Complexing of Zinc(II) in the Transamination System: α-Ketoglutarate + α-Alaninate ⇌ Glutamate + Pyruvate[1]

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Abstract: The constants for the formation of the Zn(II) Schiff base complexes of α-ketoglutarate and pyruvate, each with α-alaninate and glutamate, have been determined. The stabilities of the Schiff base complexes decrease with an increase in the size of the ligand side chains owing to increased steric interactions. Tautomerization constants have been calculated. For the uncomplexed Schiff base, β_T is 0.6 for $Pyr\cdot Glu^{3-} \rightleftharpoons Akg\cdot Ala^{3-}$; however, for complexed Schiff base, β_{ZnT} is 0.08 for $Zn(Pyr\cdot Glu)^- \rightleftharpoons Zn(Akg\cdot Ala)^-$. The relative increase in the stability of N-pyruvylideneglutamate over that of N-α-ketoglutarylidenealaninate on complexing arises from the stereochemistry imposed on the Schiff base by the configuration of the bonds about the imine group. Evidence for the formation of the quaternary complexes, Zn(Pyr,Glu,Ala) and $Zn(Pyr_2,Glu,Ala)$, has been obtained. It appears that the formation constants for these kinds of complexes may be estimated reasonably well from the binding properties of the binary and ternary species. The first of these complexes is approximately a 1:1 mixture of the coordination isomers $Zn(Pyr\cdot Glu)(Ala)^{2-}$ and $Zn(Pyr\cdot Ala)(Glu)^{2-}$. The second comprises the "mixed" Schiff base $Zn(Pyr\cdot Glu)(Pyr\cdot Ala)^{3-}$.

A series of investigations has been underway in our laboratories concerning equilibria and kinetics of reactions in aliphatic and aromatic Schiff base complex ion systems.[2-4] Data suggest[3] that tautomeric forms of isomeric Schiff base complexes may have appreciably different stabilities, but the general lack of information regarding the relative stabilities of the keto acids and amino acids for the systems studied has prevented this point from being firmly established. For the biologically important transamination

$$Akg^{2-} + HAla^{\pm} \rightleftharpoons {}^{\pm}HGlu^- + Pyr^- \qquad (1)$$

Krebs[5] has determined the equilibrium constant to be 0.67 at pH 7.4.[6] Determination of the formation constants for $Zn(Akg\cdot Ala)^-$ and $Zn(Pyr\cdot Glu)^-$, together with the constants reported for reaction 1 and the pK_a values of the amino acids, should allow the evaluation of the tautomerization constant, K_{ZnT}, for the reaction

$$Zn(Pyr\cdot Glu)^- \overset{K_{ZnT}}{\rightleftharpoons} Zn(Akg\cdot Ala)^- \qquad (2)$$

and thereby determine conclusively, for this system at least, the effect of complexing on the relative stabilities of the tautomers.

Furthermore, the interest in the catalytic effect of metal ions on nonenzymatic transamination processes[7-9] increases the importance of equilibrium data on transamination systems. Metal ions catalyze the reaction by forming stable Schiff base complexes with the reactant amino and keto acids, and, as the reaction proceeds, form complexes of a similar nature with the products. In addition the opportunity for forming cross-species, *e.g.*, between reactant amino acid and product keto acid, also increases as products build up. Another goal of the present work was to establish whether or not such species may be sufficiently important to take into consideration in nonenzymatic metal ion catalysis studies.

Experimental Section

Sodium pyruvate (Sigma Chemical Co., dimer free) was found to be 99.2 ± 0.1% pure by alkalimetric titration, after passing an aliquot through a cation exchange column in the acid form, and through absorbance measurements at 315 mμ (ε 22.1). Titration[10] of *dl*-α-alanine and *l*-α-alanine (Sigma Chemical Co.) and recrystallized *l*-glutamic acid (Matheson Coleman and Bell) yielded purities of 100.0 ± 0.1%. α-Ketoglutaric acid (Nutritional Biochemicals) was found to be 99.8 ± 0.2% by titration with standard base.

pH measurements were performed using a Radiometer instrument (pH 26) with the electrodes standardized *vs.* NBS buffers. Titration curves for the binary and ternary systems were obtained as previously described, as was also the evaluation of the stability constants.[2-4] Equilibration was rapid in the binary systems, but 20–60 min was required for the systems where Schiff base complexes were formed.

