The rate of hydrogen evolution varied according to the source of the NaH used. In one run with NaH from a commercial source, 75% of the theoretical hydrogen gas was evolved in 30 min at −78°. In another case, with the NaH made in our laboratory, 60% of the total hydrogen was evolved in 1.5 hr. Factors controlling this reaction are still under study.

**Acknowledgment.** Support of this work by the National Science Foundation through Grant No. GP-8249 to the University of Michigan and Grant No. GP-14873 to the University of Utah is gratefully acknowledged.

# Stabilities, Rates of Formation, and Rates of Transimination in Aqueous Solutions of Some Zinc(II)–Schiff Base Complexes Derived from Salicylaldehyde[1,2]

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**Abstract:** The rates of formation of Schiff bases with salicylaldehyde and ethylamine, α-alaninate, or β-alaninate have been determined in the presence and absence of Zn(II). Rate laws identical with that previously reported for the formation of *N*-salicylideneglycinate have been found. Rate constants for the reaction $sal^- + RNH_2 \rightarrow$ Schiff base increase with increasing basicity of the amine. On the other hand, rate constants for the Zn(II)-catalyzed path, $Zn^{2+} + sal^- + RNH_2 \rightarrow$ Schiff base, are almost independent of the amine, consistent with a promnastic model. Amine-exchange (transimination) reactions of Schiff bases have also been investigated. Proton-catalyzed, Zn(II)-dependent and -independent paths have been uncovered. Exchange reactions of the type H(sal-NR) + $H_2NR' \rightleftarrows$ H(sal-NR′) + $H_2NR$, as well as those which involve Zn(II), proceed at considerably faster rates than does the formation of the Schiff bases by the reaction of $R'NH_2$ with salicylaldehyde. The stabilities of the free and protonated Schiff bases correlate with the basicities of the parent amines. The stabilities of the Zn(II)–Schiff base complexes are also strongly influenced by structural factors.

Schiff bases and their metal-ion complexes find interest in organic chemistry, biochemistry, and inorganic chemistry. A program in these laboratories has been devoted to the investigation of the behavior of these substances in the presence of metal ions in aqueous media. Of particular interest has been the effect of metal ions on the rates of formation. The rate of the reaction of salicylaldehyde and glycinate to give *N*-salicylideneglycinate has been studied in the presence and absence of various divalent metal ions.[3,4] To account for the interesting pattern of metal-ion reactivities wherein polarizing metal ions such as $Co^{2+}$, $Ni^{2+}$, and $Cu^{2+}$ were found to be kinetically inactive, but where others, $Cd^{2+}$ and $Pb^{2+}$, in particular, but also $Mn^{2+}$, $Mg^{2+}$, and $Zn^{2+}$, were found to be active, the promnastic effect[4] was postulated. It was proposed that in essence the metal ion principally serves to lower the order of the reaction from the second-order reaction which is observed between the free ligands to a first-order reaction occurring within a mixed complex in which the reactants (at least the amine donor) are loosely bound to the metal ion. The highly dynamic situation in which various metal–donor atom bonds are frequently broken and re-formed in chelate[5] rings is visualized to give a sufficiently high concentration of uncoordinated amine electron pairs to enable the addition of amine to carbonyl. The lower the inherent strength of the metal–nitrogen bond, the higher the activity of the mixed complex. Furthermore, in the reaction with glycinate variation of the carbonyl compound from salicylaldehyde to pyruvate[6,7] has little effect on the rate law except to introduce a change which quantitatively arises from the difference between the binding of these two ligands to the metal ion [Zn(II)] in the mixed complex.

In the present study the nature of the amine was varied. The amines investigated were ethylamine, α-alanine, and β-alanine, which permitted the examination of the affect on the kinetics of (a) monodentate binding, (b) an alkyl side chain, and (c) six-membered chelate ring formation by the amine moiety.

