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C−H Activation in Pyridoxal-5′-phosphate and Pyridoxamine-5′-phosphate Schiff Bases: Effect of Metal Chelation. A Computational Study

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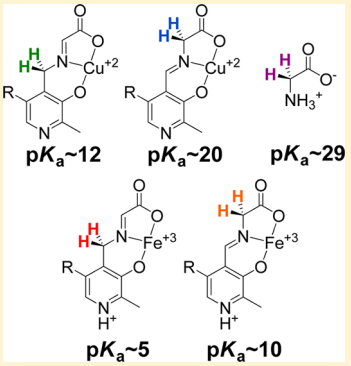
Supporting Information

ABSTRACT: This study reports the carbon acidities of Cα and C4′ atoms in the Schiff bases of pyridoxal-5′-phosphate (PLP) and pyridoxamine-5′-phosphate (PMP) complexed with several biologically available metal ions (Mg^{2+}, Ni^{2+}, Zn^{2+}, Cu^{2+}, Al^{3+}, and Fe^{3+}). Density functional theory calculations were carried out to determine the free energies of proton exchange reactions of a set of 18 carbon acids and a Schiff base used as a reference species. The experimental pK_a values of such carbon acids were used to calibrate the computed free energies in a range of 30 pK_a units. Eventually, the pK_as of the chelates were obtained by calculating the corresponding free energies against the same reference species and by considering the previous calibration. The carbon acidity of Cα in the chelates of Mg^{2+}, Ni^{2+}, Zn^{2+}, and Cu^{2+} varies between pK_a ∼22 and pK_a ∼13 whereas the pK_a values of C4′ range between ∼18 and ∼7. Chelation of trivalent metals Al^{3+} and Fe^{3+} causes further decrease of the pK_a values of Cα and C4′ down to ∼10 and ∼5, respectively. The results highlight the efficiency of the combined action of Schiff base formation and metal chelation to activate the Cα carbon of amino acids (pK_a ∼29 for zwitterionic alanine). Our results explain that the experimental increase of transamination rates by Zn^{2+} chelation is due to stabilization of the reactive Schiff base species with respect to the free ligand under physiological pH conditions. However, the increase in reactivity for transamination due to Cu^{2+} and Al^{3+} chelation is mostly due to C−H ligand activation. Each metal ion activates the Cα and C4′ carbon atoms to a different extent, which can be exploited to favor specific reactions on the amino acids in aqueous solution. Metal chelation hinders both intramolecular and intermolecular proton-transfer reactions of the imino, phenol, and carboxylate groups. This is the only apparent inconvenience of metal complexes in enzymatic reactions, which, in turn, proposes their consideration for enzyme inhibition.



INTRODUCTION

Pyridoxal-5′-phosphate (PLP) is one of the forms of vitamin B6 whose primary biological role is to act as a cofactor of more than a hundred different enzymes, mostly related to amino acid metabolism. PLP is covalently bound to the ε-amino group of a lysine residue in the active site forming a Schiff base known as internal aldimine. The first step in all the PLP-catalyzed reactions on amino acids is the replacement of such lysine residue by the substrate amino acid forming a new Schiff base called external aldimine.[1−5] Subsequently, one of the bonds of the amino acid Cα carbon undergoes a heterolytic cleavage causing the loss of one substituent and the formation of a carbanionic species (Scheme 1). This reaction is favored by the stabilization of the forming negative charge in the transition state across the delocalized π-system of the Schiff base. By means of this mechanism, PLP-dependent enzymes catalyze racemization, transamination, α-elimination, β- and γ-replacements, decarboxylation, and aldolic cleavage reactions.

Another vitamer of B6 is pyridoxamine-5′-phosphate (PMP), which also participates as intermediate of some of the PLP reactions since their Schiff base counterparts are tautomeric species (Scheme 1).[1−5] Pyridoxamine has another important biological role as inhibitor of nonenzymatic glycation of proteins and advanced glycation and lipoxydation end products (AGEs and ALEs).[6,7] Studies in our group showed that pyridoxamine forms Schiff base adducts with sugars and reactive carbonyl species (RCS), preventing their reactions with free amino groups of proteins and aminophospholipids.[8−12] However, additional studies suggest that the potential of pyridoxamine to inhibit AGEs and ALEs formation is most likely related to chelation of the metal ions that favor the oxidation of the Schiff bases and Amadori compounds formed between sugars and RCS and amino groups of proteins and aminophospholipids.[13−17]

Since all the PMP- and PLP-catalyzed reactions involve one or more proton abstractions and/or additions to a carbon atom, research on the acidities of such carbon atoms in PLP and PMP Schiff bases is of high interest and have recently been studied experimentally and computationally.[18−23] However, the experimental determination of carbon acidities is difficult and limited to significantly populated species,[19−21] so computa-

