Kinetics of Formation of N-Salicylideneglycinatonickel(II), -copper(II), and -zinc(II). Elucidation of the Template Mechanism[1]

Kyu Sun Bai and D. L. Leussing

Contribution from the Department of Chemistry, The Ohio State University, Columbus, Ohio 43210. *Received May 24, 1967*

Abstract: The kinetics of formation of N-salicylideneglycinatonickel(II), -copper(II), and -zinc(II) have been determined using a pH-Stat technique, 25°, 0.50 *M* ionic strength. The composition of each reaction solution was defined in terms of free M^{2+}, Sal^-, Gly^-, and H^+ by making use of the equilibrium conditions which apply to the rapidly formed binary species. After this was done, the rates in the Cu(II) and Ni(II) systems were found to be independent of M^{2+}. Zn(II) systems exhibited both Zn^{2+}-dependent and Zn^{2+}-independent paths. For all metal ion systems, the M^{2+}-independent path (in min^{-1}) is described by the equation, $d(MSalGly)/dt = [4.1 \times 10^{17})a_H^2 + (3.0 \times 10^{10})a_H + 300](Sal^-)(Gly^-)$. This rate law probably arises from the rate-determining reactions: $Sal^- + Gly^- \rightarrow$ Schiff base + H_2O (k_0), $HSal + Gly^- \rightarrow$ Schiff base + H_2O (k_1), and $HSal + Gly^- + H^+ \rightarrow$ Schiff base + H_2O (k_2), followed by the rapid reaction of the Schiff base with metal ions. The parallel Zn^{2+}-dependent path follows the rate law, rate = $(6.3 \times 10^6)(Zn^{2+})(Sal^-)(Gly^-)$. The template mechanism appears to hold

$$Zn^{2+} + Sal^- + Gly^- \underset{\text{fast}}{\overset{K_{11}}{\rightleftharpoons}} Zn(Sal)(Gly); \quad Zn(Sal)(Gly) \xrightarrow[\text{slow}]{k_T} Zn(SalGly) + H_2O$$

Zn(II) appears to be a better catalyst than either Ni(II) or Cu(II) owing to the greater geometric flexibility permitted in its coordination of ligands.

The kinetics of Schiff base formation as well as other carbonyl addition reactions have interested chemists for some time.[2] It has long been recognized that metal ions aid the formation of Schiff bases by forming stable complexes with them, thereby contributing favorably to the over-all free energy of reaction. In addition, it has been postulated that metal ions catalyze these reactions by serving as a reaction template.[3-6] The reactants are pictured to be first coordinated to the metal ion forming a labile ternary complex. Interligand condensation is then supposed to occur within the coordination sphere of the metal ion, where presumably, because they are held in close proximity, a better opportunity for reaction is presented to the ligands. In this reaction sequence, the metal ion functions as a rudimentary enzyme.

In the absence of metal ions, Schiff base formation involves a two-step reaction between the carbonyl compound and the amino compound. First, addition takes place to form a carbinolamine which then undergoes dehydration. Both steps exhibit general acid–base catalysis.[7-9] Relatively little quantitative information is available regarding the kinetic effect of metal ions on these reactions. A qualitative study[10] of the effects of Cu(II) and Ni(II) on the condensation of salicylaldehyde and glycinate showed that the reaction actually proceeds faster when the reactants are mixed before the addition of the metal ions than when one of the reagents is added to a mixture of the metal ion with the other reactant. Owing to the lack of information regarding the nature of the equilibria existing in these semiaqueous systems, it was not possible to obtain quantitative rate laws describing the reaction. However, the existence of an active ternary complex was postulated.

A more recent study of the kinetics of formation of N-pyruvylideneglycinatozinc(II) at pH <6.0 has been reported.[11] A two-step mechanism was found. Metal ions appeared to trap the final product and the intermediate carbinolamine, but the initial step, the addition of glycinate to pyruvate, was observed to be independent of Zn(II). Thus, the kinetic template mechanism does not seem to be operative in this system under the conditions studied.

The equilibrium properties of aqueous Ni(II), Cu(II), or Zn(II) solutions containing salicylaldehyde and glycinate have been determined in these laboratories.[12] As was observed in dioxane–water media[10] highly stable Schiff base complexes were found. This paper describes the kinetics of formation of these complexes. The first quantitative information pertaining to the kinetic template mechanism is reported.