It was also anticipated that *N*-salicylideneethylamine forms weaker Schiff base–metal ion complexes than Schiff bases derived from the bidentate amino acids, and this difference could be employed to study amine-exchange (transimination) processes in these systems. It has been reported[8] that Cu(II)–5-sulfosalicylaldehyde solutions containing ethylamine and glycinate first exhibit the absorption spectrum of the Cu(II) complex of the ethylamine Schiff base, but after 96 hr the spectrum goes over to that of the more stable glycinate Schiff base complex. This result shows that thermodynamic control determines the final distribution of species in these systems, although kinetics determine the initial distribution.

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(1) The authors wish to acknowledge support by the National Science Foundation for this research.

(2) Presented at the 2nd Central Regional Meeting of the American Chemical Society, Columbus, Ohio, June 1970.

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The kinetics for the direct replacement of an imine in a Schiff base by hydroxylamine, methoxylamine, and semicarbazide have been examined,[9–11] and an enzymatic relaxation time ($k > 10^7$ sec$^{-1}$) which presumably incorporates a transimination process has been reported.[12] The effect of metal ions on these systems does not seem to have been investigated, however. This task has been undertaken in this work. Another goal was to investigate approaches toward obtaining meaningful rate constants from systems which potentially contain all the species in solution arising from the interactions of a carbonyl compound, two different amines, and two different Schiff bases in competition for metal ions and protons.

## Experimental Section

Vacuum-distilled salicylaldehyde was freshly weighed out for each run. Ethylamine hydrochloride, glycine, α-alanine, and β-alanine were recrystallized from water–ethanol mixtures. Stock solutions of $ZnCl_2$ were prepared and standardized using accepted procedures.

The experimental and calculational procedures for obtaining the Schiff-base stability constants and formation rate constants at 25° at an ionic strength of 0.5 (KCl) were essentially the same as those previously described.[3,4] To determine the stability constants, a series of batchwise titrations were performed in which individual points were obtained by adding either standard sodium aminoacidate or NaOH to an aliquot of a solution containing accurately known amounts of metal ion and salicylaldehyde. Each of these solutions was kept under an atmosphere of nitrogen, and after standing 12–24 hr, which was sufficiently long for equilibration, the pH was determined.

Although the pH range available for investigation was found to be limited owing to precipitation, both the mono and bis Schiff base Zn(II) complexes were formed in sufficiently high concentrations to assess their formation constants with sufficient accuracy for the purposes of this study. The experimental conditions employed for several series of titrations were 0.006 $M$ $\mathrm{Zn_{tot}}$, 0.006–0.02 $M$ salicylaldehyde, 0.05 $M$ ethylamine hydrochloride, titrated with 0.10 $N$ NaOH, $\mathrm{pH_{max}}$ 7.0; 0.008 $M$ $\mathrm{Zn_{tot}}$, 0.006–0.020 $M$ salicylaldehyde, titrated with 0.10 $M$ sodium α-alaninate, $\mathrm{pH_{max}}$ 6.6. $\mathrm{pH_{max}}$ is the maximum value of pH for which reliable data could be obtained before the onset of precipitation. Attempts to obtain the ethylamine Schiff base formation constants for Co(II), Ni(II), and Cu(II) failed owing to a greater tendency for precipitation than with Zn(II). The formation constants of the uncomplexed protonated Schiff bases were determined by titrating solutions containing equimolar (0.02 $M$) concentrations of salicylaldehyde and amino acid or amine hydrochloride with standard NaOH. Formation constants of the unprotonated Schiff bases were determined spectrophotometrically in solutions at pH 12.0 by measuring the change in intensity of the 377-nm absorption maximum of salicylaldehyde anion as the amine concentration was varied. The total salicylaldehyde level was $1.6 \times 10^{-4}$ $M$ and the amine level ranged from 0 to 0.15 $M$.

Rates of reaction of salicylaldehyde with the various amines to give Schiff bases in the absence of metal ions were determined spectrophotometrically using conditions and procedures identical with those described in the glycinate study.[4] The pH-Stat technique was employed to obtain the reaction rates in the presence of Zn(II). The concentrations investigated were: 0.006–0.06 $M$ $\mathrm{Zn_{tot}}$, 0.1 $M$ ethylamine hydrochloride, 0.01–0.03 $M$ salicylaldehyde, titrated with 0.05 $M$ NaOH over the pH range 6.0–7.0; 0.01–0.04 $M$ $\mathrm{Zn_{tot}}$, 0.02 $M$ salicylaldehyde, titrated with 0.05 $M$ sodium α-alaninate over the pH range 5.9–6.7; 0.006–0.04 $M$ $\mathrm{Zn_{tot}}$, 0.01–0.03 $M$ salicylaldehyde, titrated with 0.1 $M$ sodium β-alaninate over the pH range 6.3–7.0.

