Laser-Induced Reactions in Pyridoxal Catalysis

John W. Ledbetter,* Harold W. Askins, and Raymond S. Hartman

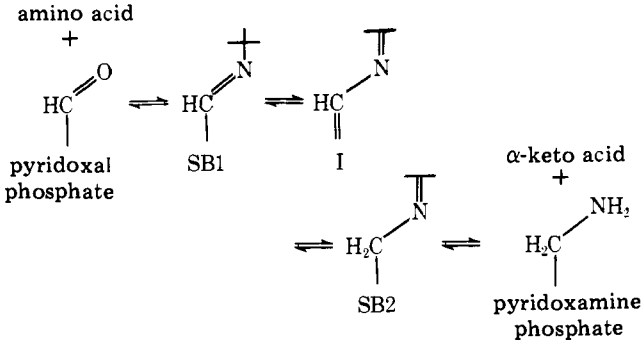
Contribution from the Department of Biochemistry, Medical University of South Carolina, Charleston, South Carolina 29403. *Received December 12, 1978*

Abstract: Excitation of a pyridoxal–amino acid complex with a nitrogen laser produced the key intermediate of transamination in the absence of enzyme. Experimental evidence showed that, when Schiff bases of pyridoxal and the methyl esters of amino acids were excited by near-ultraviolet light, the α hydrogen became more labile. After photoionization the carbanion relaxed to two structures one of which was a planar *p*-quinoid structure, the key intermediate. This intermediate was observed with a dye laser which was simultaneously pumped by the nitrogen laser. Identification was possible through its electronic absorption spectrum and structural requirements for formation. These requirements provided some interesting information on transamination. The observations required that only the *o*-hydroxyl group of the pyridoxal–amino acid Schiff base be complexed with a positive center, e.g., Al(III). Complete chelation inhibited the laser-induced reaction. The absence of an α hydrogen on the amino acid also prevented the formation of the intermediate. Further stimulation of the intermediate with the nitrogen laser resulted in a second laser-induced reaction. These results demonstrated clearly that photoexcitation of the complex produced a bona fide biochemical intermediate of metabolism and also supported a currently proposed mechanism for transamination.

Introduction

Lasers are proving useful for the stimulation of chemical reactions because of their photon specificity and intensity. In biochemical reactions specific stimulation is more difficult because of the complexity of the molecules and systems. Lasers with electronic energies have proven useful in establishing reaction mechanisms, intermediates, and kinetics in biological processess which normally rely on light-initiated reactions and which occur in electron transport. This paper is a report on the use of an ultraviolet laser to induce an intermediate of metabolism. We have found that the nitrogen laser which emits at 3371 Å will induce the key intermediate of pyridoxal 5′-phosphate (vitamin B_6) catalysis of transamination without the presence of the enzyme.

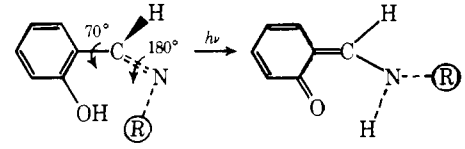
Pyridoxal 5′-phosphate (PLP) is a necessary cofactor for enzymic transamination in biological systems. Formally, a Schiff base results from reaction of the aldehyde form of the cofactor with an amino acid substrate. At the enzyme active site this complex presumably loses a proton at the α carbon of the amino acid and becomes a planar *p*-quinoid structure, the so-called key intermediate, I.[1]



This intermediate rearranges to a Schiff base between an α-keto acid and pyridoxamine 5′-phosphate (PMP). Hydrolysis then yields the α-keto acid. Concurrently, another α-keto acid reacts with PMP and the reverse reactions occur.

Evidence for the intermediate has been obtained in model systems from both kinetic[2,3] and spectral[4-6] data. A band observed near 500 nm has been assigned to this *p*-quinoid structure. This band has been observed also in enzymic systems with pseudosubstrates.[7,8] Formation of this *p*-quinoid intermediate occurs on deprotonation at the α carbon and subsequent negative charge migration toward the pyridine nitrogen of the PLP–amino acid Schiff base. Therefore, model systems which facilitate this show catalytic activity. Examples are the *N*-methyl derivative of pyridoxal and the metal ion chelates of the Schiff base. Some of those metal ions showing catalytic activity are Cu(II) > Al(III) > Fe(II) > Fe(III) > Ni(II) > Co(II).[9] This trend suggests that the weakly bonded chelates are the most active[10] and in this paper we find this to be of interest.

In opposition to this electron flow is the formation of the *o*-quinoid structure in PLP Schiff bases. In this case proton transfer occurs from the *o*-hydroxyl group to the imine nitrogen with subsequent electron migration out of the ring and toward the imine nitrogen. A band observed near 420 nm has been assigned to this tautomer.[11] This tautomerization also occurs in Schiff bases of salicylaldehyde in which the reaction has been studied in both the dark and flash photolysis.[12] As shown by the experiments of Weber[13] and later in detail by Förster[14] and Weller,[15] this hydroxyl proton is more labile in the excited state. For example, the pK_a of phenol decreases by 6.4 units in the first excited singlet state.[16] It follows that in the following reaction electronic excitation enhances proton migration from the hydroxyl group to the imine nitrogen with subsequent relaxation to a planar *o*-quinoid structure; at least that is presumed one of the possible conformations.[17,18] The



same proton migration is likely to occur on flash excitation of amino acid Schiff bases of PLP. As determined from fluorescence data the pK_a of the cation of the hemiacetal form of pyridoxal in the excited state is −3.3.[19] Compare this to the ground-state value of 4.2.[20] Since the zwitterionic structure is predominant in solution after ionization, the acidity of the *o*-hydroxyl group must be enhanced by seven orders of magnitude in the excited state. Evidence for the photoinduced tautomerism is presented here.