Experimental Section

Stock solutions of metal chlorides were prepared and standardized according to usual procedures. Salicylaldehyde was redistilled

(1) This research was supported by a grant from the National Science Foundation, GP 5396.

(2) A. Lapworth, *J. Chem. Soc.*, 83, 995 (1903); J. B. Conant and P. Bartlett, *J. Am. Chem. Soc.*, **54**, 2881 (1932).

(3) G. L. Eichhorn, Advances in Chemistry Series, No. 37, D. H. Busch, Ed., American Chemical Society, Washington, D. C., 1963.

(4) E. J. Olszewski, L. J. Boucher, R. W. Oehmke, J. C. Bailar, Jr., and D. F. Martin, *Inorg. Chem.*, **2**, 661 (1963).

(5) L. T. Taylor, S. C. Vergez, and D. H. Busch, *J. Am. Chem. Soc.*, **88**, 3170 (1966).

(6) E. J. Olszewski and D. F. Martin, *J. Organometal. Chem.* (Amsterdam), **5**, 203 (1966).

(7) W. P. Jencks, *Progr. Phys. Org. Chem.*, **2**, 63 (1964).

(8) R. B. Martin, *J. Phys. Chem.*, **68**, 1369 (1964).

(9) T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," W. A. Benjamin, Inc., New York, N. Y., 1966.

(10) L. J. Nunez and G. L. Eichhorn, *J. Am. Chem. Soc.*, **84**, 901 (1962).

(11) D. L. Leussing and C. K. Stanfield, *ibid.*, **88**, 5726 (1966).

(12) D. L. Leussing and K. S. Bai, to be reported.

at reduced pressure and stored in the dark at −10°. Gas chromatography showed the presence of no other components.

The reaction rates were obtained using the pH-Stat technique employed earlier.[11] An aliquot of a solution containing known concentrations of metal ion and salicylaldehyde, brought to an ionic strength of 0.5 with KCl, was placed in the titration cell of the pH-Stat. The instrument was set to the desired pH and the buret was actuated. A standard sodium glycinate solution (also adjusted to an ionic strength of 0.5 with KCl) flowed rapidly into the cell until the desired pH was attained. This first stage of the reaction involved the (partial) neutralization of salicylaldehyde and the formation of various complexes of salicylaldehyde and glycinate.

$$HSal + M^{2+} \xrightarrow[\text{rapid}]{\text{NaGly}} MSal^{+}, MGly^{+}, Sal^{-}, HGly, \text{etc.}$$

A slower second reaction involving Schiff base complex formation was then observed

$$M^{2+} + Sal^{-} + Gly^{-} \xrightarrow{\text{slow}} M(Sal\cdot Gly) + H_2O$$

The consumption of glycinate ions in this last reaction caused the pH of the reaction solution to decrease. The pH was restored automatically to the preset value by the addition of NaGly from the buret. A recording of the volume of added NaGly *vs.* time yielded the data from which the rates were calculated. Typical experimental volume-time curves are shown in Figure 1. The reaction rates were obtained from initial slopes observed at the onset of the second stage in the manner previously described.[11]

For each metal ion a limited pH range exists over which it is possible to measure the rates with the pH-Stat. The lower limit is determined by the free energy of the reaction $M^{2+} + HSal + HGly \rightarrow M(Sal\cdot Gly) + 2H^{+} + H_2O$ (see curve 1a of Figure 1), while the upper limit is determined by the extent of formation of the higher glycinate complexes. Over-all reactions of the type $M(Gly)_2 + Sal^{-} \rightarrow M(Sal\cdot Gly) + Gly^{-}$ reduce the sensitivity of the volume-time curve to changes within the solution. The pH "window" over which suitable data may be obtained varies with the metal ion and is determined by the relative stabilities of the Schiff base complexes. With the various metal ions a considerable pH range was investigated: pH 2.7–4.7, Cu^{2+}; pH 4.5–6.4, Ni^{2+}; pH 5.7–7.9, Zn^{2+}. In the series of experiments, salicylaldehyde was varied from 0.01 to 0.033 *M* and metal chloride was varied from 0.001 to 0.20 *M*. Occasionally HCl up to 0.03 *M* was added initially to alter the pH range in a set.