Rate determinations with other metal ions were also performed under the following conditions: 0.004 $M$ $\mathrm{Cu_{tot}}$, 0.05 $M$ alanine, 0.013 $M$ salicylaldehyde, titrated with 0.05 $M$ NaOH over the pH range 3.6–4.6; 0.004–0.016 $M$ $\mathrm{Ni_{tot}}$, 0.016 $M$ salicylaldehyde, 0.25 $M$ ethylamine hydrochloride, titrated with 0.050 $M$ NaOH over the pH range 5.2–5.8; 0.003–0.32 $M$ $\mathrm{Co(II)_{tot}}$, 0.016 $M$ salicylaldehyde, 0.30 $M$ ethylamine hydrochloride, titrated with 0.05 $M$ NaOH over the pH range 5.6–6.0.

The pH range in each case was restricted to the lower limit by an unfavorable free energy of reaction and to the upper limit by solubility considerations and by the fact that the pH-Stat becomes insensitive to the rate when high concentrations of binary complexes are formed initially. However, by varying ligand levels in the Zn(II) experiments it was possible in most cases to cause the free $Zn^{2+}$, free amine, and free salicylaldehyde anion concentrations each to vary by at least a factor of 10.

The amine-exchange reactions were studied using two different experimental approaches. In one set of experiments the rate of transimination was determined spectrophotometrically in a Durrum-Gibson stopped-flow apparatus. One of the solutions to be mixed contained a low concentration of salicylaldehyde ($\sim$0.00015 $M$) in an excess of either ethylamine hydrochloride or another of the amino acids. The solution was adjusted to a slightly alkaline pH ($\sim$8) by the addition of NaOH and, depending on the amine, allowed to stand for 30 min to several hours until equilibration was attained. At equilibrium, at least 95% of the salicylaldehyde was combined as the Schiff base in most experiments.

The second solution to be mixed contained the exchanging amino acid and Zn(II). Prior to filling the drive syringe, the pH of this solution had been adjusted with NaOH to a value which caused the pH of the mixed solutions to attain some desired value. The required experimental conditions were determined through preliminary calculations.

The absorbance of the mixed solution was monitored either at 390 nm (the wavelength of the absorption maximum of the monoprotonated form of the Schiff bases[13–15]) or at 350 nm (the absorption maximum of the Zn(II) complexes[15]). The absorption spectra of the protonated forms of the Schiff bases are practically identical in this wavelength region, as are also the spectra of their Zn(II) complexes. Because of this it was possible to observe the course of the reaction spectrophotometrically *only* when conditions could be arranged so that the ratio of protonated to complexed forms of the initial Schiff base differed from that in the exchange product. Initial values of absorbance and initial slopes were used to calculate the pseudo-first-order rate constants for the rate of change of absorbance.

The rate of amine exchange was also determined in pH-Stat experiments by determining the effect of added ethylamine hydrochloride (in the range 0.1–0.5 $M$) on the rate of uptake of sodium glycinate or sodium α-alaninate at constant pH by Zn(II)–salicylaldehyde solutions. Measurable catalytic effects were indeed observed. To obtain the rate parameters from the data a numerical integration procedure was employed. This procedure is described in more detail below.

Measurements of pH were made using Radiometer PHM 26 and Orion 801 pH meters. The glass electrodes were calibrated against NBS standards. Although concentration units are employed below to represent hydrogen ion terms in the equilibrium and rate expressions, the actual constants were evaluated without conversion of the measured hydrogen ion activities to concentration units; *i.e.*, the constants given are "practical" constants. Concentration units are employed for other species, however.

