Kinetics of Formation of N-Pyruvylideneglycinatozinc(II). Zinc(II) Catalysis under Approximately Neutral Conditions[1]

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Abstract: The rate of reaction of pyruvate (P^-) with glycinate (G^-) in the presence of Zn(II), pH range 4.5–7.6, ionic strength 0.5, 25°, is described by the rate law: $d(ZnPG)/dt = k_{PG}(P^-)(G^-) + k_{HPG}(H^+)(P^-)(G^-) + k_{ZnPG}\cdot(Zn^{2+})(P^-)(G^-) + k_{ZnPGH}(Zn^{2+})(P^-)(G^-)(H^+) + k_{ZnPG_2}(Zn^{2+})(P^-)(G^-)^2$, where $k_{PG} = 0.15\ M^{-1}\ sec^{-1}$, $k_{HPG} = 9.2 \times 10^7\ M^{-2}\ sec^{-1}$, $k_{ZnPG} = 3.4 \times 10^3\ M^{-2}\ sec^{-1}$, $k_{ZnPGH} = 1.03 \times 10^9\ M^{-3}\ sec^{-1}$, and $k_{ZnPG_2} = 3.4 \times 10^7\ M^{-3}\ sec^{-1}$. In these reactions Zn(II) appears to play a promnastic role rather than serve as a template; *i.e.*, the metal ion brings the ligands into proximity by forming a labile ternary complex but imposes a minimum geometric constraint upon them.

A recent study[3] of the kinetics of formation of N-pyruvylideneglycinatozinc(II) under acidic conditions (pH <6) showed that this complex was formed *via* two parallel paths when sodium glycinate was added to a solution containing pyruvate and zinc(II) ions. Both of these paths were first order in each of hydrogen ion, pyruvate, and glycinate, but only one path showed metal ion dependence. This latter path seemed to involve two steps: reaction of pyruvate and glycinate bound to a single zinc ion to give complexed carbinolamine followed by dehydration to yield the Schiff base complex.

The data at higher pH values indicated the existence of still another path which was not amenable to investigation using the pH-stat technique employed. In view of the interest in the role played by metal ions in aiding Schiff base formation, it was deemed worthwhile to define this reaction system more rigorously. The metal ion mediated paths were examined in the present study under more neutral conditions of pH. In addition, the reaction of pyruvate with glycinate in the absence of divalent metal ions was sudied under alkaline conditions where the Schiff base is sufficiently stable to form in appreciable quantities. The reaction rates were examined spectrophotometrically using a stopped-flow apparatus which enabled examination of the reaction within a few milliseconds after mixing.

Experimental Section

A stopped-flow apparatus similar to that described in ref 4 was employed except that the cell was modified to give a 2.1-cm optical path length. Experimental conditions were arranged so that in an experiment a solution containing sodium pyruvate was mixed with a solution containing glycine and sodium glycinate. Depending on the experimental conditions this latter solution may or may not have also contained zinc chloride. The concentration of pyruvate after mixing was 0.0050 *M*, and its disappearance was followed at 335 mμ (ϵ 20.2 $M^{-1}\ cm^{-1}$). At this wavelength the absorption of the Schiff base can be safely neglected. All runs were performed at 25°. The ionic strength of the final solution was adjusted to 0.5 with KCl. Both of the solutions being mixed were brought to the same initial KCl level to minimize the effects of density and viscosity differences on the mixing rate.

The sodium pyruvate used was dimer free as supplied by Sigma Chemical Co. Solutions of this salt were freshly prepared. Glycine was recrystallized from ethanol, and sodium glycinate solutions were generated by carefully neutralizing weighed amounts of glycine with standardized NaOH.

The initial rate of formation of Schiff base was calculated using the expression

$$\frac{d(SB)}{dt} = -\frac{1}{\epsilon l}\frac{dA}{dt}$$

where dA/dt is the experimentally determined initial rate of change of absorbance.