It is generally recognized that the ground state of A is probably not planar. The rotations and angles shown begin with a planar trans structure where R is the phenyl group and result from a PCILO calculation.[17] This conformation, assumed for the ground state of the aniline Schiff base of salicylaldehyde, is not that assumed for the amino acid Schiff bases of PLP. Recently, it was suggested[21] on the basis of NMR data that the C=N bond is coplanar with the pyridoxal ring. Accord-

0002-7863/79/1501-4284$01.00/0

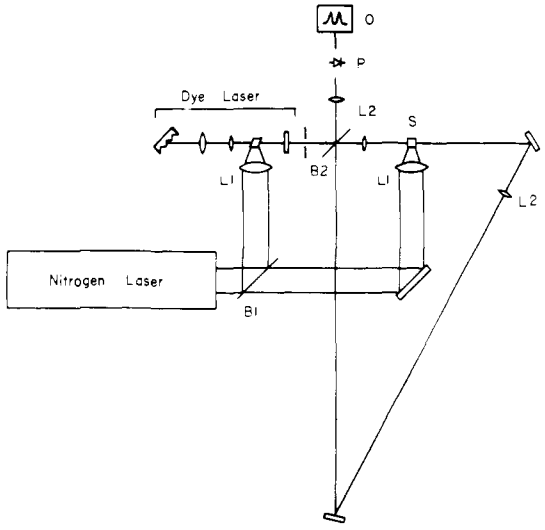
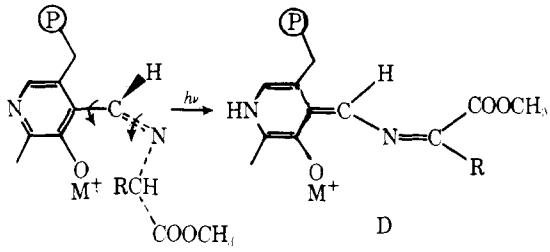


Figure 1. The experimental apparatus. L, lens; B, beam splitter; P, photodiode; O, oscilloscope; S, sample.

ingly, in the following uncertainty is noted for the ground-state conformation.

To facilitate transamination by laser excitation it was necessary to prevent the formation of the *o*-quinoid structure on excitation and to encourage negative charge migration toward the pyridine nitrogen. To accomplish this we reacted the *o*-hydroxyl group with Al(III) in methanol at low temperature. This eliminated proton transfer from the hydroxyl group on excitation and allowed negative charge migration toward the ring nitrogen on relaxation of the carbanion. Using the ester of the amino acid prevented full chelation which inhibited the photoinduced reaction. When this solution was excited with



C D

the nitrogen laser, an electronic absorption spectrum nearly identical with that obtained spontaneously in the dark was produced. Further experiments demonstrated that the key intermediate of metabolism involving the vitamin B_6 complex was generated.

Experimental Section

Figure 1 shows the simple experimental design. A nitrogen laser which emits at 3371 Å was used simultaneously to excite the sample and to pump a dye laser which acted as an analyzing light beam. With the latter I_0 and I were measured to determine the absorbance of the sample which was unexcited by the nitrogen laser. Then I_0 and I again were measured while the sample was being excited. From the two measurements repeated at different wavelengths a difference spectrum, ΔA against wavelength, was plotted.

The nitrogen laser was constructed according to the design of Schenck and Metcalf[22] and the dye laser was built in the typical Hänsch configuration.[23] The energy deposited in the sample in the 10-ns ultraviolet pulse was 0.6 mJ. The tunable dye laser pulse of no more than about 6 μJ had a spectral bandwidth of 3 Å and a pulse width of 5 ns. The leading edge of this pulse at the sample followed that of the ultraviolet pulse by 3 ns. In order to measure I_0 a microscope slide (B2) deflected a small percentage of the dye laser beam onto a Hewlett-Packard 4220 photodiode (P). The intensity of the

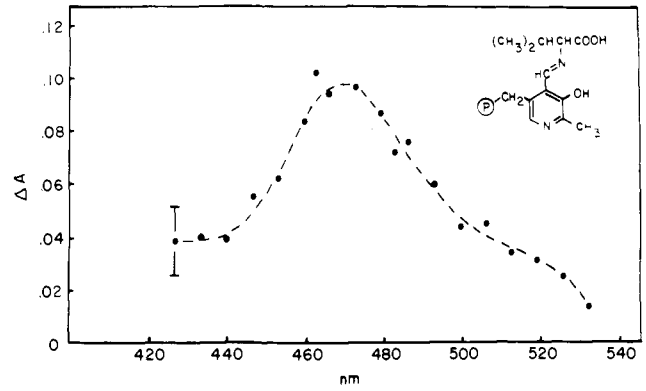


Figure 2. Difference spectrum of a laser-excited solution of 1×10^{-3} M pyridoxal 5′-phosphate and 1×10^{-2} M valine in methanol at room temperature.

pulse was recorded on a Tektronix 7623 oscilloscope (O). The main beam then traversed the sample, was reflected across the room and back, and was focused onto the same photodiode. This pulse, now I, was observed on the same sweep of the oscilloscope. The maximum peak height of each pulse was used to measure I_0 and I, respectively. In over 2500 recorded measurements, the average of the standard deviations for the I_0/I determinations at different wavelengths was 0.031. This average error is reflected in all the plots.