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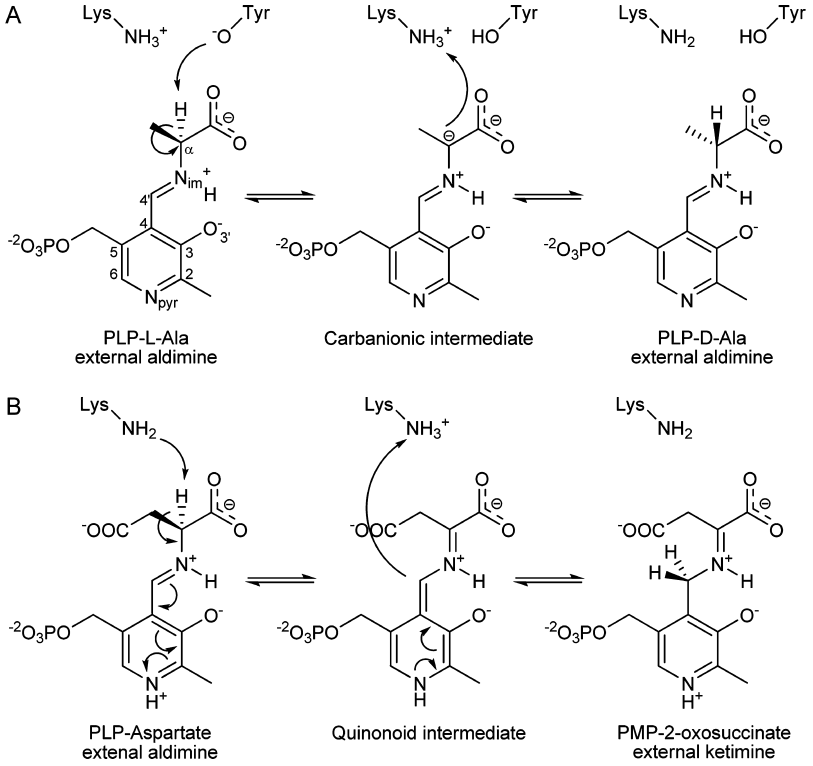
Scheme 1. Formation and Evolution of the Carbanionic Intermediates of PLP Schiff Bases According to Racemization (A) and Transamination (B) Reactions



tional studies are of high interest as they provide information that is inaccessible otherwise.[21]

Under physiological conditions of pH and also in enzyme active sites, PLP and PMP Schiff bases exhibit carbon acidity pK_a values between ∼12 and ∼23 depending on the protonation state of the phenol group, pyridine and imine nitrogen atoms.[18−23] Furthermore, such protonation state strongly modulates the pK_a difference of Cα and C4′ carbons between 1.5 and 7 units.[21] These data, together with examination of crystallographic active sites of different PLP-dependent enzymes, point out that high reaction specificity is achieved by controlling the protonation state of the external aldimine Schiff bases.[20,21,24,25] On the other hand, remarkably low pK_a values (i.e., ∼6 and ∼11) were reported for the Cα hydrogens of PLP-Gly Schiff bases at low pH by Richard and co-workers.[19] However, it should also be noted that Schiff bases are unstable in acidic conditions, which means that very low concentrations of the reactive form are available in solution.[19]

The early studies on vitamin B6 showed that PLP and PMP are also capable of catalyzing reactions on amino acids in nonenzymatic media.[4,5] Therefore, a significant effort was made to unravel the mechanisms of Schiff base formation and its dependence on the polarity of the environment.[26−34] In addition, transition metals were reported to form complexes with these Schiff bases by replacing the proton of the imine−phenol moiety, increasing their reactivity for transamination and other reactions on the Cα and C4′ atoms.[4,5,35] It was pointed out that metal ions favor PLP and PMP reactions by increasing the concentration of Schiff base, whose formation is required to initiate the transformations on the amino acids.[35] However, real activation of the Schiff base ligands by metal ions is not completely understood.[5,35] Considering the number of possible metals and their potential effects on the ligand carbon activation after complex formation, the application of our methodology for pK_a calculations[21] will help to clarify the origins of metal activation by comparison with the previously reported pK_a values of the free ligands. This will also provide interesting information concerning the general reactivity of PLP and PMP Schiff bases, particularly to explain the reason why there is no evidence of such Schiff base−metal complexes in any of the known PLP enzymes. In addition, the study of Schiff base−metal complexes is of interest as catalysts in organic synthesis as these compounds catalyze a broad number of reactions in aqueous solution and in a wide range of pH.[35]

This study reports the aqueous pK_a values of Cα and C4′ carbon atoms of the PLP and PMP Schiff bases formed with glycine and glyoxylic acid, respectively, complexed with some biologically available metals. Schiff bases with both protonated and unprotonated pyridine nitrogen forming coordination complexes with Mg^{2+}, Al^{3+}, Fe^{3+}, Ni^{2+}, Cu^{2+}, and Zn^{2+} metals were considered for the pK_a calculations.

Methodology and Computational Details. The pK_a calculations were performed by considering an isodesmic reaction (Scheme 2). According to this procedure, the free energy of a proton exchange reaction is calculated (eq 1) and related to the pK_a difference of the two acids involved in the reaction. Therefore, as shown in eq 2, it is straightforward to

Scheme 2. Proton Exchange Reaction between an Acid Species (AH) and a Reference Acid Molecule (RefH)[a]

$$AH^{q}_{(aq)} + Ref^{q'-1}_{(aq)} \xrightarrow{\Delta G_{aq}} A^{q-1}_{(aq)} + RefH^{q'}_{(aq)}$$

[a]The formal charges of the acids and the conjugate bases are represented by q/q' and $q - 1/q' - 1$, respectively.

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obtain the pK_a value of an acid (AH) by considering the pK_a of acid RefH and the calculated free energy of proton exchange.

$$\Delta G_{aq} = G_{aq}(\text{RefH}) + G_{aq}(\text{A}) - G_{aq}(\text{Ref}) - G_{aq}(\text{AH}) \quad (1)$$

$$\text{p}K_a(\text{AH}) = \frac{\Delta G_{aq}}{RT \ln 10} + \text{p}K_a(\text{RefH}) \quad (2)$$

An effective strategy to obtain good precision in the calculated pK_a values is to use an experimental pK_a value for RefH, which, in fact, is known as the reference acid species. It can be deduced from eq 1 that the isodesmic reaction scheme is designed to favor cancellation of systematic errors present in the individual free energies of each species. Besides, maximum accuracy in the pK_a predictions of the AH species is expected for those reactions that show ΔG_{aq} values close to zero (i.e., those species close in acidity with RefH) according to eq 2.