When Zn(II) was present the concentrations of Zn^{2+}, HG, P^-, G^-, ZnP^+, ZnP_2, ZnG^+, ZnG_2, and ZnG_3^- were calculated from known *total* concentrations of Zn(II), P, G, and H at the time of mixing (zero time) using the constants reported earlier[3] ($P^- = CH_3COCO_2^-$, $G^- = H_2NCH_2CO_2^-$). The rate data were analyzed and interpreted in terms of free Zn^{2+}, P^-, G^-, and H^+, where H^+ represents the measured activity of hydrogen ions rather than their concentration. The hydrogen ion activity was determined using a pH meter which was standardized against NBS buffers.

Results and Discussion

Values of the second-order rate constant, k_{PG}, calculated for the disappearance of pyruvate in the absence of zinc(II) are presented in Table I. The data show that within the experimental error k_{PG} does not depend on the buffer ratio or buffer concentration. Under the present conditions only the solvent catalyzed reaction of pyruvate with glycinate is observed, and the rate law is simply

$$d(PG^{2-})/dt = k_{PG}(P^-)(G^-)$$

Table I. Rate of Pyruvate Reaction in the Absence of Zn(II)[a]

HG, *M*	NaG, *M*	k_{PG},[b] $M^{-1}\ sec^{-1}$	Std dev	No. of points
0.075	0.075	0.156	0.021	5
0.10	0.10	0.147	0.017	5
0.15	0.15	0.159	0.017	5
0.20	0.20	0.175	0.028	3
0.20	0.15	0.129	0.014	4
0.10	0.15	0.129	0.014	4
0.075	0.15	0.130	0.021	5
		Av 0.15 ± 0.02		

[a] Temperature 25°, ionic strength = 0.5 (KCl), $NaP_{initial}$ = 0.00500 *M*. [b] k_{PG} = $rate_{initial}/(P^-)(G^-)$.

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[2] Supported by a NSF Undergraduate Research Fellowship, Summer 1967.

[3] D. L. Leussing and C. K. Stanfield, *J. Am. Chem. Soc.*, **88**, 5726 (1966).

[4] G. Dulz and N. Sutin, *Inorg. Chem.*, **2**, 917 (1963).

Table II. Rate of Formation of ZnPG[a]

Zn_{tot}, M	NaG_{tot}, M	HG, M	Zn^{2+}, M	P^-, $M \times 10^3$	G^-, $M \times 10^5$	pH	Rate, M $sec^{-1} \times 10^5$
0.1514	0.1250	0.025	0.0473	2.70	2.19	6.64	1.21
0.0883	0.0625	0.025	0.0337	3.10	1.68	6.53	1.05
0.0757	0.0625	0.025	0.0234	3.51	2.21	6.65	0.84
0.0757	0.0625	0.0125	0.0234	3.51	2.21	6.95	0.70
0.0568	0.03125	0.0250	0.0275	3.33	1.14	6.36	0.62
0.1202	0.0938	0.0250	0.0406	2.88	1.98	6.60	1.05
0.0938	0.1562	0.060	0.00217	4.81	16.8	7.15	2.58
0.0569	0.03125	0.0125	0.00277	3.33	1.14	6.66	0.56
0.1328	0.1712	0.060	0.00194	4.83	22.0	7.27	3.16
0.0708	0.1115	0.060	0.00262	4.78	12.6	7.02	1.73
0.1012	0.1723	0.060	0.00185	4.85	19.3	7.21	2.57
0.1113	0.1926	0.060	0.00175	4.85	21.2	7.25	2.80
0.1012	0.1824	0.060	0.000945	4.92	28.4	7.38	2.11
0.0911	0.1723	0.060	0.000379	4.98	44.0	7.57	2.07
0.1113	0.1926	0.0250	0.00175	4.86	21.2	7.64	1.81
0.0708	0.1115	0.0250	0.00262	4.78	12.6	7.41	1.06

[a] Temperature 25°, ionic strength = 0.5 (KCl), P_{tot} = 0.0050 M.

The experimental conditions and observed rates for the zinc-containing solutions are presented in Table II. The rate law proposed earlier[3] for proton-catalyzed reactions in the range pH 4.5–6.0 is

$$\frac{d(ZnPG)}{dt} = \frac{k_1(k_2 + [Zn^{2+}])[H^+][P^-][G^-]}{k_3 + [Zn^{2+}]} \quad (1)$$

where $k_1 = 3.3 \times 10^8\ M^{-2}\ sec^{-1}$, $k_2 = 0.0275\ M$, and $k_3 = 0.106\ M$.