In order to resolve the rate laws from the rate data, it was first necessary to determine the solution compositions at onset of the second stage of the reaction. Since the rates of equilibration of the binary metal ion complexes in these systems are very fast[13] compared to slow rates of formation of the Schiff base complexes, it was possible to calculate the free ligand and free metal ion distributions using the binary formation constants previously determined[12] and the gross solution compositions. In this way, the solution compositions were defined in terms of the concentrations of the free metal ions, free salicylaldehyde anions, free glycinate ions, and hydrogen ions.

As the Schiff base complexes form, a redistribution of the solution species occurs. To compensate for these changes, the rates of formation of the Schiff bases, $d(M(Sal\cdot Gly))/dt$ were calculated by differentiating with respect to time the four mass-balance expressions

$$M_{tot} = M^{2+} + MSal^{+} + MSal_2 + MGly^{+} + MGly_2 + MGly_3^{-} + M(Sal\cdot Gly)$$

$$Gly_{tot} = H_2Gly^{+} + HGly + Gly^{-} + MGly^{+} + 2MGly_2 + 3MGly_3^{-} + M(Sal\cdot Gly)$$

$$Sal_{tot} = HSal + Sal^{-} + MSal^{+} + 2MSal_2 + M(Sal\cdot Gly)$$

$$H_{tot} = H^{+} - OH^{-} + 2H_2Gly^{+} + HGly + HSal$$

Using the equilibrium condition, the derivatives of the binary species were expressed in terms of the rates of change of the free species, *e.g.*

$$d(MGly_2)/dt = K_{form}\left[(Gly^{-})^2\frac{d(M^{2+})}{dt} + 2(M^{2+})(Gly^{-})\frac{d(Gly^{-})}{dt}\right], \text{ etc.}$$

(13) M. Eigen and R. G. Wilkins, Symposium on Mechanisms of Inorganic Reactions, University of Kansas, June 21–24, 1964.

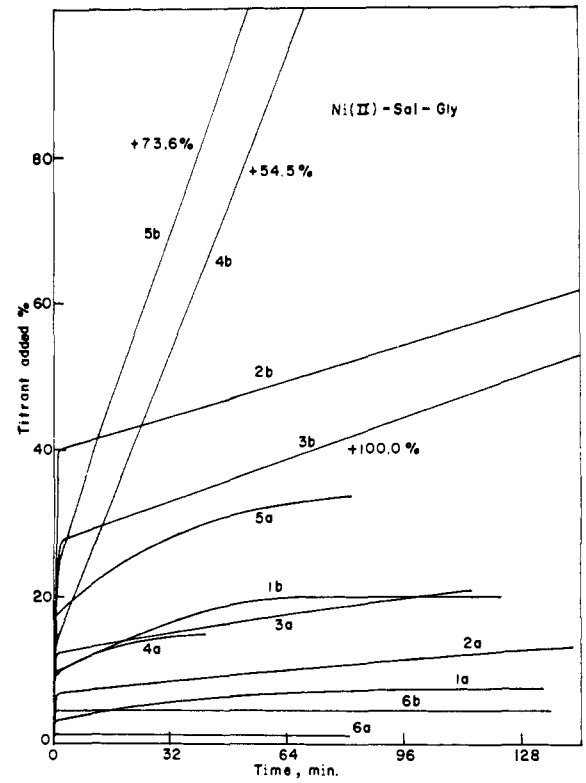


Figure 1. Some Ni(II) rate curves. For the curves given, the following values, $NiCl_2$ (*M*), HSal (*M*), and pH, are listed, respectively: (1a) 0.00100, 0.0300, 5.75; (1b) 0.00100, 0.0300, 6.35; (2a) 0.0100, 0.0100, 5.47; (2b) 0.0100, 0.0100, 6.27; (3a) 0.0300, 0.0100, 5.17; (3b) 0.0300, 0.0100, 6.27; (4a) 0.0300, 0.033, 4.65; (4b) 0.0300, 0.033, 5.45; (5a) 0.0500, 0.033, 4.64; (5b) 0.0500, 0.033, 5.34; (6a) 0.0000, 0.01, 6.47; (6b) 0.0000, 0.01, 6.97. Test solution, 20.0 ml initially; titrant, 0.1000 *M* NaGly; ordinate, 100% = 2.500 ml.