The electronic absorption spectra of samples at equilibrium were recorded with a Cary Model 14 recording spectrophotometer.

Low temperatures for the samples were achieved with an Andonian Associates cryostat.

The biochemicals were purchased from Sigma Chemical Co. To allow time for Schiff base formation in the samples, the solutions were mixed a few hours prior to the experiment. After the sample was placed in the cryostat, the temperature was lowered. Then, when desired, a solution of aluminum or other metal nitrate in methanol was introduced by means of a capillary tube.

Results and Discussion

The previously assigned spectrum of the *p*-quinoid intermediate for both enzymic and nonenzymic reactions consists of a maximum near 500 nm and a shoulder on the short-wavelength side of the maximum. When a solution of PLP and valine in methanol was subjected to the ultraviolet laser the difference spectrum shown in Figure 2 was observed. This spectrum with a maximum at 470 nm is very much like that observed for the Schiff bases of salicylaldehyde. It is presumed, therefore, that the spectrum is that of an *o*-quinoid tautomer of the molecule. Whether or not a band exists near 500 nm in Figure 2 is impossible to verify at this time. Owing to the shape of the band, it appears possible; but it would be difficult to say if electronic excitation in this experiment is producing the *p*-quinoid structure.

The Primary Absorbing Species. When Al(III) was added to an equilibrated solution of pyridoxal (PL) and the ester of an amino acid in methanol, an electronic absorption band developed at 488 nm. This band has been attributed to the key *p*-quinoid intermediate in the metal-mediated catalysis.[6] However, we found that, when the Al(III) was added at −65 °C, the band at 488 nm did not develop because the responsible reaction must be very slow at this temperature. As seen in the electronic absorption spectra in Figure 3, the Al(III) did react at low temperature, however. What was observed was an increase in the band at 420 nm along with changes at shorter wavelengths. On warming to 0 °C additional bands developed, the one at 485 nm and two others which appeared as shoulders at about 450 and 315 nm. The same observations occurred with a PL and phenylalanine methyl ester mixture. The increase in the intensity of the band at 420 nm has to be the result of a shift in the enol imine ⇄ keto enamine equilibrium toward the keto structure because of the isosbestic point formed at 360 nm and

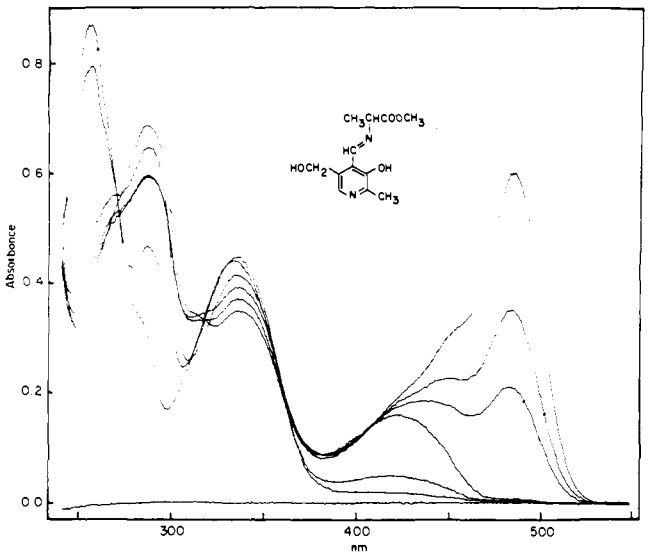


Figure 3. Absorbance of a solution 1×10^{-4} M in each of pyridoxal, alanine, and Al(III) in methanol. The solution represented by the lowest curve at 400 nm is at −65 °C and contains no Al(III). The next lowest curve reflects the addition of Al(III) at this temperature. The higher curves at 400 nm occur on warming.

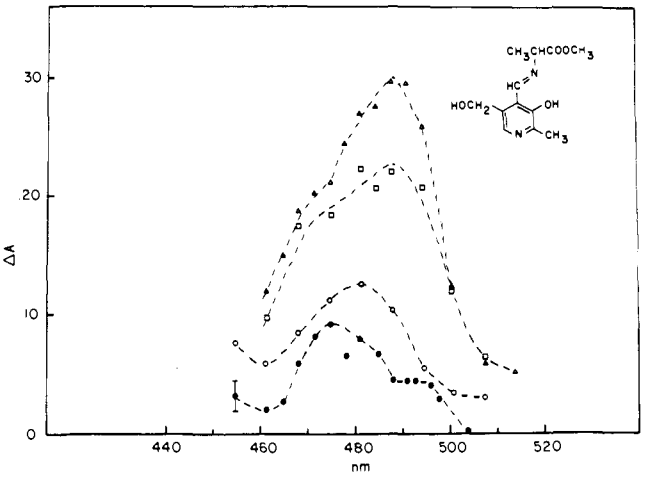


Figure 4. Difference spectra of a laser-excited solution 2×10^{-3} M in each of pyridoxal, alanine methyl ester, and Al(III) in methanol at −65 °C. The lowest curve represents the addition of Al(III) at this temperature. Higher curves represent increased reaction with Al(III) which occurs on warming.

not simply increased Schiff base formation. This shift toward the keto structure (*o*-quinoid) which occurred when Al(III) was first added at −65 °C probably resulted from some Al(III) complexation of the pyridine nitrogen. The placement of a positive charge here surely will result in a more acidic *o*-hydroxyl which will enhance formation[24] of the *o*-quinoid structure. Prominent at shorter wavelengths was the immediate decrease in the band at 255 nm along with the increase in the band at 286 nm, the latter of which is expected for the *o*-quinoid structure.[11,25] This was, therefore, commensurate with the shift in the equilibrium.