In the various titrations, levels of keto acid were varied in the range 0.01 to 0.05 *M* at 0.01 *M* Zn(II). Typically good[2-4] fits of calculated to observed titration curves were obtained at all keto acid levels, indicating the negligible effect of keto acid dimerization.[11,12] The negligible effect of pyruvate dimerization under these experimental conditions was verified theoretically using the constants determined for this system.[11] Long range (day to day) irreversible changes accompanied by CO_2 release may, however, arise from slow keto acid polymerization.

Results and Discussion

The protonation constants found in this work for the ligands and the formation constants for their Zn(II) complexes are given in Table I, along with pyruvate

(1) This work was supported by a grant from the National Science Foundation.

(2) D. Hopgood and D. L. Leussing, *J. Amer. Chem. Soc.*, **91**, 3740 (1969), and references cited therein.

(3) D. L. Leussing and E. Hanna, *ibid.*, **88**, 693 (1966).

(4) D. L. Leussing and D. C. Schultz, *ibid.*, **86**, 4846 (1964).

(5) H. A. Krebs, *Biochem. J.*, **54**, 82 (1953).

(6) Abbreviations used in this article: Pyr^-, pyruvate, $CH_3COCO_2^-$; Ala^-, α-alaninate, $H_2NCH(CH_3)CO_2^-$; Akg^{2-}, α-ketoglutarate, $^-O_2CCH_2CH_2COCO_2^-$; Glu^{2-}, glutamate, $^-O_2CCH_2CH_2CH(NH_2)CO_2^-$; $Pyr\cdot Ala^{2-}$, N-pyruvylidenealaninate, $^-O_2CC(CH_3)=NCH(CH_3)CO_2^-$; $Pyr\cdot Glu^{2-}$, N-pyruvylideneglutamate, $^-O_2CC(CH_3)=NCH(CO_2^-)CH_2CH_2CO_2^-$; $Glu\cdot Ala^{3-}$, N-α-glutarylidenealaninate, $^-O_2CCH_2CH_2C(CO_2^-)C=NCH(CH_3)CO_2^-$.

(7) A. E. Martell and Y. Matsushima, "Pyridoxal Catalysis," E. E. Snell, A. E. Braunstein, E. S. Severin, and Yu. M. Torchinsky, Ed., John Wiley and Sons, New York, N. Y., 1968, p 33.

(8) O. A. Gansow and R. H. Holm, *J. Amer. Chem. Soc.*, **90**, 5629 (1968).

(9) D. E. Metzler, M. Ikawa, and E. E. Snell, *ibid.*, **76**, 648 (1954), and other papers in this series.

(10) M. S. Dunn and A. Loshakoff, *J. Biol. Chem.*, **113**, 359 (1936).

(11) D. E. Tallman and D. L. Leussing, *J. Amer. Chem. Soc.*, **91**, 6253, 6256 (1969).

(12) A. E. Martell and E. H. Abbott, Proceedings of the XII International Conference on Coordination Chemistry, Sydney, Australia, Aug 1969.

Table I. Log of Protonation and Zinc Complex Stability Constants[a]

A. Binary Species

	Protonation constants[b]			Zinc constants[c]		
	K_1	K_{12}	K_{13}	β_1	β_2	β_3
Pyruvate, Pyr^-	2.35			1.258	1.98	
α-Ketoglutarate, Akg^{2-}	4.592	1.997		1.13	1.7	
dl-α-Alanine, Ala^-	9.818	2.440		4.564	8.558	10.578
l-Glutamate, Glu^{2-}	9.556	4.153	2.254	4.487	8.251	9.8

B. Ternary Species[d]

$$Zn^{2+} + iO{=}jA \rightleftharpoons Zn(O)_i(A)_j + iH_2O, \quad \beta_{ij} = [Zn(O)_i(A)_j]/[O]^i[A]^j$$

$$O + A \rightleftharpoons OA, \quad \beta_{OA} = [OA]/[O][A]$$

O / A	Pyruvate				α-Ketoglutarate			
	β_{11}	β_{12}	β_{22}	β_{OA}	β_{11}	β_{12}	β_{22}	β_{OA}
dl-α-Alanine	7.09 ± 0.02	11.18 ± 0.07	13.28 ± 0.03	0.06	5.97 ± 0.02	10.00 ± 0.02		−0.11
l-Glutamate	6.974 ± 0.015	10.64 ± 0.05	12.38 ± 0.05	0.03	5.64 ± 0.04			