The pK_a values of PLP and PMP Schiff bases and other related carbon acids span over a range of ~20 units,[19−21] so it is expected that the acidities of Schiff base−metal complexes can also show considerably different pK_a values. In order to guarantee the accuracy of the predicted values in the entire pK_a range, eq 1 was used to calculate the free energies of proton exchange (ΔG_{aq}) of 18 carbon acids[19,36−41] (Scheme 3). Then, the experimental pK_a values of such carbon acids were plotted against the calculated free energies (ΔG_{aq}) to obtain the coefficients a and b that correct the terms in eq 2 for the studied species in the considered pK_a range (eq 3).

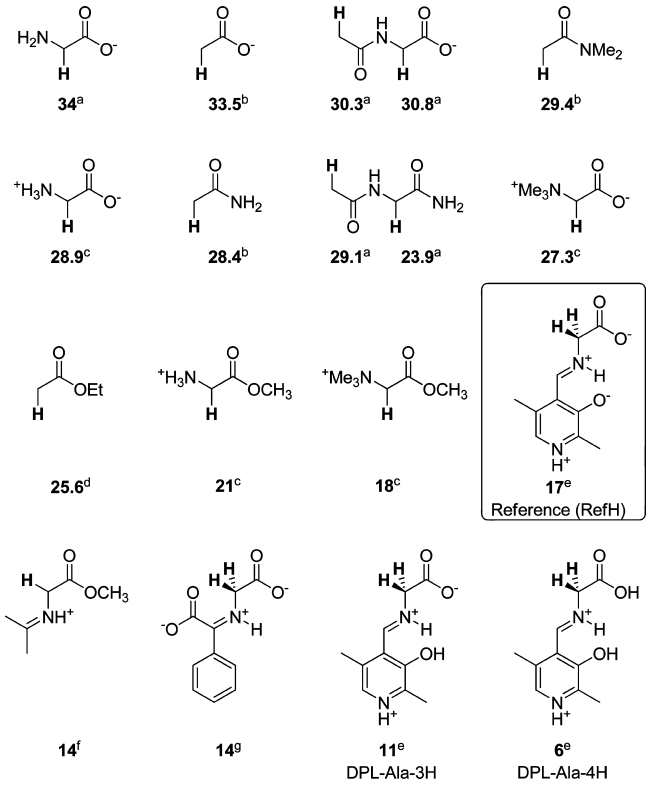
$$\text{p}K_a(\text{AH}) = a\frac{\Delta G_{aq}}{RT \ln 10} + b\text{p}K\text{a(RefH)} \quad (3)$$

This methodology was reported to provide pK_a values within ~0.5 units of error with respect to experimentally derived NMR values.[21] Both the set of carbon acids with experimental pK_a values and the reference acid species (RefH) used in this study (Scheme 3) are the same as the one previously used for the pK_a calculation of the uncomplexed Schiff base counterparts in solution.[21]

Density functional theory (DFT) calculations were carried out with the Gaussian09 package.[42] The M06 functional, recommended for accurate thermochemistry and calculations with transition metals,[43] was used in all the calculations in combination with the SMD solvent model.[44] Geometry optimizations and vibrational analyses were performed with the 6-31+G(d,p) basis set without using pseudopotentials for the metal ions. After characterization of all the structures as energy minima by the absence of imaginary frequencies, single-point energy calculations were carried out with the all-electron AUG-cc-pVDZ basis set. Finally, individual free energies in solution for each species were calculated by adding up thermal corrections to the free energies calculated at the M06/6-31+G(d,p) level without including the translational and rotational entropic contributions from the gas-phase partition functions[45] to the M06/AUG-cc-pVDZ energies. No scaling factor was used in computing the thermal corrections. NBO analyses were performed with module NBO 3.1 in G09 program to study the charge distributions of the metal complexes.[46]

Octahedral coordination geometries were considered for the chelates of Mg^{2+}, Zn^{2+}, Ni^{2+}, Al^{3+}, and Fe^{3+} metals, which were all considered as high-spin complexes in the case of Fe^{3+}, and square-planar coordination geometries for Cu^{2+} complexes. Since the Schiff bases of PLP and PMP are bonded as tridentate ligands, the three remaining coordination positions (one in the case of Cu^{2+} complexes) were completed with water molecules.

Scheme 3. Set of Carbon Acids and Their Experimental pK_a Values Used for the Linear-Regression Fit with the Calculated Proton Exchange Energies with Respect to the Acid Reference Species (RefH)[a]



[a]The experimental pK_a values were taken from refs 36 (a), 37 (b), 38 (c), 39 (d), 19 (e), 40 (f), and 41 (g).

RESULTS AND DISCUSSION

The plot of experimental pK_a values for the species in Scheme 3 against the calculated free energies of proton exchange reactions (ΔG_{aq}) shows a good linear correlation between such magnitudes (Figure 1). The regression equation obtained from the least-squares method provides a good coefficient of determination (i.e., R^2 = 0.981), which is in accordance with previous analogue correlations made for pK_a determinations of carbon acids in solution with the B3LYP and M06-2X functionals[21] and with a study of Klamt et al.[47] who reported an R^2 value of 0.984 for a set of 64 organic and inorganic acid species.