Of more interest was the band (shoulder) formation at about 315 nm which occurred on warming along with the increased absorption at 485 nm. The band at 286 nm also became more intense. The band at 315 nm does not result from the same structure responsible for the 485-nm absorption because it could be produced with pyridoxal Schiff bases of amines other than amino acids (no labile proton on an α carbon). For example, the tris(hydroxymethyl)aminomethane (Tris) Schiff base of pyridoxal formed a band at 315 nm in methanol when Al(III) was first added at room temperature. This band with time gave way to one at 365 nm. During this time, the band at 420 nm also rose and fell. The band at 315 nm, in fact, is characteristic of the absorption obtained when the *o*-hydroxyl is methylated to form the methoxy derivative. When the *o*-hydroxyl of PLP is methylated, the bands shift from 340 and 289[11,25] to 313 and 279 nm.[26] When the *o*-hydroxyl of salicylaldehyde is methylated the band in dioxane shifts from 326 to 315 nm.[27] This band for the valine Schiff base of 3-hydroxy-4-pyridinecarboxaldehyde in methanol shifts from 323 to 310 nm for the 3-methoxy derivative.[27] To compare the effect of complexation, we added Al(III) to a solution of phenol in methanol and observed a 1-nm blue shift in the absorption spectrum. This agrees with the shift of the spectrum of phenol in methanol from 271 to 270 nm for the methoxy structure.[28] What we observed here was a shift from 355 to 315 nm and that can be explained by the reaction of Al(III) with the *o*-hydroxyl to give $-OAl^{2+}$. This also means that chelation with the imine nitrogen is not occurring at this time. When it does, for the amino acid[6] and Tris Schiff bases of PL a band develops at 365 nm. Other ions such as Zn(II), Cu(II), Ni(II), Co(II), and Mn(II) which chelate with the valine Schiff base of PL form a band between 382 and 393 nm.[25] No evidence has been observed thus far for the presence of the $-OZn^{+}$ species. Another reason to conclude that Al(III) has reacted with the hydroxyl group but has not fully chelated is that, as we shall show, only the solutions demonstrated by Figure 3 will show the laser-induced intermediate. Solutions of the fully chelated species (365 nm) will not. Structure C is probably, therefore, the primary absorbing species which leads to the laser-induced intermediate, D.

The Laser-Induced Intermediate. Excitation of these solutions containing Al(III) at low temperatures resulted in the difference spectra shown in Figure 4. Initially, we saw a spectrum which was similar to that taken at room temperature without the addition of Al(III), except that a band around 490 nm appeared clearly. On increased reaction of the Al(III), which occurred on warming, the two bands increased in intensity while the excitation energy remained constant. Eventually, a band became prominent at 488 nm while a shoulder appeared at 475 nm. Noted clearly was the almost identical character of the band and the shoulder with that produced in the dark (ref 6 and Figure 3), a difference being a bathochromic shift in the shoulder.

The same observations, Figure 5, were made for the phenylalanine and valine methyl ester Schiff bases of PL in methanol. These observations were also made with PLP, but were less intense. Note that in the case of phenylalanine the maximum was at about 480 nm. The shoulder at 475 nm did not appear, but two bands still seemed to be the case.

The question of whether or not the band at 488 nm or even the shoulder at 475 nm represents a key intermediate in transamination has gained support in the past from the fact that when an α hydrogen was not present on the amino acid, the band did not occur.[29] The same rationale was used here. A solution of PL and α-methyltyrosine methyl ester in the presence of Al(III) was excited with the nitrogen laser. The results are shown in Figure 6. In the case of no Al(III) a curve was found resembling that of Figure 2. But, interestingly, it was much sharper than the former. When Al(III) was added, the difference spectrum decreased and at equilibrium at room temperature it was quite small. Conclusively, observations of the band at 488 nm and the shoulder at 475 nm did require an α hydrogen on the amino acid. Also evident was that the Al(III) served one of its purposes, namely, that of preventing the formation of the *o*-quinoid structure by complexing with the *o*-hydroxyl group.

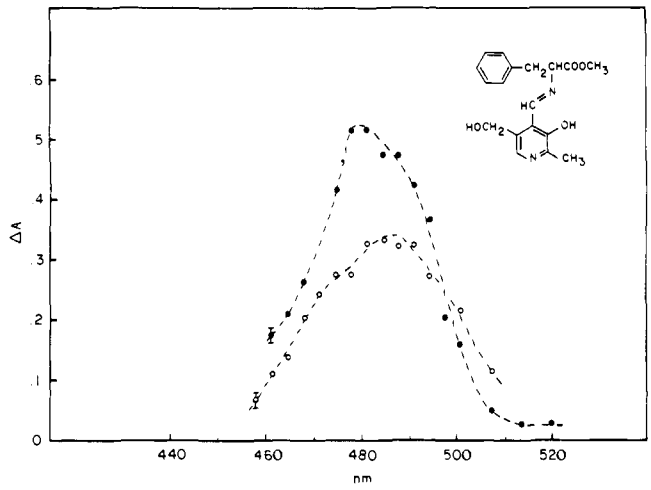


Figure 5. Difference spectra of laser-excited solutions 2×10^{-3} M in each of pyridoxal, amino acid methyl ester, and Al(III) in methanol at −66 °C. ●, phenylalanine methyl ester; ○, valine methyl ester.