[a] 25°, μ = 0.5 (KCl). [b] $K_1 = [HL]/a_H[L]$. [c] $\beta_n = [ZnL_n]/[Zn^{2+}][L]^n$. a_H represents hydrogen ion activity, brackets designate concentration. [d] O = oxoacid anion, A = amino acid anion.

values from ref 4. Weak complexing prevented an accurate assessment of the higher Akg–Zn(II) species due to interference from hydroxide precipitation. In accordance with previous observations[13] no effect was observed on substituting *l*-alanine for the *dl* mixture.

The uncomplexed Schiff bases investigated in this work are unstable with respect to hydrolysis, as shown by the small β_{OA} values in Table I. The stabilities are lower even than that found for N-pyruvylideneglycinate[4] ($\log \beta_{Oa} \sim 0.3$). The trend of decreasing stability with increasing size of side chain probably arises from the combined effects of increased steric hindrance, which is more pronounced with aliphatic Schiff bases than with aromatic, with a parallel decrease in entropy, entropy becoming more negative with increasing molecular volume. Interaction in the Akg^{2-}–Glu^{2-} system was too slight to be detected.

Pyruvate, Pyr^-, is seen to form more stable Schiff base–zinc(II) complexes than does Akg^{2-} with both the amino acid anions Ala^- and Glu^{2-}. These amino acid anions form a typical series of complexes in which the Zn(II):Pyr^-:amino acid anion ratios are 1:1:1, 1:1:2, and 1:2:2. Comparison with glycinate[4] shows that the stabilities of the 1:2:2 complexes, the bis Schiff base complexes, fall off relative to the 1:1:1 species with increasing size of the α substituent of the amino acid. This would seem to arise from steric interactions. The 1:1:1 complexes also appear to exhibit the effect of steric interactions since the ligand enhancement factors (LEF = $\log \beta_{11} - \log \beta_{01} - \log \beta_{10}$) are 1.46, 1.31, and 1.22 for Zn(Pyr·Gly), Zn(Pyr·Ala), and Zn(Pyr·Glu)$^-$, respectively. A decrease in LEF indicates a decrease in the stability of the condensed ligands relative to the mixed complex in which the ligands are independently bound to the metal ion. The stability of this latter type of complex is less sensitive to ligand–ligand interactions, owing to greater ligand–ligand distances. The differences in the stabilities of the monopyruvate Schiff base complexes are similar to the differences in the stabilities of the corresponding free Schiff bases, indicating that in these lower complexes the effect has its origin in the Schiff bases themselves rather than in Schiff base–coordinated water molecule interactions.

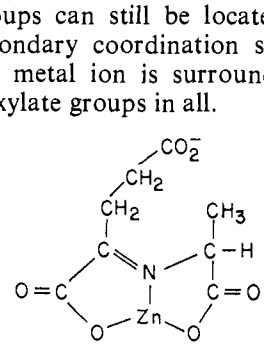
(13) R. D. Gillard, *Inorg. Chim. Acta*, 1, 69 (1967).

Owing to much weaker binding in the Akg^{2-} series, not only are the 1:2:2 species not observed, but the LEF values are calculated to be 0.28 for Zn(Akg·Ala)$^-$ and 0.02 for Zn(Akg·Glu)$^{2-}$. This last result indicates that the ligands for the most part are independently bound as Zn(Akg)(Glu)$^{2-}$ rather than as the Schiff base, Zn(Akg·Glu)$^{2-}$.