The regression coefficients a (0.68 ± 0.02) and b (0.97 ± 0.02) are also very similar to those found for B3LYP and M06-2X calculations.[21] The parameter a is important to ensure that the relative acidities are reliable in the entire pK_a range as its largest correction is to the calculated free energies of proton exchange reactions which involve much more basic or acidic species than the reference molecule (i.e., large ΔG_{aq} values). The coefficient b is very close to unity; that is, the y-intercept is close to the experimental pK_a value of the reference species. By using these regression coefficients, a mean absolute deviation of

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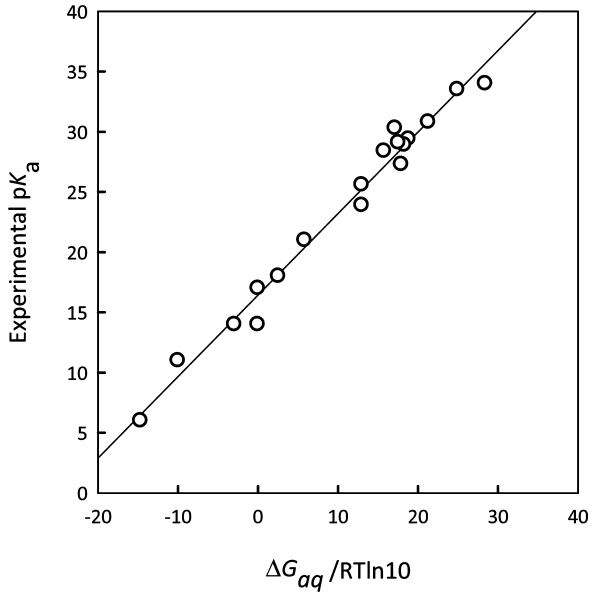

Figure 1. Experimental pK_a values vs the calculated free energies of proton exchange for the carbon acids depicted in Scheme 3.

0.8 units is found for the predicted pK_a values of the species depicted in Scheme 3. Therefore, the errors in the predicted acidities are of the same magnitude as the experimental uncertainties of carbon acid pK_a values.[19,21,36−41]

Additional regression analyses were carried out by considering the free energies of proton exchange obtained with the 6-31+G(d,p) basis set and the M06[43] or M06-L[48] functionals. In both cases, the coefficients of determination R^2 and the regression coefficients were very similar (see Table S1 and Figures S1 and S2 in the Supporting Information), which points out that most of the systematic errors originated in the choice of functional and basis set are efficiently canceled out in the calculation of reaction free energies due to the isodesmic reaction scheme (eq 1), and that the regression procedure principally corrects the errors caused by the solvent model.

pK_a Values of Schiff Base−Metal Complexes. The predicted pK_a values for the PLP and PMP Schiff bases complexed with metal ions are depicted in Scheme 4A. Additionally, the pK_a values of the uncomplexed Schiff base ligands obtained in previous work[21] are depicted in Scheme 4B for comparison purposes. In order to keep a consistency in the nomenclature of the species in Scheme 4, A and B, all PLP and PMP Schiff bases are labeled as Cα or C4′, respectively, designating the acidic carbon atoms. The H label stands for the pyridine protonated species. There is an additional label "M" only in the metal complexes that stands for the corresponding metal ion. The free ligand Schiff bases are numbered as "1" or "2" depending on whether the proton of the imine moiety is attached to the phenol oxygen or the imine nitrogen.

The optimized structures for Mg^{2+}, Ni^{2+}, Al^{3+}, and Fe^{3+} show octahedral geometries with minor distortions for both the PLP and PMP Schiff bases (Cα−M, CαH−M, C4′−M, and C4′H−M) and also for their carbanionic counterparts as exemplified in Figure 2 for three Fe^{3+} chelates. In the case of Zn^{2+} complexes, all the structures show octahedral geometries except for the common carbanionic species of CαH−Zn and C4′H−Zn. For such species, the metal atom is pentacoordinated according to a square pyramid where one apical water molecule is lost from the coordination sphere, remaining hydrogen-bonded to the carboxylate group of the Schiff base. On the other hand, all the Cu^{2+} chelates exhibit square planar geometries (Figure 3).

Examination of the optimized structures shows that the Hα−Cα−N_{im}−C4′ dihedral adopts values between 60° and 89° in all the chelates formed with PLP Schiff bases (Figures 2A and 3A). Similarly, the H4′−C4′−C4−C3 dihedral in the chelates formed with PMP Schiff bases adopts values between 58° and 74° (see Supporting Information for detailed values of each chelate) (Figures 2B and 3B). This result indicates that the most stable conformations after metal chelation show C−H bonds that are properly oriented for proton abstraction since the forming negative charge in the carbon atom can be easily delocalized across the π-system. Additionally, the entropic cost associated to reorganization of the chelate prior to the abstraction process is minimal. On the other hand, all the chelates resulting from deprotonation of either the Cα or the C4′ atoms exhibit geometries where the pyridine ring, the imine moiety and the metal ion are coplanar (Figures 2C and 3C). Therefore, the presence of the metal atom does not hinder the formation of a complete delocalized π-system, which is required for a maximum stabilization of the formed carbanion.

As shown in Scheme 4, the pK_a of Cα atom in Cα−M complexes with Mg^{2+}, Zn^{2+}, and Ni^{2+} divalent cations is approximately 22, very similar to the pK_a of the free Schiff bases Cα-1 and Cα-2. Protonation of the pyridine nitrogen in the metal complexes reduces the pK_a of Cα by 6−7 units down to values between 15 and 16, which are intermediate values with respect to CαH-1 and CαH-2 species. On the other hand, Cα−Cu and CαH−Cu copper complexes show lower pK_a values than the free Schiff bases shown in Scheme 4B and the complexes formed with other divalent cations.