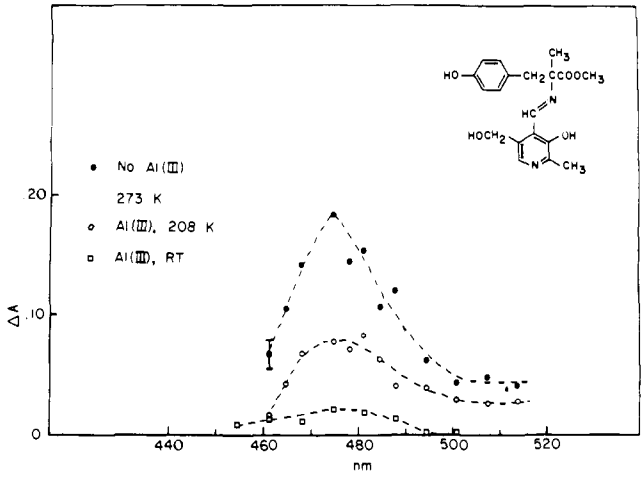


Figure 6. Difference spectra of a laser-excited solution 2×10^{-3} M in each of pyridoxal, α-methyltyrosine methyl ester, and Al(III) in methanol. Closed circles represent no Al(III) at 0 °C. Open circles represent the addition of Al(III) of 0 °C and the solution cooled to −65 °C. Boxes represent the addition of Al(III) and equilibrium at room temperature.

We think it is very probable that photoexcitation by the nitrogen laser resulted in a more labile α hydrogen and an absorption band which others and ourselves are attributing to the key *p*-quinoid intermediate of transamination. Of the experiments which failed to yield the band at 488 nm, some were informative. After a solution of Al(III) and the aldimine reached equilibrium at room temperature and exhibited the band at 365 nm, no laser-induced band was observed. When the amino acid was used instead of the methyl ester or when Zn(II) was used instead of Al(III), no laser-induced band was observed. The interpretation of all this is that, to have observed the intermediate, only complexation of the *o*-hydroxyl was necessary and that chelation actually prevented formation of the photoinduced intermediate. The reasons why the complexation is effective have been stated. The reason why the $-OAl^{2+}$ complex can be discerned in the overall chelation is that the chelation rate is slow. Gansow and Holm[30] have found that on the ^1H NMR time scale exchange between chelated and free aldimine in D_2O is fast for Zn(II) and slow for Al(III). Their aldimine was formed from PL and alanine. For Zn(II) in our system the chelation was not slowed to the point that the $-OZn^+$ complex was present in amounts sufficient to have observed the *p*-quinoid structure. The carbonyl of the amino acid methyl ester in the aldimine would be expected to have less affinity for chelation than the carboxylate group. This would further reduce the rate of chelation and sustain the formation of the $-OAl^{2+}$ complex.

Laser Stimulation of the Intermediate. It was observed that at a higher pulse rate the absorbance of the laser-induced intermediate at first increased and then decreased below an initial value. This indicated that the intermediate may have exhibited a photoinduced reaction. To investigate this phenomenon the intermediate was approached from the other side of the dark equilibrium, i.e., from pyridoxamine (PM) plus ethyl pyruvate in the presence of Al(III). The same experimental approach was utilized. Since the 3371-Å excitation of the laser was removed too far from the absorption band at shorter wavelengths of a solution of Al(III) and the ketimine, absorption of the laser was not appreciable. Consequently, we did not observe the photoinduced band at 488 nm. In the presence of Al(III), however, on warming to 0 °C spontaneous formation of the *p*-quinoid structure did occur in the dark[6] and, after it was allowed to do so, photoexcitation produced the difference spectra shown in Figure 7. A band which decreased in intensity had a maximum at 495 nm while a band which had a maximum at 385 nm appeared. It was also observed that the

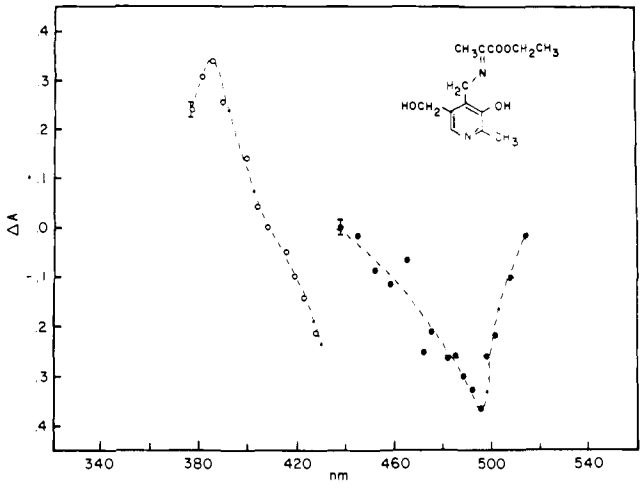


Figure 7. Difference spectrum of a repetitively laser-excited solution 2×10^{-3} M in each of pyridoxamine and Al(III) and 6×10^{-3} M in ethyl pyruvate in methanol at −50 °C. The pulse period is 120 ms. While both curves represent Al(III) binding, the open circles represent increased reaction.

increase at 385 nm did not occur within the 10-ns ultraviolet pulse. Clearly, something was consumed and something was produced in what must be a second laser-induced reaction in this system. The same observations were made in solutions of PL plus amino acid methyl ester in the presence of Al(III). The results are shown in Figure 8. Note that in the case of phenylalanine the maximum occurred at 488 nm, again a difference from the other of about 7 or 8 nm. The new band also appeared at 385 nm. Interestingly, the bands at the long wavelengths in Figures 7 and 8 were sharper than the previously observed band at 488 nm, i.e., they did not appear to have a shoulder on their short-wavelength side.