Thus, four linear equations in the four unknowns $d(M^{2+})/dt$, $d(Gly^{-})/dt$, $d(Sal^{-})/dt$, and $d(M(Sal\cdot Gly))/dt$ were obtained. The derivatives of $d(M_{tot})/dt$, $d(Sal_{tot})/dt$, and $d(H_{tot})/dt$ are small and arise from dilution. The term $d(Gly_{tot})/dt$ was evaluated using the observed slope of the volume-time curve, the titrant concentration, and the volume of the test solution. Substitution of the known (H^{+}) and previously calculated values of (M^{2+}), (Sal^{-}), and (Gly^{-}) into the resulting expressions allows the evaluation of the derivatives.

Results and Discussion

As is generally observed with carbonyl addition reactions,[7-9] the rates were found to be first order in the concentrations of both the carbonyl compound and the amine. With Cu^{2+} and Ni^{2+} the rate divided by the product $(Sal^{-})(Gly^{-})$ yields a result which is independent of the concentration of free metal ion (see Figure 2). Neither free metal ion nor any metal-containing species is involved in the rate-determining step in the Cu(II) and Ni(II) systems. This explains the inhibition of the rates observed by Nunez and Eichhorn[10] since these ions remove the reactants by complexing. On the other hand, the Zn(II) system exhibits both metal-ion-dependent and metal-ion-independent paths. In Figure 3 a plot of $[d(ZnSalGly)/dt]/(Sal^{-})(Gly^{-})$ *vs.* (Zn^{2+}) is seen to yield a family of almost parallel straight lines. The Zn^{2+}-independent path as indicated by the changes in the ordinate shows a pH dependence, while the Zn^{2+}-dependent path as indicated by the parallel lines is pH independent.

The reduced metal-ion-independent rates for all three systems are shown plotted *vs.* pH in Figure 4.

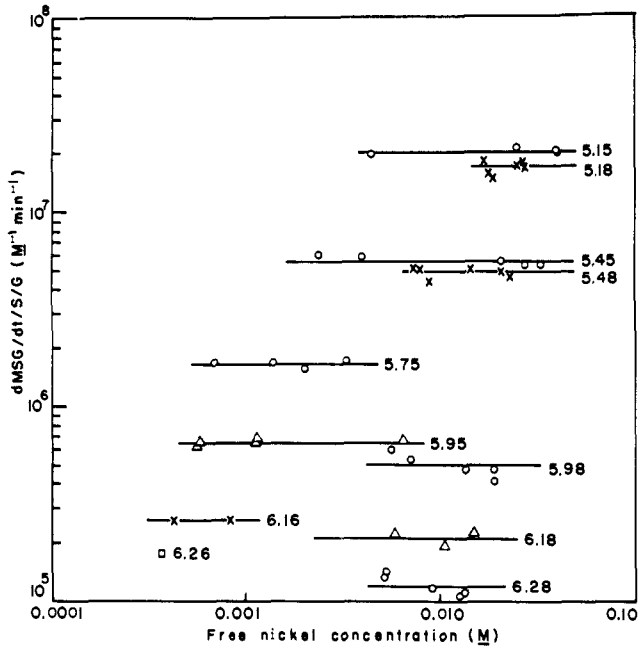


Figure 2. Ni(II) rates as a function of free Ni^{2+}.

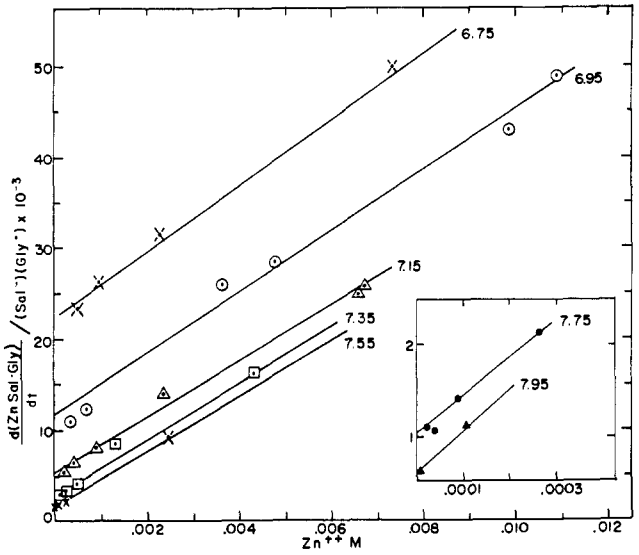


Figure 3. Zn(II) rates as a function of free Zn^{2+}.

