for the SAL–Ala–Zn(II) complex is in good agreement with that reported by Leussing and Bai.[5] The first-order rate constants (k_c) of the complex formations were determined by recording the increases in absorbances (A) at the wavelengths of the absorption maxima with respect to time, and by plotting $-\ln(A_\infty - A)$ *vs.* time. A_∞ is the absorbance at $t=\infty$. The activation energies ($E_{a,c}$) were calculated from the Arrhenius plot of the rate constants obtained.

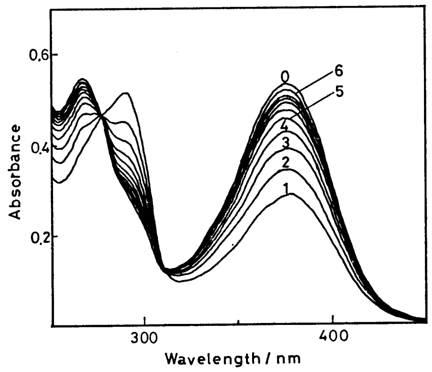


Fig. 1. Spectral changes of the PLP–Ala–Zn(II) complex in 0.05 mol dm^{-3} borate buffer at 25 °C after UV irradiation.
0: Before irradiation, 1: immediately after irradiation of 3 min, 2—5: thermal UV spectral changes at intervals of 1.5 min, 6: 30 min after irradiation. Other conditions: see the footnote in Table 1.

UV Irradiation and Thermal Back Reaction. The equilibrated solutions of the Zn(II) complexes were deaerated by repeated evacuation and flushing with argon gas seven or more times. The solutions were then placed in a 1-cm quartz spectrophotometric cell, thermostated at desired temperatures, and irradiated with a 500-W super high-pressure Hg lamp (Ushio USH-500D) through glass filters (Toshiba UV-39 transmitting less than 10% light below 380 nm, for PLP complexes; UV-D36A transmitting less than 10% light below 330 nm, for SAL complexes). The progress of a thermal back reaction was followed by determining the absorption spectra. The kinetic data (k_b and $E_{a,b}$) of this process were obtained by the method described above.

Results and Discussion

The irradiation of a buffered solution of the PLP–Ala–Zn(II) complex in an argon atmosphere caused the immediate decrease of the complex absorption band at 378 nm (Fig. 1, 0→1). When the photolysate was kept in the dark, a thermal reaction occurred; the UV spectrum of the photolysate returned more than 98% to its initial figure within 30 min at 25 °C (Fig. 1, 1→6), and showed two isosbestic points at 278 and 311 nm. The spectral changes during the thermal reaction were the same in shape as those during the complex formation. In addition, more than 95% of the original absorbance at 378 nm was restored even after the photochemical and thermal processes were repeated three times. These results suggest that the

Table 1. Kinetic data of the Schiff base-Zn(II) complex formation and the thermal back reaction[a)]

Schiff base	Temp/°C	$10^3\ k_c/\mathrm{s}^{-1}$ b)	$E_{a,c}$/kJ mol^{-1} b)	$10^3\ k_b/\mathrm{s}^{-1}$ b)	$E_{a,b}$/kJ mol^{-1} b)
PLP-Ala	10.0	1.12±0.03	61.5±2.0	—c)	64.5±2.6
	15.0	1.70±0.01		1.68±0.04	
	20.0	2.62±0.01		2.60±0.04	
	25.0	4.00±0.03		3.95±0.07	
PLP-AIB	25.0	1.48±0.01		1.44±0.02	
SAL-Ala	15.0	1.95±0.02	34.6±0.6	1.93±0.02	35.6±3.0
	20.0	2.47±0.02		2.37±0.04	
	25.0	3.05±0.03		2.88±0.02	
	30.0	3.95±0.05		4.02±0.08	
SAL-AIB	20.0	1.80±0.01		1.80±0.02	

a) All the experiments were carried out in 0.05 mol dm^{-3} borate buffer, pH 9.5 (0.5 mol dm^{-3} KCl). The initial concentration of PLP or SAL was 1.00×10^{-4} mol dm^{-3}. The initial concentrations of amino acid and Zn^{2+} were 1.0×10^{-2} and 7.0×10^{-4} for PLP-Ala, 5.0×10^{-2} and 5.0×10^{-3} for PLP-AIB, 1.0×10^{-2} and 1.5×10^{-3} for SAL-Ala, and 5.0×10^{-2} and 5.0×10^{-3} mol dm^{-3} for SAL-AIB systems, respectively. b) Errors given are standard deviations. c) Not determined.