Since the first laser-induced reaction produced a band at 488 nm and the second laser-induced reaction resulted in a decrease in a band at 495 nm, which did not have the shoulder, we must consider in more detail the nature of the absorption band at 488 nm. Matsumoto and Matsushima[6] observed a band at 488 nm which they assigned to the *p*-quinoid structure and a shoulder at 452 nm which they assigned to a benzenoid species not protonated at the pyridine nitrogen but which had undergone proton labilization at the α carbon. This was done on the basis

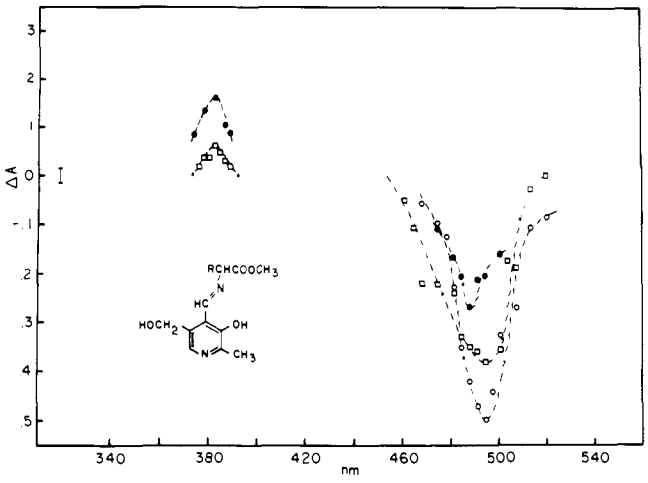


Figure 8. Difference spectra of repetitively laser-excited solutions 2×10^{-3} M in each of pyridoxal, amino acid methyl ester, and Al(III) in methanol at −50 °C. The pulse period is 120 ms. ●, phenylalanine; ○, valine; □, alanine.

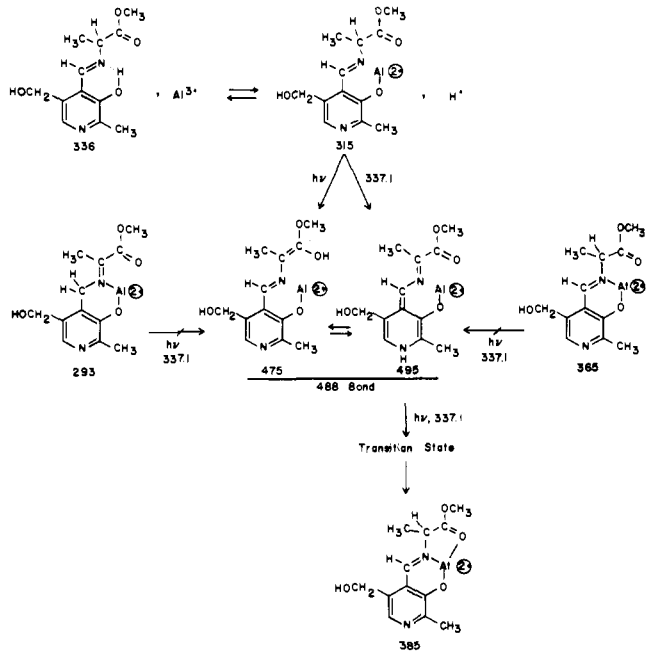


Figure 9. Scheme of laser-induced reactions in transamination. Numbers immediately beneath the figures represent identifying electronic absorption bands in nanometers. Chelates are 1:1 but may also be 2:1.

of concentration effects and acid–base behavior which demonstrated the 452-nm absorption not as a shoulder but clearly as a band more intense now than a band at 500 nm. In other words, the band at 488 nm with a shoulder appeared as two bands, one at 452 nm and one at 500 nm, each assigned to different structures. In several PLP–enzyme systems it also has been suggested that the shoulder at about 470 nm on the short-wavelength side of the intense band near 500 nm is due to vibronic fine structure.[31] In addition, another quinoid structure has been suggested as responsible for the shoulder.[32]

It is probable in the system considered here that, if two different species are responsible for the shoulder and the main absorption, they also will absorb coincidentally at 3371 Å, the laser emission. If the shoulder represents vibrational structure, then it also will be affected. What we observed was a decrease in a band at 495 nm and no change in the shoulder. It is likely, therefore, that two species are present. This band at 495 nm is closer to 500 nm where it was observed when clearly separated[6] and brings our results to near complete agreement.

Nature of the Laser-Produced Species. Since the Al(III) ion inhibited formation of the *o*-quinoid structure and since an α-methyl group on the amino acid prevented any further observable molecular changes on laser excitation, the products of the excitation of the complexed aldimine probably involved the α hydrogen. Once the proton is photolabilized, relaxation of the carbanion may proceed through the remaining three bonds, the C_α–C_β, C_α–$COOCH_3$, and C_α–NCH bonds. For alanine, phenylalanine, or valine the groups at C_β are not good leaving groups and would not be expected to contribute to the stability of the carbanion.[33] However, through the other two bonds, and associated groups, stabilization may be achieved with the structures shown in Figure 9. In this figure, generally the dark reactions are on the horizontal axis while the photoinduced reactions are on the vertical axis. At the top the complexation of the aldimine with Al(III) is shown. The structures expected after relaxation of the photoinduced carbanion are assigned to the bands indicated. The assignment by others of the band near 500 nm to the *p*-quinoid structure largely has been due to chemical evidence obtained in enzyme systems.[7,34] This has been supported with arguments[31] on the narrowness of the band being typical of "quinoid" bands. The 495-nm bands here qualify with an average width of 1.1×10^3 cm^{-1} at half height. It is also reasonable to assign the *p*-quinoid structure to the 495-nm band over the 475-nm band when the lengths of the conjugated systems are compared.