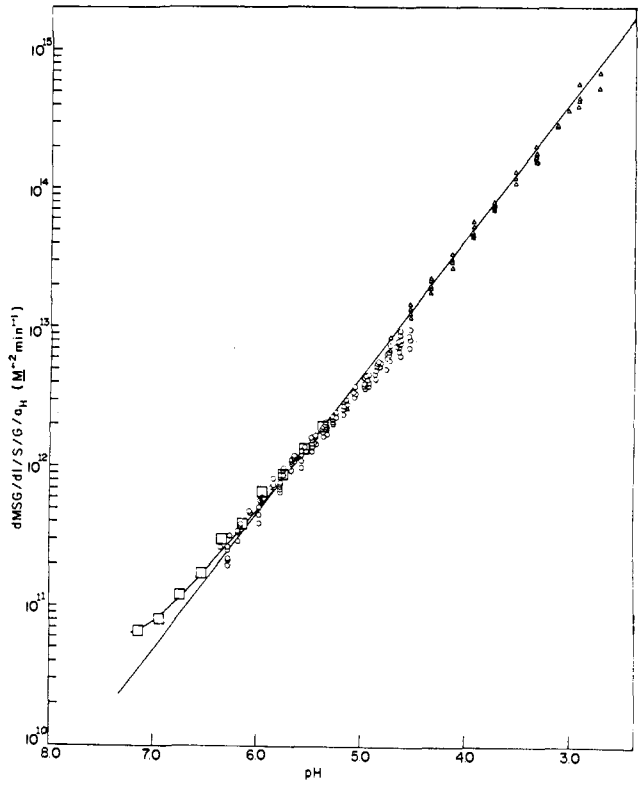


Figure 4. The pH dependence of the metal-independent paths: Δ, Cu(II); ○, Ni(II); □, Zn(II), extrapolated to $Zn^{2+} = 0$.

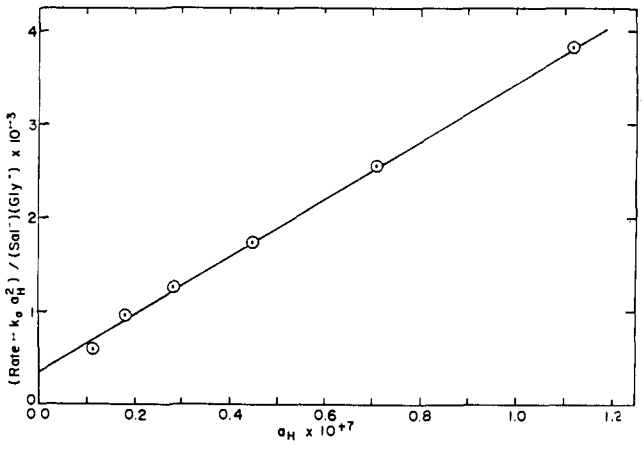


Figure 5. The pH dependence of the metal-independent path in the higher pH range after a correction has been made for the second-order H^+ term.

The Zn(II) points are the values of the ordinate obtained from plots as illustrated in Figure 3, while the Ni(II) and Cu(II) points have been calculated directly from the rates observed in the presence of these ions. It is seen in Figure 4 that the points for all three metal ions fall along a single curve. The linear right-hand branch,[14] which extends for about four pH units with a unit positive slope, indicates a rate law of the form

$$\text{rate} = k_a a_H^2(\text{Sal}^-)(\text{Gly}^-)$$

where k_a is evaluated to be $4.1 \times 10^{17}\ M^{-3}\ \text{min}^{-1}$.