Values of eq 1 for the conditions described in Table II show that in the present study contributions to the observed rates from these proton-catalyzed paths are small although not entirely negligible. Thus, it was possible to make an analysis of the rates shown in Table II to a good approximation regardless of the accuracy of the earlier conclusions. After correcting the rates in Table II for the k_{PG} path described above and the proton-catalyzed paths given by eq 1, it was found that a plot of $rate_{cor}/[Zn^{2+}][P^-][G^-]$ *vs.* $[G^-]$ gave a straight line with a positive intercept and a positive slope. This behavior is shown in Figure 1 where $rate_{cor}$ was actually obtained by subtracting the k_{HPG} and k_{ZnPGH} terms of eq 2 as well as k_{PG} from the observed rates. The plot which was first obtained, however, was essentially the same as that shown in Figure 1, having only a slightly different slope and intercept.

The linear behavior shown in Figure 1 is evidence for two parallel paths: $Zn^{2+} + P^- + G^- \rightarrow$ products, $Zn^{2+} + P^- + 2G^- \rightarrow$ products. The intercept is the value of k_{ZnPG} and the slope gives k_{ZnPG_2}. In view of the relatively low concentrations of the mixed species, $Zn(P)(G)_2^-$, estimated for the reaction conditions of Table II, the relatively large contribution of the term second order in glycinate is surprising. Further comment on this point is made below.

The preliminary values found for k_{ZnPG} and k_{ZnPG_2} were in turn used to correct the rates below pH 6 found earlier.[3] A large and significant effect was found. Originally as shown in ref 3 a plot of $rate/[P^-][G^-]a_H$ *vs.* $[Zn^{2+}]$ caused the points in the pH range 4.5–5.5 to fall along a single curve, albeit with some scatter. However, subtracting out the contribution of the k_{ZnPG} term (that due to k_{ZnPG_2} is negligible at low pH) now causes the points in such a plot to fall along separate pH-dependent lines. Furthermore, these lines show no tendency for curvature as was evidenced earlier, but instead linear behavior with respect to Zn^{2+} is indicated (see Figure 2).

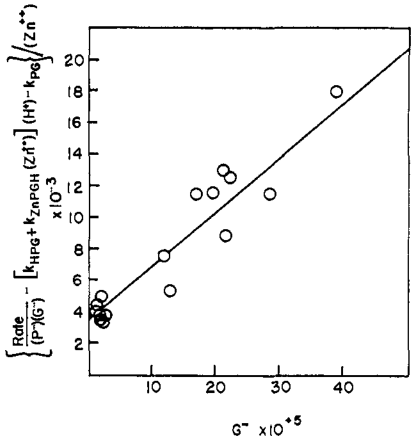

Figure 1. Corrected rate as a function of free glycinate.

Since it was necessary to invoke a two-step metal ion path in order to account for the apparent curvature in the original plot, a simpler rate law can now be used to describe the results for acid media. The metal ion independent proton-catalyzed path is still shown, but only a single rate-determining step is indicated for the Zn(II)-dependent path. This path involves Zn^{2+}, P^-, G^-, and H^+ each to the first order.

The former acid media data and those obtained in this work were combined and a least-squares fit to the rate equation

$$\frac{d(ZnPG)}{dt} = k_{ZnPGH}(Zn^{2+})(P^-)(G^-)(H^+) + k_{ZnPG}(Zn^{2+})(P^-)(G^-) + k_{ZnPG_2}(Zn^{2+})(P^-)(G^-)^2 + k_{HPG}(H^+)(P^-)(G^-) + k_{PG}(P^-)(G^-) \quad (2)$$

was made to obtain the rate constants for all but the last term for which the value given in Table I was used. The results are given in Table III. How well the data conform to eq 2 may be seen from Figures 1 and 2 where the solid lines have been calculated using the constants reported in Table III. The most important