On the other hand, complexation of Mg^{2+}, Zn^{2+}, and Ni^{2+} with PMP Schiff bases causes the carbon C4′ to show pK_a values between 17 and 18 for C4′−M species, and between 11 and 13 for C4′H−M species. Similarly to the previous observations of PLP Schiff base complexes, the pK_a of C4′ atoms in C4′−M and C4′H−M complexes are close to those in the free ligands C4′-1, C4′-2 and C4′H-1, C4′H-2, respectively. It is worth noting that, in C4′−Cu and C4′H−Cu complexes, the pK_a of C4′ is significantly lower (i.e., 12.5 and 6.9 units, respectively) than that observed in the rest of divalent metal complexes or free ligands. In fact, copper is the only divalent metal that forms complexes in which the Cα and C4′ atoms are always more acidic than the free ligands shown in Scheme 4.

Trivalent metals Al^{3+} and Fe^{3+} have a much larger effect than divalent metals on the pK_a values of Cα and C4′ atoms (Scheme 4). For the Cα−Al and Cα−Fe complexes, carbon Cα has a pK_a of 16, which is 5−6 units more acidic than free Schiff bases or divalent metal complexes. In pyridine-protonated Al^{3+} and Fe^{3+} complexes, carbon Cα exhibits a pK_a value of ∼10, thus being 3−7 pK_a units more acidic than Cα in the free ligands and complexes with divalent metals, and comparable to common organic acids such as phenols or amines. These results are in agreement with early studies which report that, in aqueous solution, PLP Schiff bases complexed with Al^{3+} are deprotonated, yielding a carbanion species in concentrations high enough to be measured by NMR methods.[5,49]

Chelates of PMP Schiff bases with Al^{3+} and Fe^{3+} (i.e., C4′−M and C4′H−M in Scheme 4) exhibit pK_a values around 10 and 5, respectively, for C4′ carbons. These values are remarkably low for carbon atoms and, also, between 5 and 8 units more acidic than C4′ in free Schiff bases and complexes with Mg^{2+}, Zn^{2+}, and Ni^{2+}. However, it should be noted that copper

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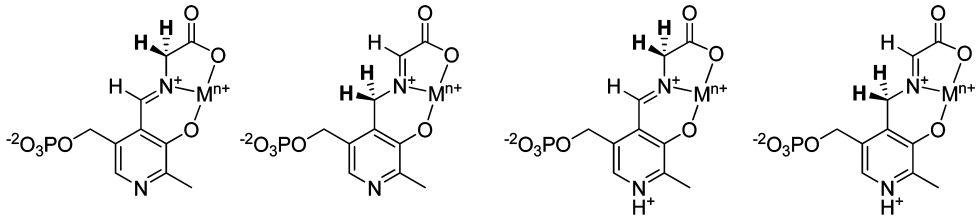
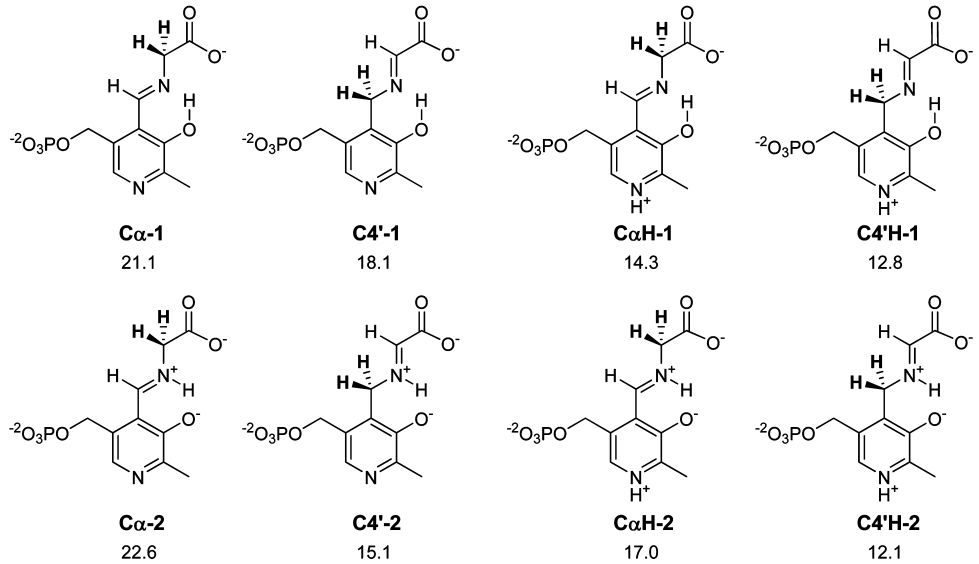
Scheme 4. Calculated pK_a Values of PLP and PMP Schiff Base Metal Complexes (A) and pK_a Values of Free PLP and PMP Schiff Bases (B)[a]

A



M^{n+}	Cα-M	C4′-M	CαH-M	C4′H-M
Mg^{2+}	22.3	17.9	15.8	12.9
Zn^{2+}	21.9	17.4	14.8	11.6
Ni^{2+}	21.7	17.1	15.5	11.0
Cu^{2+}	20.3	12.5	13.1	6.9
Al^{3+}	16.0	9.3	9.4	4.8
Fe^{3+}	16.0	10.2	10.6	4.9

B



Cα-1	C4′-1	CαH-1	C4′H-1
21.1	18.1	14.3	12.8
Cα-2	**C4′-2**	**CαH-2**	**C4′H-2**
22.6	15.1	17.0	12.1

[a]Cα and C4′ stand for the PLP and PMP Schiff bases, respectively; the M label stands for each specific metal ion in the complexes whereas labels "1" and "2" stand for the tautomers protonated at the phenolic oxygen (O3′) or at the imine nitrogen atoms, respectively, of the free Schiff bases. The "H" label designates the structures protonated at the pyridine nitrogen. The acidic protons are depicted in bold. The pK_a values of the free Schiff bases are taken from ref 21 (obtained from DFT/B3LYP calculations and the same statistical procedure of the present work).

complexes C4′−Cu and C4′H−Cu show pK_a values at C4′ which are closer to aluminum and iron complexes than to other divalent cations.

