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PHOTODECARBOXYLATION OF PYRIDOXAL-α-AMINO ACID SCHIFF BASES. INTERMEDIATE SPECIES INDUCED BY FLASH EXCITATION

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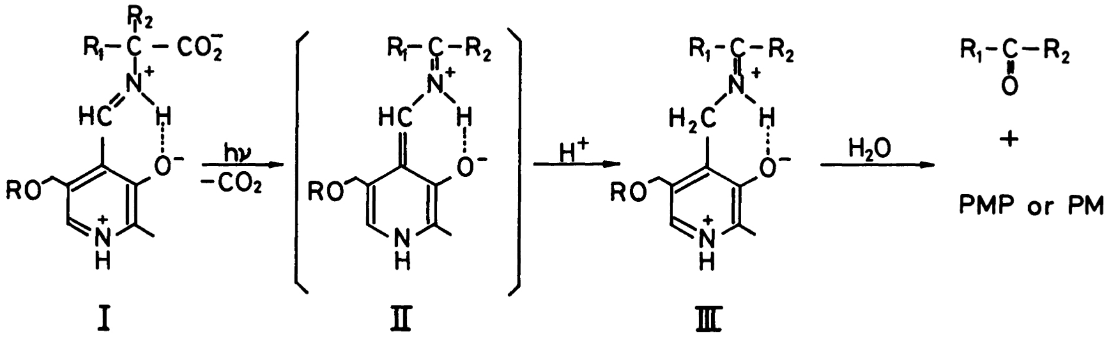
Pyridoxal Schiff bases of α-amino acids, phenylalanine and α-aminoisobutyric acid underwent photochemical decarboxylation in methanol to give pyridoxamine and carbonyl compounds after hydrolysis. Flash photolysis of the Schiff bases induced transient absorptions which were assigned to the decarboxylated quinonoid intermediates.

Enzymatic pyridoxal catalysis proceeds *via* the Schiff base formation from pyridoxal 5'-phosphate (PLP) and amino acids, and subsequent cleavage of one of three bonds attached to the α-carbon of an amino acid in the Schiff base to give a quinonoid intermediate.1) Evidence for the intermediate has been obtained in nonenzymatic model reactions,2) as well as in enzymatic reactions.3) Most of the intermediates, however, are the deprotonated species and have been observed in transamination, racemization, and γ-elimination or γ-replacement reactions.

We previously reported that the photoexcitation of the PLP Schiff bases in an aqueous phosphate buffer induced a half-reaction of decarboxylation-dependent transamination to give pyridoxamine 5'-phosphate (PMP) and the aldehyde or the ketone (Scheme).4) In this communication, we wish to report that an intermediate (II) in PLP-catalyzed decarboxylation, as proposed by O'Leary and Baughn,5) was detected by flash photolysis of a similar system, pyridoxal (PL)-α-amino acid Schiff bases in

Scheme



R=$-PO_3^{2-}$ for PLP Schiff bases, R=-H for PL Schiff bases.

methanol.

The steady state photochemical reactions were investigated in the same manner as described previously.[4)] Figure 1 shows the spectral changes of an equilibrated solution of L-phenylalanine (Phe) Schiff base (PL•HCl, 1.0×10^{-4}; Phe, 5.0×10^{-4}; KOH, 6.0×10^{-4} mol dm^{-3}) in methanol containing acetic acid (1.0×10^{-3} mol dm^{-3}) during irradiation in an argon atmosphere. The Schiff base absorption bands with maxima at 420 and 335 nm gradually decreased, while a new band with a maximum at 283 nm appeared. Two isosbestic points were observed at 277 and 294 nm. The irradiation under air hardly caused the reduction of the Schiff base bands.

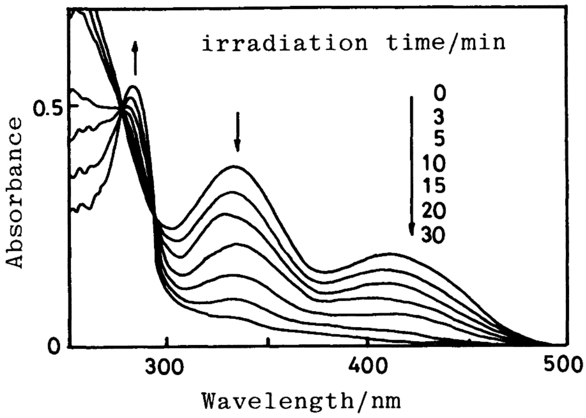


Fig. 1. Spectral changes of PL-Phe Schiff base during irradiation.

Table Photoreactions of the PL Schiff bases in methanol[a)]

Amino acid	Irradiation time/min	Yield/%[b)]		
		$PhCH_2CHO$	$(CH_3)_2CO$	PM
Phe	40	45		51
	60	56		62
AIB	60		48	50
	120		70	72

a) Irradiations were carried out with the same merry-go-round apparatus as previously described.[4)]
b) Based on PL used.