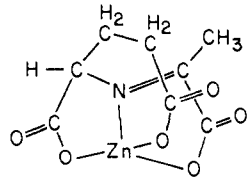
From the value reported for the constant of reaction 1[5] and the β_{OA} values of Table I it is calculated that the free Schiff base anions have comparable stabilities with respect to tautomerization, *i.e.*, $\beta_T = 0.6$ for the reaction

$$Pyr{\cdot}Glu^{3-} \overset{\beta_T}{\rightleftharpoons} Ala{\cdot}Akg^{3-}$$

On the other hand, a value of 0.08 is calculated for β_{ZnT} for reaction 2. This large difference in the stabilities of the complexed tautomers appears to have its origin in the stereochemistry imposed by the bond angles about the carbon–nitrogen double bond. The planar arrangement about the unsaturated carbon causes the propionate group of the Akg^{2-} moiety of (Ala·Akg^{3-}) to be directed away from the metal ion, whereas the propionate substituent on the tetrahedral α carbon of (Pyr·Glu^{3-}) may actually partake in chelate ring formation with the Zn(II) ion, leading to weak tetradentate coordination. Although tetradentate coordination is unlikely in the bis complex, Zn(Pyr·Glu)$_2^{4-}$, models show that the propionate carboxylate groups can still be located near the metal ion in its secondary coordination sphere. In this arrangement the metal ion is surrounded by a cluster of six carboxylate groups in all.



Zn(AKG·ALA)$^-$



Zn(PYR·GLU)$^-$

A Schiff base system undergoing transamination will contain two different carbonyl compounds and two different amino acids after the reaction is underway. Such a system will not only yield the tautomeric reactant

and product Schiff base complexes when a suitable metal ion is present, but also cross Schiff base complexes (involving, *e.g.*, product amino acid and reactant keto acid) will also be formed. Furthermore, under conditions when the bis Schiff base complexes become appreciable, it is expected that it may be necessary to take into account quaternary species. Pertaining to the present work, in solutions containing all the components shown in reaction 1 and zinc(II) it is expected that species such as Zn(Pyr, Glu, Ala)$^{2-}$ and Zn((Pyr)$_2$, Glu, Ala)$^{3-}$ will be present (bis-Akg^{2-} species being unstable). To ascertain the importance of these quaternary complexes, a series of experiments were run in which varying amounts of Zn(II) were added to a series of solutions representing reaction 1 at equilibrium. The details and results are given in Table II. Observed values of pH are shown along with various calculated values. The pH calculations were made using the constants given in Table I for all the species including the cross Schiff bases and various assumed values of $\beta_{Zn,Pyr,Glu,Ala}$ and $\beta_{Zn,(Pyr)_2,Glu,Ala}$, the constants for the quaternary species. When these last two constants are taken to be equal to zero, the calculated values of the pH are seen quite clearly to be too high, indicating the formation of additional stable complexes other than the binary and ternary species characterized in Table I. Reasonable fits to the data are achieved equally well for the combinations 0, 4×10^{13}; 3×10^{11}, 0; and 2×10^{11}, 1×10^{13} for $\beta_{Zn,Pyr,Glu,Ala}$ and $\beta_{Zn,(Pyr)_2,Glu,Ala}$, respectively.

Table II. Observed and Calculated pH Values for Simulated Transamination Mixtures as a Function of $[Zn]_{tot}$[a,b]

	$[Zn]_{tot}$		
	0.005 *M*	0.0075 *M*	0.010 *M*
pH_{obsd}	8.12	7.29	6.93
pH_{calcd}[c]	8.30	7.43	7.01
pH_{calcd}[d]	8.18	7.25	6.89

Calculated distribution of major species ($\geq 10\%$ $[Zn]_{tot}$) using $\beta_{Zn,Pyr,Glu,Ala} = 2 \times 10^{11}$ and $\beta_{Zn,(Pyr)_2,Glu,Ala} = 1 \times 10^{13}$. Values in parentheses are per cent of $[Zn]_{tot}$

$[Zn]_{tot} = 0.005$: Zn(Pyr·Glu)(Ala)$^{2-}$ + Zn(Pyr·Ala)(Glu)$^{2-}$ (26); Zn(Pyr·Glu)(Pyr·Ala)$^{3-}$ (13); Zn(Pyr·Ala)$_2^{2-}$ (12); Zn(Pyr·Glu)(Glu)$^{2-}$ (12); Zn(Pyr·Ala)(Ala)$^-$ (10)

$[Zn]_{tot} = 0.0075$: Zn(Pyr·Glu)$^-$ (22); Zn(Pyr·Ala) (15); Zn(Pyr·Glu)(Ala)$^{2-}$ + Zn(Pyr·Ala)(Glu)$^{2-}$ (12)