Deeper inspection of the optimized geometries shows that the C−H activation is directly related to the ligand polarization caused by the metal ion. The distances between the metal ion and the O3′ or the carboxylate oxygen atoms of the Schiff bases show the following trend: $d(Mg^{2+}) \sim d(Zn^{2+}) > d(Ni^{2+}) > d(Cu^{2+}) \sim d(Fe^{3+}) > d(Al^{3+})$, while the distances between the metal and the imine nitrogen decrease according to $d(Mg^{2+}) > d(Fe^{3+}) > d(Zn^{2+}) > d(Ni^{2+}) > d(Al^{3+}) > d(Cu^{2+})$ (see Supporting Information for a detailed report of the bond distances). These data explain why the pK_a values of Cu^{2+} chelating Schiff bases are intermediate to those of Schiff bases chelated with divalent and trivalent metals (Scheme 4).

Examination of the formal charges calculated in the NBO analysis indicates that the charge on each metal is approximately constant (i.e., $q(Mg^{2+}) = 1.71$, $q(Zn^{2+}) = 1.58$, $q(Ni^{2+}) = 1.42$, $q(Cu^{2+}) = 1.34$, $q(Al^{3+}) = 2.49$, and $q(Fe^{3+}) = 2.04$). So, the average transferred charges from the ligand to each metal are easily calculated from such values: $\Delta q(Mg^{2+}) = -0.29$, $\Delta q(Zn^{2+}) = -0.42$, $\Delta q(Ni^{2+}) = -0.58$, $\Delta q(Cu^{2+}) = -0.66$, $\Delta q(Al^{3+}) = -0.51$, and $\Delta q(Fe^{3+}) = -0.96$, where the negative sign indicates transference of negative charge (see Supporting Information for further details on the formal charges of each complex). These results show that carbon acidities of Schiff base ligands increase according to the charge

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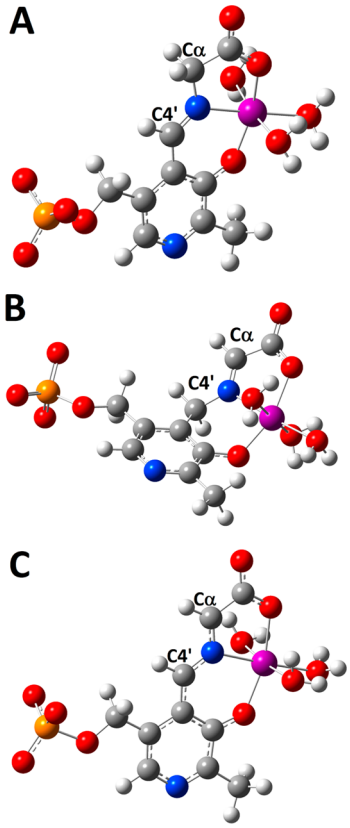

Figure 2. Optimized geometries of Cα–Fe (A), C4′–Fe (B), and carbanion–Fe (C) complexes.

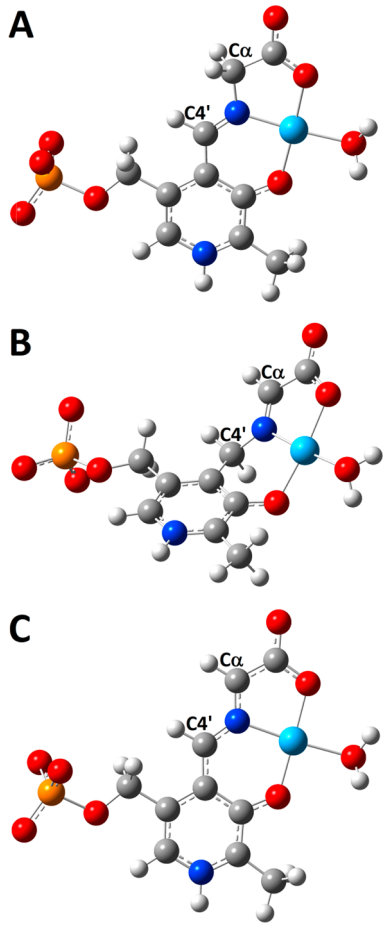

Figure 3. Optimized geometries of CαH–Cu (A), C4′H–Cu (B), and carbanionH–Cu (C) complexes.

transferred to the metal ion. Additionally, these values also explain that the C–H activation by Cu^{2+} is the largest among the studied divalent metals. Eventually, it is worth noting that similar pK_a values are found for Al^{3+} and Fe^{3+} chelates despite their differences in polarization of the ligand. The formal charges indicate that ligand polarization (i.e., covalent effects) is more important in Fe^{3+} chelates, whereas electrostatic effects are predominant in their Al^{3+} counterparts.