Twenty times concentrated solutions, as compared with the spectroscopic studies, were used for the identification or determination of the products. Formation of pyridoxamine (PM) was verified by high-performance liquid chromatography (JASCOREX CV-01 and Zipax SCX, 0.1 mol dm^{-3} KH_2PO_4) and paper chromatography (n-BuOH-AcOH-H_2O=4:1:4) after acid treatment of the photolysate. The product from the amino acid moiety was identified as phenylacetaldehyde by isolation of the 2,4-dinitrophenylhydrazone. Neither phenylpyruvic acid, the product expected from transamination, nor phenethylamine, the product expected from normal decarboxylation was detected. The Schiff base of α-aminoisobutyric acid (AIB) also showed almost the same spectral changes; the formation of PM and acetone was confirmed in a similar manner. These two products, PM and phenylacetaldehyde or acetone were formed in approximately equimolar amounts as shown in Table.[6)] Thus, these results suggest that the PL Schiff bases (I), as well as the PLP Schiff bases,[4)] were photochemically decarboxylated to give ketimine Schiff bases (III), which were hydrolyzed to PM and the aldehyde or ketone partly *in situ* and partly during post-treatment with acid.

The quantum yield of PM formation increased with increasing the concentration of acetic acid: for example, 0.065 with 6.0×10^{-2} mol dm^{-3} acetic acid; 0.18 with 2.4×10^{-1} mol dm^{-3}. Generally, protonation of this kind of Schiff bases on the ring nitrogen has a minor effect on the UV spectra. For example, the protonated ketoenamine form of 5'-deoxy PL-leucine Schiff base has a band at 414 nm, while the nonprotonated does at 420 nm.[7)] In the case of the PL Schiff bases, however, no clear distinction was observed. On the other hand, the fluorescence spectra were significantly affected by the addition of acetic acid. The fluorescence maximum of PL-Phe Schiff base shifted from 514 nm to 495 nm together with great increase in intensity on the addition of acetic acid.[8)] In addition, the titration curve of the relative fluorescence intensity at 495 nm (excitation at 450 nm) was nearly proportional to that of the quantum yield of PM formation. Thus, it is quite likely that the protonated excited singlet species participates in the photo-induced decarboxylation, although it is difficult to conclude at the present stage that the singlet is the reactive exited state.

Flash photolysis was applied to detect the decarboxylated intermediate (II), a new intermediate in pyridoxal catalysis. Flash excitation (Xe lamp: 200 J, 20 µs pulse; 1 dm length quartz cell; >310 nm) of PL-Phe Schiff base (PL•HCl, 2.0×10^{-3}; Phe, 1.0×10^{-2}; KOH, 1.2×10^{-2} mol dm^{-3}) in methanol containing acetic acid (2.4×10^{-1} mol dm^{-3}) under argon at room temperature gave a transient absorption with a maximum at 485 nm (curve a in Fig. 2). A similar transient (λ_{max}=490 nm, curve b in Fig. 2) was also observed in the case of AIB. The decay of the transient absorption showed the reaction kinetics with rate constants of $5.9 \pm 0.3 \times 10^{2}$ s^{-1} (at 490 nm) for Phe and $5.7 \pm 0.3 \times 10^{2}$ s^{-1} (at 500 nm) for AIB.

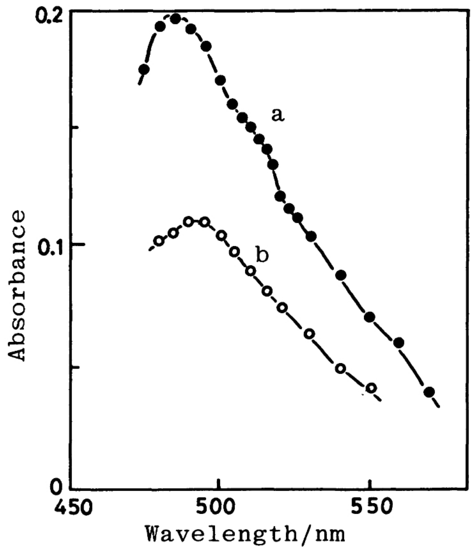
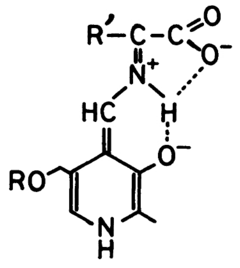


Fig. 2. Transient spectra in the flash photolysis of PL Schiff bases of Phe (a) and AIB (b) recorded 0.4 ms after the pulse.

A deprotonated intermediate (IV) in pyridoxal catalysis has been reported to show an absorption band at around 500 nm,[2),3)] which is not far from the absorption maxima for the transients in the present experiments (Fig. 2). The present reaction, however, leads to the specific photodecarboxylation of the Schiff bases as described above. In addition,



IV

the same transient absorption was also observed with the AIB system, where no α-hydrogen is present. On the basis of these reasons, the observed transient absorption bands are assigned to the decarboxylated species (II), which affords the ketimine (III) on protonation.

The positions of the absorption bands (485-490 nm) are not in conflict with this assignment since the species II bears the extended π-system of the quinonoid structure like the deprotonated species IV, and the presence of the carboxylate π-electrons in IV is not estimated to affect very greatly the position of the maximal absorption. Furthermore, the absorption intensities increased with increasing the concentration of acetic acid, and the presence of the transients was not observed under air. These characteristics were also observed in the steady state photochemical reaction as described above, and supported the above assignment.

The specificity in a large number of PLP catalyzed reactions has been interpreted in terms of a steric controlled mechanism, proposed by Dunathan,1) in which the C_α bond to be cleaved lies in a plane perpendicular to the plane of π-system of the conjugated Schiff base to achieve the maximum σ-π overlap. The simplest explanation for the specific decarboxylation described above is that in the reactive excited state, the carboxylate group can be located exclusively at the reactive position and undergo decarboxylation by delocalizing the bonding electron pair into the conjugated Schiff base system.

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