$[Zn]_{tot} = 0.010$: Zn(Pyr·Glu)$^-$ (25); Zn(Pyr·Ala) (17); $[Zn^{2+}]$ (14)

[a] 25°, μ = 0.5 (KCl). [b] Total concentrations: $[H]_{tot} = 0.020$, $[Ala]_{tot} = 0.0143$, $[Akg]_{tot} = 0.0143$, $[Pyr]_{tot} = 0.0157$, $[Glu]_{tot} = 0.0157$ *M*. [c] $\beta_{Zn,Pyr,Glu,Ala} = 0$, $\beta_{Zn,(Pyr)_2,Glu,Ala} = 0$. [d] When $\beta_{Zn,Pyr,Glu,Ala} = 0$, 3×10^{11}, 2×10^{11}; $\beta_{Zn,(Pyr)_2,Glu,Ala} = 4 \times 10^{13}$, 0, 1×10^{13}, respectively.

The following line of reasoning was employed to decide which of these sets of constants most nearly corresponds to the true distribution of species in the experiments of Table II. The complex having the general formula, Zn(Pyr, Glu, Ala)$^{2-}$, may be comprised of the coordination isomers Zn(Pyr·Glu)(Ala)$^{2-}$ and Zn(Pyr·Ala)(Glu)$^{2-}$. The logs of the stepwise constants are 3.98 for Zn(Pyr·Ala) + Ala$^-$ $\rightleftharpoons$ Zn(Pyr·Ala)(Ala)$^-$ and 3.67 for Zn(Pyr·Glu)$^-$ + Glu^{2-} $\rightleftharpoons$ Zn (Pyr·Glu)(Glu)$^{3-}$. Differences in steric hindrance would serve to reduce the former for the addition of Ala$^-$ to Zn(Pyr·Glu)$^-$ and increase the latter for the addition of Glu^{2-} to Zn(Pyr·Ala). Assuming an intermediate value of 3.8 for both of these reactions, $\beta_{Zn,Pyr,Glu,Ala}$ is estimated to be 1.3×10^{11} from the relationship

$$\beta_{Zn,Pyr,Glu,Ala} = (\beta_{11_{Zn,Pyr,Ala}} + \beta_{11_{Zn,Pyr,Glu}}) \times 6 \times 10^3$$

The same stepwise addition constant of 6×10^3 for the formation of each of the quaternary species implies that the equilibrium ratio of the coordination isomers Zn(Pyr·Glu)(Ala)$^{2-}$ to Zn(Pyr·Ala)(Glu)$^{2-}$ is the same as the ratio of the β_{11} values for the ternary species, *i.e.*, close to unity.

Similarly, log *K* is 6.20 for Zn(Pyr·Ala) + Pyr$^-$ + Ala$^-$ $\rightleftharpoons$ Zn(Pyr·Ala)$_2^{2-}$ + H_2O, and 5.40 for Zn(Pyr·Glu)$^-$ + Pyr$^-$ + Glu^{2-} $\rightleftharpoons$ Zn(Pyr·Glu)$_2^{4-}$ + H_2O. Assuming an intermediate value of 5.8

$$\beta_{Zn,(Pyr)_2,Glu,Ala} = (\beta_{11_{Zn,Pyr,Ala}} + \beta_{11_{Zn,Pyr,Glu}}) \times 6 \times 10^5 = 1.3 \times 10^{13}$$

These estimated values are in good agreement with the last set of constants which were found to fit the pH data of Table II. Similar estimates for Akg quaternary complexes were also made. Calculations showed that these species are formed in negligible amounts and can be safely ignored.

The distribution of Zn(II) among the major species calculated using $\beta_{Zn,Pyr,Ala,Glu}$ equal to 2×10^{11} and $\beta_{Zn,(Pyr)_2,Ala,Glu}$ equal to 1×10^{13} is also given in Table II. It can be readily seen that, unless relatively high levels of zinc are employed to suppress the formation of the higher complexes, quaternary species may readily be formed at appreciable levels in a transamination system. With studies involving pyridoxal or pyridoxamine and those oxo and amino acids with the shorter side chains, it is possible that even quinternary complexes may be formed. It would be best to arrange conditions to repress such species, but if this is not possible it appears likely that rough corrections can be made for their presence using constants estimated from studies on the separate ternary systems. Cross ternary complexes are probably important under most experimental conditions, however.