Considering that the reported pK_a value of zwitterionic alanine in aqueous solution is approximately 29,[37] the calculated pK_a values of the complexes show the high efficiency of the combined action of Schiff base formation and metal chelation in enhancing the acidity of the Cα of free amino acids. However, it is important to note that none of the metal ions in the complexes reduces the pK_a of the carbon atoms as efficiently as the addition of the equivalent amount of positive charge in the form of individual protons to the imine moiety. The Schiff bases of 5′-deoxypiridoxal and glycine with the protonated pyridine, imine, and phenol groups (DPL-Ala-3H in Scheme 3) exhibit pK_a 11 at the Cα carbon[19] whereas the predicted pK_a values of Cα in all complexes with divalent metal ions are between 13 and 16 (CαH–M in Scheme 4A). Likewise, the Schiff bases that additionally have a protonated carboxylate group (DPL-Ala-4H in Scheme 3) show pK_a 6 at the Cα carbon[19] whereas that of CαH–M complexes with Al^{3+} and Fe^{3+} is approximately 10 (Scheme 4). These results are in agreement with previous suggestions of Martell[49] who reported that a single proton bonded to the imine nitrogen stabilizes the negative charge of the carbanionic intermediates more efficiently than divalent transition metals and hypothesized that trivalent transition metals should have larger effects.

The carbon acidities of the free Schiff bases shown in Scheme 4B and their complexes with Mg^{2+}, Zn^{2+}, and Ni^{2+} metals (Scheme 4A) are very similar. Therefore, the experimentally observed increase in transamination rates by Zn^{2+} complexation[5] has to be attributed to an increase in the concentration of the Schiff base due to its stabilization as a ligand in the chelate. Likewise, the experimentally measured increase in transamination rates after Cu^{2+} complexation[5] is also partly attributable to an increase in concentration of the reactive species. Nevertheless, according to our calculations, the Cu^{2+} ion causes real ligand C–H activation, especially for the Schiff bases of PMP and keto acids (Scheme 4). With respect to complexes with trivalent metals, our pK_a calculations indicate that metal chelation increases the carbon acidity of the ligand with respect to the uncomplexed Schiff bases that bear a single proton in the imine moiety (Scheme 4).

As observed in Scheme 4, the C4′ carbons of PMP Schiff bases are more acidic than the Cα carbons of PLP Schiff bases both in the free ligands and in the complexes. This means that the PLP Schiff bases are the more stable tautomers in aqueous solution. This result is in agreement with the reported observation that, due to the spontaneous 1,3-prototropic shift of the Schiff bases of PMP–pyruvate and PLP–alanine complexed with Al^{3+}, the latter species is the major tautomer in solution.[49]

In another study, the PMP^{3-} and pyruvate Schiff base complex with Cu^{2+} was reported to undergo spontaneous transamination whereas the equivalent Zn^{2+} complex does not

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unless the pyridine nitrogen is protonated.[5] According to our results (Scheme 4A), C4′−Cu species exhibits a pK_a value of 12.5, acidic enough to be deprotonated by common bases in aqueous solution whereas the pK_a of C4′−Zn is 17.4, significantly higher. However, the pyridine protonated C4′H−Zn complexes show pK_a values of 11.6, close to the acidity of C4′−Cu complexes.

Additional studies show that Schiff bases of PMP^{3-} and pyruvate protonated on the imine nitrogen have slower transamination rates than Schiff bases complexed with Zn^{2+} protonated on the pyridine nitrogen, but similar to those of free Schiff bases protonated on both the imine and pyridine nitrogens.[5] As shown in Scheme 4, the pK_a value of C4′-2 species is approximately 3.5−5 units higher than that of C4′H−Zn, which explains that the protons at C4′ are more available to be removed by bases in the latter complexes. Besides, the acidity of C4′ carbon in C4′H−Zn and C4′H-2 species is similar and, therefore, similar deprotonation rates are expected.

Implications on Enzyme Catalysis. Nonenzymatic complex formation of PLP and PMP Schiff bases with Zn^{2+}, Ni^{2+}, Cu^{2+}, Al^{3+}, and Fe^{3+} was studied in numerous experiments to model PLP-catalyzed reactions.[4,5,35,49−51] Here, we have also considered chelates of PLP and PMP Schiff bases with Mg^{2+} because it is a bioavailable abundant ion. However, it has to be noted that there is no evidence of PLP acting as enzyme cofactor in the form of metal chelates.[4,5,35,51]

The presence of metal ions is clearly inconvenient for some reactions as decarboxylations at the Cα carbon since the carboxyl group chelates the metal in the PLP Schiff bases, preventing its cleavage.[51] However, comparison of the pK_a values of Schiff bases in enzymes (i.e., pK_a 19 for Cα in Alanine racemase[22]) and our results indicates that Schiff base metal complexes exhibit adequate carbon acidity in reactions involving deprotonation and reprotonation of carbon atoms.

Several factors that should be taken into account point out that Schiff base complexes are more advantageous than the free Schiff bases: (1) metal ions stabilize the reactive Schiff base species;[4,5,35,49,51] (2) metal complexation causes a significant decrease in the pK_a values of Cα and C4′ with respect to the free Schiff bases under physiological pH conditions (Scheme 4); and (3) metal ions are good anchor points for the external aldimines by interaction with nucleophile side chains in a hypothetical active site. Contrarily, according to Toth and Richard,[19] the free Schiff bases that exhibit the lowest pK_a values (1) have lower formation constants than those of lesser protonated species (i.e., those shown in Scheme 4B); (2) full protonation of the imine moiety involves a very large thermodynamic barrier under physiological pH conditions; and (3) full protonation of the imine moiety would prevent interactions with oppositely charged residues in the active site.