The curvature at the high-pH end of Figure 4 indicates the increasing importance of additional paths which involve fewer hydrogen ions. A plot shown in Figure 5 of the quantity (rate $- k_a a_H^2$)/(Sal⁻)(Gly⁻) *vs.* a_H indicates the additional terms

$$\text{rate} = k_b a_H(\text{Sal}^-)(\text{Gly}^-) + k_c(\text{Sal}^-)(\text{Gly}^-)$$

where $k_b = 3.0 \times 10^{10}\ M^{-2}\ \text{min}^{-1}$ and $k_c = 300\ M^{-1}\ \text{min}^{-1}$.

The rather simple rate law which is observed for the reaction of salicylaldehyde with glycinate indicates that only a single step in the formation of the Schiff base is rate determining. Similar results have been reported by French, Auld, and Bruice[15] for the rate of formation of Schiff bases formed between 3-hydroxypyridine-4-aldehyde and various amino acids. These authors

(14) The negative deviation of the Ni(II) points at their upper pH limit results from the influence of the back-reaction on the earliest observations possible with these data. The effect may be seen in curve 1a of Figure 1.

(15) (a) T. C. French, D. S. Auld, and T. C. Bruice, *Biochemistry*, 4, 77 (1965); (b) see also D. S. Auld and T. C. Bruice, *J. Am. Chem. Soc.*, 89, 2083 (1967).

have concluded that the addition reaction is rate determining, followed by the rapid elimination of water. They postulated that dehydration of the carbinolamine is catalyzed by the presence of the *o*-phenolate group. Our results are consistent with this interpretation.

The second-order rate term which yields the parameter k_c clearly represents the attack of salicylaldehyde anion by glycinate ion. The expressions involving k_a and k_b, which are first and second order in a_H, most likely arise from glycinate attack on salicylaldehyde rather than on the anion, however. A comparison of the derived rate constants for these reactions compared with those obtained for 3-hydroxypyridine-4-aldehyde is given in Table I. Conversion was made using pK_a = 8.215 for HSal.[12]

Table I. Glycinate Reaction Rates (min^{-1})

Reaction	Salicylaldehyde	3HO-pyridine-4al
$^-$OArCHO + Gly$^-$	k_0 = 300	200
HOArCHO + Gly$^-$	k_1 = 180	1.4×10^4
HOArCHO + Gly$^-$ + H$^+$	$k_2 = 2.5 \times 10^9$	5.7×10^6 [a]

[a] $H_2OArCHO^+$ + Gly$^-$ → products.

The rate constants for the reaction of the phenolate forms of the two aromatic aldehydes are seen in Table I to be similar. Relative to these reactions the reactivities of the neutral aldehydes show marked differences which are attributable to differences in their modes of protonation. The phenolate oxygen of salicylaldehyde anion is the only protonatable group in this pH range, whereas, with 3-hydroxypyridine-4-aldehyde, the more basic ring nitrogen is protonated to a greater extent than is the phenolate oxygen. The charged pyridinium group, which is thought to play an important role in pyridoxal activity,[15b-17] is seen to have a profound effect on the ability of the *p*-formyl group to undergo condensation. Through resonance the *p*-carbon atom of the pyridinium ring assumes a partial positive charge which facilitates the nucleophilic attack on the formyl carbon atom by the amine.

The value of k_2 for the remaining term is too large and the basicity of the carbonyl group is too small for this reaction to involve the preequilibrium: SalH + H$^+$ $\rightleftharpoons$ $SalH_2^+$. A concerted attack, SalH + H$^+$ + Gly$^-$ → product, is indicated. The proton may either be donated by the solvent or by the carboxylic acid group of an attacking molecule of the nonpolar form of neutral HGly.

The Zn(II)-dependent rate terms were obtained by subtracting the calculated metal-ion-independent rates from the total observed rates. A plot of the quantity log [residual rate/(Sal$^-$)(Gly$^-$)] *vs.* the log of the free zinc concentration (Figure 6) yields a straight line having a slope of 1.08. This shows a reaction which is first order in (Zn^{2+}) as well as in (Sal$^-$) and (Gly$^-$). The constant k_d for the expression

$$\text{rate} = k_d(Zn^{2+})(Sal^-)(Gly^-)$$

is evaluated to be $6.3 \times 10^6\ M^{-2}\ min^{-1}$ from the data of Figure 6.