The assignment of the shoulder to the structure shown agrees with the assignment by others,[6] with some variances being the state of complexation and an estimated 23-nm bathochromic difference in the shoulder reported here. The assignment of a band at 467 nm to the similarly conjugated structure of an α-aminoacrylate Schiff base of PL[32] has been made. It is not well understood why this shoulder appears near 470 nm in some systems[31] and nearer 450 nm in others. It could be that the origin is different in different systems. In comparing the laser-induced reactions here with those in the dark[6] it could be that either bond rotations and angles are different or that association with the metal ion is different in the dark and photoinduced structures. The former alternative has been required for the explanation of spectra in photoinduced and dark reactions of Schiff bases of salicylaldehyde.[17]

The final product of the dark reaction of the ketimine with a metal ion in methanol is the chelated aldimine structure.[35] It is interesting that of the common ions which catalyze the transamination in methanol[6,35] only Al(III) exhibited the 488-nm band and formed ultimately a band at 365 nm, some 20 nm less than the final band of the other metal chelates. The Al(III) chelate of the Tris Schiff base of PL also absorbs at 365 nm. We observed that the chelate of the alanine Schiff base, however, absorbs at 377 nm, which is now in better agreement with the bands of the other chelates. This same band also was reported at 380 nm in H_2O.[36] We have already shown that the laser-induced intermediate does not form when chelation occurs. The fact that the observations could be made at all is because the chelation with Al(III) is slow, thus making the $-OAl^{2+}$ complex observable. We propose further that at equilibrium at room temperature the carbonyl group of the methyl ester of the chelated aldimine in methanol is not bound to the Al(III) and that only bidentate chelation occurs through the *o*-hydroxyl and the imine nitrogen. In fact it is probably better said that the slow chelation is the result of the decreased affinity of the ester carbonyl for the Al(III). For Zn(II) and for the carboxylate, the affinity being greater, tridendate chelation does result and the band occurs at or nearer 385 nm. The simple explanations here do not attempt to explain the complex equilibria involving both the 1:1 and 2:1 chelates.

Gansow and Holm[30] reported unbound carboxylate in D_2O solutions of the Al(III) chelate of the PL and alanine aldimine in equilibrium with the fully chelated structure. For the Zn(II) ion they found only the tridentate chelate. These results parallel ours. Because of the lower dielectric constant of methanol, the carboxylate group in this solvent will have greater affinity for the metal ion and is probably more bound than not in the Al(III) chelate of the PL and alanine aldimine. The ester carbonyl of the Al(III) chelate in methanol, however, remains unbound.

In the second laser-induced reaction which was from the ketimine side of the equilibrium, we searched to see if the photoexcitation of the intermediate would produce a band at 365 nm, indicative of the same product as the dark reaction. What was observed was a band at 385 nm. This band could also be produced by excitation of the intermediate formed from the aldimine side of the equilibrium. This is strong evidence that the structure responsible for the 385-nm band does result from the *p*-quinoid intermediate and not from something peculiar to either the aldimine or the ketimine structure. The formation of the band at 385 nm is, therefore, concomitant with the decrease in the band at 495 nm. Because of its position, its absorptivity relative to that of the 488-nm band, and $\Delta A = 0$ at 407 nm[6] we suspect that it is a fully chelated aldimine structure. That the laser excitation can cause the ester carbonyl to become chelated to the metal ion can be explained by the increased basicity of this group in the excited state. For example, the pK_a of ethyl-1-naphthoate-H^+ increases by eight orders of magnitude in the excited state.[16] In addition, since the *p*-quinoid intermediate is planar, conditions in the excited state are good for chelation. The enol group of the benzenoid structure which absorbs at 475 would be expected to be more acidic in the excited state and, therefore, would not favor chelation. This structure is similar in this regard to that which absorbs at 315 nm and which exhibits the photolabilization of an acidic hydrogen. An alternative structure could only result from photoionization of the Al(III). Since it did not in the first laser-induced reaction, it is not likely now and the anionic structure cannot be supported. It remains to be shown if this band results from a stable chelate. Kinetic studies on this and other structures are now being pursued.

Conclusions

Photoexcitation by the nitrogen laser of amino acid methyl ester Schiff bases of pyridoxal resulted in the formation of a *p*-quinoid structure which appeared identical with the key intermediate in transamination systems. Photolabilization of the α hydrogen of the amino acid was made possible by first complexing only the *o*-hydroxyl group of PL with Al(III). The photoexcitation produced an additional benzenoid structure whose absorption appeared as a shoulder at 475 nm on the 495-nm band of the *p*-quinoid structure. Subsequent laser excitation of the benzenoid and quinoid intermediates resulted in the conversion of the quinoid structure to another species which appeared to be that of a chelated aldimine.