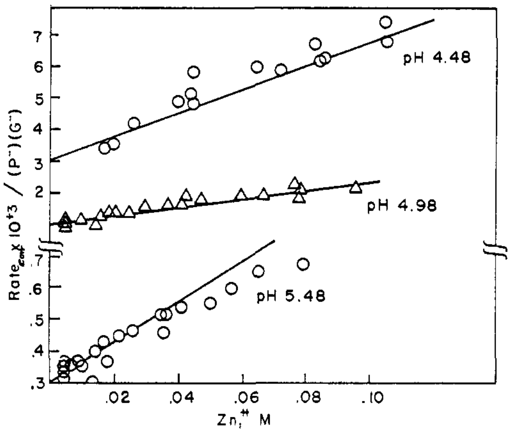

Figure 2. Rates in acid media as a function of pH and free Zn^{2+}.

factor in causing the scatter is very likely the difficulty in obtaining accurate values for the equilibrium concentrations of Zn^{2+}, P^-, and G^-. Nevertheless considering the range of solution concentrations employed satisfactory agreement between the calculated solid lines and the experimental points is observed. The value of k_{HPG}, $9.2 \times 10^7\ M^{-2}\ sec^{-1}$, agrees closely with the ratio k_1k_2/k_3, $8.0 \times 10^7\ M^{-2}\ sec^{-1}$, found earlier[3] for the metal-independent proton-catalyzed path. Also, the ratio k_1/k_3, $3.1 \times 10^9\ M^{-3}\ sec^{-1}$, is similar to k_{ZnPGH}, $1.03 \times 10^9\ M^{-3}\ sec^{-1}$, although the quantities are not strictly comparable. Thus, the principal features proposed earlier are still found to hold here.

Equation 2 describes a rate law which is identical with that observed for the reaction of glycinate with salicylaldehyde in the presence of Zn(II),[5] but the rate constants for the Zn(II)-dependent terms are somewhat over an order of magnitude greater with salicylaldehyde than those found for pyruvate. On the other hand, the rate constants for the direct reaction of the anionic ligands are about the same ($k_{SG} = 0.18\ M^{-1}\ sec^{-1}$ and $k_{PG} = 0.15\ M^{-1}\ sec^{-1}$). The difference between the former constants appear to arise from the difference between the stabilities of pyruvate and salicylaldate complexes. This point is discussed below.

The extensive investigation with a variety of divalent metal ions and over a wide pH range with salicylaldehyde reported in ref 5 indicates that of the two steps involved in Schiff base formation, addition followed by dehydration, addition is rate determining in these systems. Furthermore, the data suggest that the metal ion paths involve a rapid preequilibrium between the reactants and the metal ion yielding a ternary complex in which the oxo and amine donors are independently bound to the metal ion, $M^{2+} + O^- + A^- \rightleftharpoons M(O)(A)$. The rate-determining step in the formation of the Schiff base complex is the reaction of these bound ligands to give a carbinolamine followed by rapid dehydration

$$M(O)(A) \xrightarrow{\text{slow}} M(\text{carbinolamine}) \xrightarrow{\text{fast}} M(\text{Schiff base}) + H_2O$$

Assuming that a similar mechanism holds for the metal-dependent terms in the reaction of pyruvate with glycinate, the first-order rate constants for the reaction of the ternary complexes to give the carbinolamine complex are obtained by dividing k_{ZnPG} by $\beta_{11}{}^*$[6] and by dividing k_{ZnPG_2} by $\beta_{12}{}^*$. $\beta_{11}{}^*$ is the equilibrium constant for $Zn^{2+} + P^- + G^- \rightleftharpoons Zn(P)(G)$ and $\beta_{12}{}^*$ is the equilibrium constant for $Zn^{2+} + P^- + 2G^- \rightleftharpoons Zn(P)(G)_2^-$. The second-order rate constant for $Zn(P)(G) + H^+ \rightarrow$ product is obtained by dividing k_{ZnPGH} also by $\beta_{11}{}^*$.