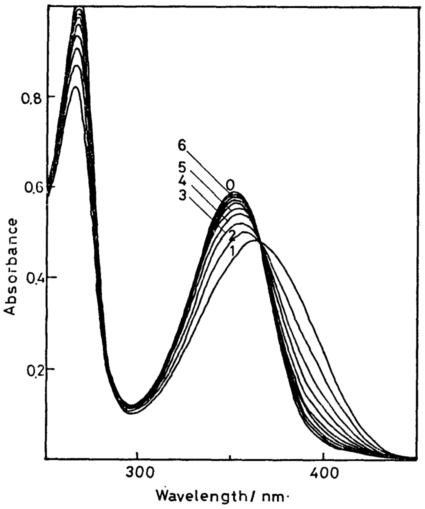
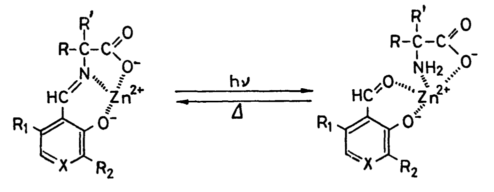


Fig. 2. Spectral changes of the SAL–Ala–Zn(II) complex in 0.05 mol dm^{-3} borate buffer at 25 °C after UV irradiation.
The numbers represent the order of the spectral changes at the same intervals as those in Fig. 1. Other conditions: see the footnote in Table 1.

photoexcitation of the complex caused the hydrolysis of the Schiff base ligand followed by the thermal regeneration of the complex (Scheme 1), and that a very minor side reaction took place only during irradiation. When other amino acids, Val and AIB were used, almost the same spectral changes were observed. The Zn(II) complex of SAL–Ala or SAL–AIB Schiff base also showed the same photochromic behavior (Fig. 2 for SAL–Ala–Zn(II) complex). The photochemical hydrolysis of these Zn(II)–Schiff base complexes was confirmed by the excellent agreement between the observed kinetic data (k_b and $E_{a,b}$) of the thermal back reaction and those of the complex formation (k_c and $E_{a,c}$), as shown in Table 1.

It has been proposed on the basis of kinetics of SAL–amino acid–divalent metal systems by Leussing *et al.*[6)] that rapid preequilibrium just after mixing the three components gives a mixed complex such as **2**, which is slowly transformed into the Schiff base complex (**1**) *via* an α-hydroxy amine complex. Under the conditions of excess Zn^{2+} and amino acid concentrations and at pH 9.5 employed in this work, the *o*-hydroxy aromatic aldehydes of UV-absorbing species are considered to exist in the mixed complex form after the photochemical hydrolysis. The first-order rate constants observed are probably those for the proton-independent formation path of the Schiff base complexes from the mixed complexes.

Scheme 1



1 **2**

PLP systems: X=N, $R_1=CH_2OPO_3^{2-}$, $R_2=CH_3$
SAL systems: X=CH, $R_1=R_2=H$

Metal-free PLP–α-amino acid Schiff bases underwent photochemically decarboxylation.[2)] Evidently the coordination of the carboxylato group to Zn(II) depresses this photo-decarboxylation.

An irreversible absorption band due to a by-product appeared around 330 nm on irradiation of the PLP–Ala–Zn(II) complex in air. It seems to us that the by-product, probably an oxidation product, corresponds to a photoproduct from PL–Val–Zn(II) complex[3)] having a similar absorption at 330 nm.

References

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4) Amino acids except for α-aminoisobutyric acid are of L configuration.

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