(16) E. E. Snell, "Chemical and Biological Aspects of Pyridoxal Catalysis," E. E. Snell, P. M. Fasella, A. Braunstein, and A. Rossi Fannelli, Ed., The Macmillan Co., New York, N. Y., 1963, p 1.
(17) B. Pullman, ref 16, p 103.

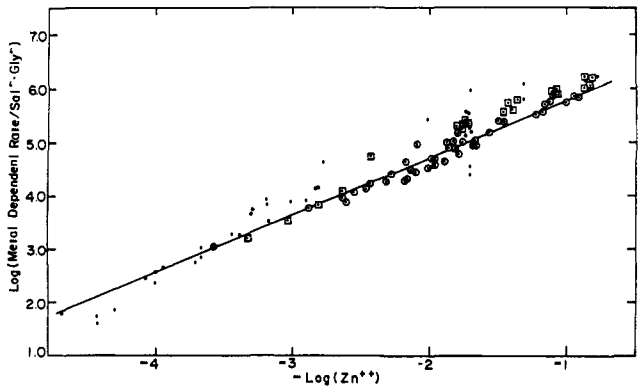


Figure 6. Log residual rate *vs.* log free Zn^{2+} (residual rate = observed rate − $k_a a_H^2 - k_b a_H - k_c$: ○, residual rate > obsd rate/2; □, obsd rate/2 > residual rate > obsd rate/3; ●, all other points.

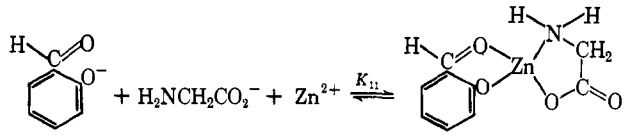
Ruling out an improbable ternary collision, this last rate law must arise from a preequilibrium between any two or all three of the species involved followed by a rate-determining reaction. Preequilibrium between Sal$^-$ and Gly$^-$ followed by a slow reaction with Zn^{2+} is shown to be inconsistent with this interpretation when the known value of k_c (300 min^{-1}) is compared with the bimolecular rate constants of about 10^6–10^7 commonly observed for Zn^{2+}–ligand reactions.[13] Rate-determining attack of Sal$^-$ on $ZnGly^+$ can also be ruled out since the amine electron pair necessary for the attack on the carbonyl carbon is coordinated to the metal ion. Coordination of the amine electron pair should result in deactivation.

A plausible mechanism is the nucleophilic attack of glycinate ion on coordinated salicylaldehyde. The value of k_p, the bimolecular rate constant for the reaction

$$ZnSal^+ + Gly^- \xrightarrow{k_p} Zn(Sal\cdot Gly) + H_2O$$

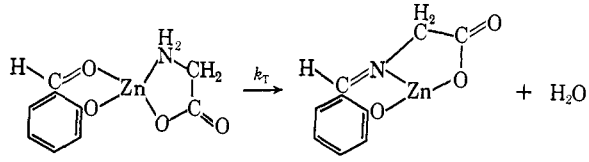
is obtained by dividing the observed value of k_d by the known formation constant of $ZnSal^+$ ($k_{form} = 7.4 \times 10^2\ M^{-1}$).[12] The result is $k_p = 8.5 \times 10^3\ M^{-1}\ min^{-1}$. Comparison with the results reported in Table I shows that if this mechanism prevails, the polarizing effect of Zn^{2+} on the formyl group is considerable. The effect of Zn(II) coordination on Sal$^-$ is almost as great as the effect of the pyridinium ring nitrogen in 3-hydroxypyridine-4-aldehyde. Furthermore, since polarization effects follow the stabilities of the complexes,[18-20] it is expected that Cu(II) and Ni(II) which form more stable Sal$^-$ complexes should exert even more pronounced catalysis than does Zn(II). The observed unreactivities of these ions and the unlikely high rate constant for Zn^{2+} are strong arguments against a polarization mechanism.