These experimental observations and related conditions provide interesting thoughts on the transamination process. Since the first laser-induced reaction only occurred for the $-OAl^{2+}$ complex, we suspect that this complex is also active in the ground state metal catalysis. Excitation results in increased proton labilization but this did not occur for the more fully chelated aldimines. The suggestion that the weakly bonded chelates are more active in the catalysis[10] probably finds its truth in that the $-OM^+$ species is responsible and not the strongly bound chelate. It is highly interesting to compare our results with those obtained with the temperature-jump method by Hammes and Haslam.[32] They found in the interaction of *erythro*-β-hydroxyaspartic acid with aspartate aminotransferase that the quinoid species absorbing at 492 nm was a key intermediate, and that a species absorbing at 460 nm was in rapid equilibrium with the *p*-quinoid structure, but was not an additional intermediate in the main aldimine $\rightleftarrows$ ketimine pathway. We observed that a laser-induced reaction produces both benzenoid and quinoid structures absorbing at 475 and 495 nm, respectively, and that a second laser-induced reaction generated an aldimine only from the latter. We believe that these parallel results are more than coincidental. It appears that the laser stimulation, in general, facilitates the proton transfer required for transamination. Further experiments to include the kinetic studies are planned.

Acknowledgment. We are grateful for a grant-in-aid from the South Carolina Heart Association which supported part of this research and for assistance from a National Institutes of Health General Research Support Grant.

References and Notes

(1) D. E. Metzler, M. Ikawa, and E. E. Snell, *J. Am. Chem. Soc.*, **76**, 648 (1954).
(2) Y. Matsushima and A. E. Martell, *J. Am. Chem. Soc.*, **89**, 1331 (1967).
(3) D. S. Auld and T. C. Bruice, *J. Am. Chem. Soc.*, **89**, 2098 (1967).
(4) L. Schirch and R. A. Slotter, *Biochemistry*, **5**, 3175 (1966).
(5) J. R. Maley and T. C. Bruice, *J. Am. Chem. Soc.*, **90**, 2843 (1968).
(6) S. Matsumoto and Y. Matsushima, *J. Am. Chem. Soc.*, **96**, 5228 (1974).
(7) W. T. Jenkins, *J. Biol. Chem.*, **239**, 1742 (1964).
(8) Y. Morino and E. E. Snell, *J. Biol. Chem.*, **242**, 2800 (1967).
(9) D. E. Metzler and E. E. Snell, *J. Am. Chem. Soc.*, **74**, 979 (1952).
(10) F. P. Dwyer in "Chelating Agents and Metal Chelates", F. P. Dwyer and D. P. Mellor, Eds., Academic Press, New York, 1964, p 367.
(11) R. J. Johnson and D. E. Metzler, *Methods Enzymol.*, **18**, 433 (1970).
(12) For a review see J. D. Margerum and L. J. Miller in "Photochromism," G. H. Brown, Ed., Wiley, New York, 1971, p 557.
(13) K. Weber, *Z. Phys. Chem., Abt. B*, **15**, 18 (1931).
(14) T. Förster, *Naturwissenschaften*, **36**, 186 (1949).
(15) A. Weller, *Prog. React. Kinet.*, **1**, 189 (1961).
(16) J. F. Ireland and P. A. H. Wyatt, *Adv. Phys. Org. Chem.*, **12**, 131 (1976).
(17) T. Rosenfeld, M. Ottolenghi, and A. Y. Meyer, *Mol. Photochem.*, **5**, 39 (1973).
(18) W. F. Richey and R. S. Becker, *J. Chem. Phys.*, **49**, 2092 (1968).
(19) J. W. Bridges, D. S. Davies, and R. T. Williams, *Biochem. J.*, **98**, 457 (1966).
(20) D. E. Metzler and E. E. Snell, *J. Am. Chem. Soc.*, **77**, 2431 (1955).
(21) M.-D. Tsai et al., *Biochemistry*, **17**, 3177 (1978).
(22) P. Schenck and H. Metcalf, *Appl. Opt.*, **12**, 183 (1973).
(23) T. W. Hänsch, *Appl. Opt.*, **11**, 895 (1972).
(24) J. W. Ledbetter, *J. Phys. Chem.*, **72**, 4111 (1968).
(25) Y. Matsushima and A. E. Martell, *J. Am. Chem. Soc.*, **89**, 1322 (1967).
(26) A. Pocker and E. H. Fischer, *Biochemistry*, **8**, 5181 (1969).
(27) D. Heinert and A. E. Martell, *J. Am. Chem. Soc.*, **85**, 183 (1963).
(28) F. M. Beringer and I. Lillien, *J. Am. Chem. Soc.*, **82**, 5135 (1960).
(29) G. G. Hammes and J. L. Haslam, *Biochemistry*, **7**, 1519 (1968).
(30) O. A. Gansow and R. H. Holm, *J. Am. Chem. Soc.*, **91**, 573 (1969).
(31) L. Davis and D. E. Metzler in "The Enzymes", Vol. 7, 3rd ed., P. D. Boyer, Ed., Academic Press, New York, 1972, Chapter 2.
(32) G. G. Hammes and J. D. Haslam, *Biochemistry*, **8**, 1591 (1969).
(33) Y. Karube and Y. Matsushima, *J. Am. Chem. Soc.*, **98**, 3725 (1976).
(34) L. Schirch, M. Mason, and W. T. Jenkins, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **22**, 534 (1963).
(35) A. E. Martell and Y. Matsushima in "Pyridoxal Catalysis: Enzymes and Model Systems", E. E. Snell, A. E. Braunstein, E. S. Severin, and Yu. M. Torchinsky, Eds., Interscience, New York, 1968, p 33.
(36) L. Davis, F. Roddy, and D. E. Metzler, *J. Am. Chem. Soc.*, **83**, 127 (1961).