Comparison of enzymatic and nonenzymatic studies indicates that carbon acidities of PLP Schiff bases in aqueous solution provide a reasonably good description of carbon acidities in enzymatic active sites.[19−24,52] For example, the carbon acidity of the PLP-Ala Schiff base in alanine racemase is very similar to that of PLP-Gly in aqueous solution.[19−22] It is worth noting that crystallographic studies show high presence of polar and charged residues in the active sites of many PLP enzymes, resembling polar/protic solvent environments with highly specific interactions.[1−3,22−24]

In fact, the latter studies on PLP-catalyzed reactions suggest that high portion of the catalytic power in enzymatic reactions resides in the PLP cofactor.[1,19−25,51,53] Therefore, apart from reducing the free energy barriers, the role of the enzymes would be to drive the reprotonation of carbanionic species by favoring certain protonation states of the Schiff bases in the active sites.[1,19−25,51,53] Particularly, the pK_a difference between Cα and C4′ is mostly determined by the protonation of the imine moiety. A protonated imine and deprotonated phenoxide groups favor the reprotonation of the carbanionic intermediates at Cα since this carbon is up to 7 pK_a units more basic than C4′ (Cα-2 versus C4′-2 in Scheme 4B). If the proton is transferred from the imine nitrogen to the phenol oxygen, the pK_a difference is reduced down to 1.5 units for pyridine protonated species (CαH-1 versus C4′H-1 in Scheme 4B), which favors the reprotonation at C4′ carbon as occurs in transamination reactions.[21,25,52]

On the other hand, this mechanism of reactivity modulation is not possible for the chelates as the metal ion is bonded simultaneously to the imino, phenoxide, and carboxylate groups. However, as pointed out by the results in Scheme 4A, each metal ion has a different effect on the acidities of Cα and C4′. Consequently, in principle there is no need to modulate the relative acidity of such carbon atoms by proton transfer between the imino and phenoxyde groups because a specific metal can be chosen to establish the proper ΔpK_a between Cα and C4′ for each reaction. For example, complexes of Mg^{2+} and Zn^{2+} would be appropriate for transamination reactions since the ΔpK_a is low (i.e., approximately varies between 3 and 4.5 units) whereas Cu^{2+} is clearly convenient to achieve high specificity in racemization reactions since the Cα carbon is 6−8 pK_a units more basic than C4′.

Intramolecular proton-transfer reactions between the imine and phenol groups as well as intermolecular proton transfer with water solvent molecules or enzyme residues are ubiquitous in PLP and PMP reactions. In fact, their relevance was highlighted by different studies on nonenzymatic and enzymatic PLP Schiff base formation,[54−56] nonenzymatic and enzymatic transimination,[57,58] nonenzymatic PMP Schiff base formation,[12] and decarboxylation reactions.[59] However, such proton exchange reactions are blocked by the metal ions in the Schiff base complexes. Consequently, the previous reactions are expected to be hindered for the chelates.

Considering the advantages in stability and carbon acidity of the complexed Schiff bases, the absence of chelates in enzymes points out that proton-transfer reactions involving imino and phenol groups are crucial in the PLP and PMP chemistry. This also suggests potential strategies of inhibition of PLP-dependent enzymes based on the cancellation of proton-transfer reactions in the imine moiety by means of modification of the contiguous enzymatic residues or Schiff base formation with artificial substrates that block the imine and phenol groups. Eventually, complexation with metal ions can either enhance or inhibit enzyme activity depending on (1) the stabilization of the reactive Schiff base with respect to the uncomplexed state; (2) the proper Schiff base activation for the main reaction; and (3) the modification of the kinetics of Schiff base formation and hydrolysis due to the blockade of proton-transfer reactions on the imine moiety.

■ CONCLUSIONS

Metal ions activate Cα and C4′ carbons in the complexes of PLP and PMP Schiff bases, although none of the studied M^{n+} metals activates the Schiff bases as efficiently as the addition of the same amount of positive charge in the form of protons to the imine moiety. At physiological pH, Schiff bases are

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monoprotonated in the imine moiety and, with respect to these species, the complexes of divalent metals Mg^{2+}, Zn^{2+}, and Ni^{2+} show similar pK_a values. Therefore, the experimental enhancement of transamination rates should be attributed to the stabilization of the complexed Schiff base and the resulting increase in concentration. Complexes with Cu^{2+} exhibit higher intrinsic ligand activation, especially at the C4′ position in PMP Schiff bases. Trivalent metals Al^{3+} and Fe^{3+} increase the acidity of Cα and C4′ atoms up to the point that the complexes are as acidic as the imine-moiety-diprotonated Schiff bases. On the other hand, each metal activates the Cα and C4′ carbons to a different extent, so it is possible to select specific metals to favor particular reactions. The only apparent inconvenience of complexes for enzymatic reactions is that chelation of metal ions hinders proton-transfer reactions on the imine moiety. However, this can be considered for enzyme modulation and/or inhibition.

■ ASSOCIATED CONTENT

Supporting Information

Regression analyses and pK_a values of the complexed Schiff bases calculated from the SMD/M06/6-31+G(d,p) and SMD/M06L/6-31+G(d,p). Cartesian coordinates of the optimized geometries of the complexed Schiff bases and their corresponding thermodynamic data. Potential energies calculated at the SMD(water)/M06/aug-cc-PVDZ level corresponding to the optimized geometries at the SMD(water)/M06/6-31+G(d,p) level. NBO formal charges of the imine, pyridine ring, and metal moieties of each complex. O3′−M, Nim−M, and O(COO)−M metal ligand bond distances. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Notes

The authors declare no competing financial interest.

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