The remaining likely reaction sequence involves the rapid preequilibrium



(18) A. E. Dennard and R. J. P. Williams, "Transition Metal Chemistry," Vol. 2, R. L. Carlin, Ed., Marcel Dekker, Inc., 1966, p 116.
(19) M. L. Bender, Advances in Chemistry Series, No. 37, American Chemical Society, Washington, D. C., 1963.
(20) M. M. Jones, Symposium on Mechanisms of Inorganic Reactions, ref 13.

followed by the rate-determining reaction



This path possesses the features of the template mechanism.

The value of K_{11} is estimated to be about 1×10^7 M^{-2} (either by multiplying K_1 for the formation of $ZnGly^+$ by K_2 for the formation of $ZnSal_2$, or, *vice versa*, by multiplying K_1 for $ZnSal^+$ by K_2 for $ZnGly_2$).[21] Allowance for a statistical effect gives 2×10^7. The value of k_T obtained from the quotient k_d/K_{11} is then about 0.2–0.3 min^{-1}.

Bruice and Benkovic[22] estimate that in an ideal enzyme system, where the rapid formation of a ternary complex causes the order of a reaction to be reduced by one unit, the entropy gain accounts for a factor of 10^3 to 10^4 favoring the first-order rate constant over the second-order rate constant observed in the absence of the enzyme. In the present case, instead of showing an increase, the value of k_T is actually lower than k_0 by a factor of about 10^3. This decrease must result to a large extent from perturbation from the optimum reaction geometry by the coordination of the functional groups to the metal ion. It appears then that the factors which favor a high value of K_{11} (strong metal–ligand interaction) tend to operate unfavorably upon k_T. Since the observed rate constant is the product $K_{11}k_T$, there ought to be some metal ion for which the combination of polarizability and charge density yields a maximum catalytic effect, *i.e.*, a maximum value of $K_{11}k_T$. This interplay between K_{11} and k_T as a function of the metal ion is being further investigated in these laboratories.

Stringent coordination geometries also contribute to the unreactivities of Cu(II) and Ni(II). Values of K_{11} for these ions are appreciably higher than that for Zn^{2+}, about 6×10^{12} for Cu^{2+} and 6×10^8 for Ni^{2+}. Using these estimates, it is possible to set upper limits upon k_T of 0.010 min^{-1} for Cu(II) and 0.002 min^{-1} for Ni(II). These low rate constants must reflect the influence of ligand field forces. Ligand field forces, which are present in the Cu(II) and Ni(II) ions owing to the unfilled d orbitals, cause the ligands to be bound to well-defined coordination sites. Ligands coordinated to these sites will tend to remain separated and be prevented from reacting unless additional energy is expended to allow for less stable configurations. Zn(II) with its filled d shell is free from these influences.

Deviations of metal ion reactivities from the normal order of complex ion stabilities have been reported for several other biologically significant systems. Co(II) and Zn(II) readily activated carbonic anhydrase while Cu(II) is inactive.[23] Metal ion activity with carboxypeptidase[24] is found to follow the order Co > Zn > Ni, Cu = 0. The ability of Zn(II) to degrade soluble polynucleotides is markedly greater than is observed for other divalent metal ions.[25] In each of these cases, the unusual reactivity is attributed not only to the coordination ability of the metal ions but also to special geometrical properties of the active metal ions.[18,25] The system described in the present work furnishes a simple model wherein the joint requirements of metal–ligand bond strengths and coordination geometries may be examined in detail. Another valuable result of this work is the finding that it is possible through the use of metal ions to extend downward the pH range over which the kinetics of carbonyl addition reactions may be examined.

(21) A tendency for a slight excess of NaGly to be added to the solution in the first stage of the kinetic runs is consistent with a value of K_{11} equal to 3×10^7. The results have been corrected for the slight amount of Zn(Sal)(Gly) formed rapidly in the first phase of the reaction.

(22) T. C. Bruice and S. J. Benkovic, *J. Am. Chem. Soc.*, **86**, 418 1964).

(23) S. Lindskog, *J. Biol. Chem.*, **238**, 945 (1963).

(24) B. L. Vallee, J. F. Riordarand, and J. E. Coleman, *Proc. Natl. Acad. Sci. U. S.*, **49**, 109 (1963).

(25) J. J. Butzow and G. L. Eichhorn, *Biopolymers*, **3**, 95 (1965).