Table III. Rate Constants for the Reaction of Pyruvate with Glycinate[a,b] (25°, $\mu = 0.5$ (KCl))

Reaction	Rate constant
$P^- + G^- \longrightarrow PG^{2-} + H_2O$	$0.15\ M^{-1}\ sec^{-1}$
$H^+ + P^- + G^- \xrightarrow{Zn^{2+}} Zn(PG) + H^+ + H_2O$	$9.2 \times 10^7\ M^{-2}\ sec^{-1}$
$Zn^{2+} + P^- + G^- \longrightarrow Zn(PG) + H_2O$	$3.4 \times 10^3\ M^{-2}\ sec^{-1}$
$Zn^{2+} + P^- + 2G^- \longrightarrow Zn(PG) + G^- + H_2O$	$3.4 \times 10^7\ M^{-3}\ sec^{-1}$
$Zn^{2+} + P^- + G^- + H^+ \longrightarrow Zn(PG) + H_2O + H^+$	$1.03 \times 10^9\ M^{-3}\ sec^{-1}$
$Zn(P)(G) \longrightarrow Zn(PG) + H_2O$	$5.7 \times 10^{-3}\ sec^{-1}$
$Zn(P)(G)_2^- \longrightarrow Zn(PG) + H_2O + G^-$	$0.3\ sec^{-1}$
$Zn(P)(G) + H^+ \longrightarrow Zn(PG) + H_2O + H^+$	$1.7 \times 10^3\ M^{-1}\ sec^{-1}$
$S^- + G^- \longrightarrow SG^- + H_2O$	$0.18\ M^{-1}\ sec^{-1}$
$Zn(S)(G) \longrightarrow Zn(SG) + H_2O$	$2.6 \times 10^{-3}\ sec^{-1}$
$Zn(S)(G)_2^- \longrightarrow Zn(SG) + G^- + H_2O$	$0.37\ sec^{-1}$
$Zn(S)(G) + H^+ \longrightarrow Zn(SG) + H^+ + H_2O$	$1.6 \times 10^3\ M^{-1}\ sec^{-1}$

[a] P^- = pyruvate. [b] S^- = salicylaldehyde anion, ref 5.

Taking[7] $\beta_{11}{}^*$ as $2\sqrt{\beta_{02}\beta_{20}}$, a value of $6 \times 10^5\ M^{-2}$ is obtained. The estimation of $\beta_{12}{}^*$ is somewhat more uncertain owing to the reluctance of Zn^{2+} to achieve hexacoordination with saturated bidentate ligands; for example, the stepwise constant, K_{03}, for the addition of a third glycinate is two orders of magnitude less than K_{01} and K_{02}. Multiplying $\beta_{11}{}^*$ by K_{03} and correcting for statistical effects yields a value of $\beta_{12}{}^*$ equal to about $1 \times 10^8\ M^{-3}$. The calculated first-order rate constants (second order for proton catalysis) for the addition reactions within the ternary complexes are given in Table III.

Comparison of the reduced first- (and second-) order pyruvate constants with those obtained for salicylaldehyde anion, and also given in Table III, shows a striking similarity between the two systems. The close agreement no doubt has its origins in the similar rates shown by the two oxo anions toward glycinate. In addition the markedly parallel behavior strongly suggests that the same mechanisms apply in both cases. The difference between the two systems as observed for the constants applying to eq 2 merely reflects the effect on $\beta_{11}{}^*$ of the stronger affinity of salicylaldehyde anion compared to pyruvate for Zn(II) ions; β_{10} is 740 and 18, respectively.

Variation of the divalent metal ion[5] showed that the reactivity of the ternary preequilibrium complex increases as the metal ion–amine bond strength decreases. This is in agreement with the view that the nitrogen electron pair must be free to attack the carbonyl carbon atom. The high reactivity of the thermodynamically unstable species, $Zn(P)(G)_2^-$, is additional evidence for this type of mechanism. Here, the second glycinate, which is very loosely bound, is observed to have enhanced reactivity.

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(5) D. Hopgood and D. L. Leussing, *J. Am. Chem. Soc.*, **91**, 3740 (1969).

(6) β_{ij} is defined for the reaction $M^{2+} + iP^- + jG^- \rightleftharpoons MP_iG_j$.

(7) Y. Kanemura and J. I. Watters, *J. Inorg. Nucl. Chem.*, **29**, 1710 (1967).