## Results

The formation constants which have been determined here or elsewhere[4,8,16] for the species pertinent to this work are presented in Table I.[17] It is seen that the

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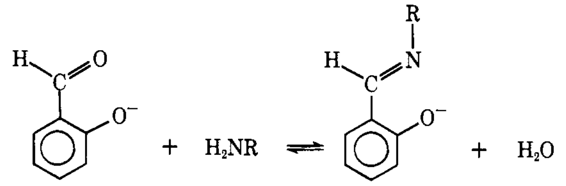
(17) The abbreviations used are $\mathrm{sal^-}$ = salicylaldehyde anion; $\mathrm{gly^-}$ = glycinate; α-$\mathrm{ala^-}$ = α-alaninate; β-$\mathrm{ala^-}$ = β-alaninate; eta = ethylamine; xyz = one of the previously listed amine or amino acid anions, omitting the charge; (sal–xyz) = a Schiff base formed by the combination of $\mathrm{sal^-}$ and xyz, *e.g.*, (sal–eta) = *N*-salicylideneethylamine.

**Table I.** Equilibrium Constants for the Schiff-Base Systems at 25°, $\mu = 0.5$ (KCl)

| Reaction[a] | Log $K$ | Reaction | Log $K$[c] |
|---|---|---|---|
| | | Ethylamine | |
| $\mathrm{H^+ + eta \rightleftarrows H(eta)^+}$ | 10.81 | $\mathrm{sal^- + eta \rightleftarrows (sal{-}eta)^- + H_2O}$ | 1.05 |
| $\mathrm{Zn^{2+} + eta \rightleftarrows Zn(eta)^{2+}}$ | 2.30 | $\mathrm{H^+ + sal^- + eta \rightleftarrows H(sal{-}eta) + H_2O}$ | 12.60 |
| $\mathrm{Zn^{2+} + eta \rightleftarrows Zn(eta)_2{}^{2+}}$ | 4.33 | $\mathrm{2H^+ + sal^- + eta \rightleftarrows H_2(sal{-}eta)^+ + H_2O}$ | 17.56[d] |
| $\mathrm{Zn^{2+} + 3\ eta \rightleftarrows Zn(eta)_3{}^{2+}}$ | 6.0 | $\mathrm{Zn^{2+} + sal^- + eta \rightleftarrows Zn(sal{-}eta)^+ + H_2O}$ | 7.78 |
| $\mathrm{Zn^{2+}{-}sal^- + eta \rightleftarrows Zn(sal)(eta)^+}$ | 4.7[b] | $\mathrm{Zn^{2+} + 2sal^- + 2eta \rightleftarrows Zn(sal{-}eta)_2 + 2H_2O}$ | 14.9 |
| | | $\mathrm{Zn^{2+} + sal^- + eta + gly^- \rightleftarrows Zn(sal{-}eta)(gly) + H_2O}$ | 12.7[b] |
| | | $\mathrm{Zn^{2+} + sal^- + eta + \alpha{-}ala^- \rightleftarrows Zn(sal{-}eta)(\alpha{-}ala)}$ | 12.7[b] |
| | | $\mathrm{Zn^{2+} + sal^- + eta + \beta{-}ala \rightleftarrows Zn + H_2O(sal{-}eta)(\beta{-}ala)^-}$ | 11.7[b] |
| | | α-Alaninate | |
| $\mathrm{H^+ + \alpha{-}ala^- \rightleftarrows H(\alpha{-}ala)}$ | 9.82 | $\mathrm{sal^- + \alpha{-}ala^- \rightleftarrows (sal{-}\alpha{-}ala)^{2-} + H_2O}$ | 0.78 |
| $\mathrm{2H^+ + \alpha{-}ala^- \rightleftarrows H_2(\alpha{-}ala)^+}$ | 12.26 | $\mathrm{H^+ + sal^- + \alpha{-}ala^- \rightleftarrows H(sal{-}\alpha{-}ala)^- + H_2O}$ | 11.75 |
| $\mathrm{Zn^{2+} + \alpha{-}ala^- \rightleftarrows Zn(\alpha{-}ala)^+}$ | 4.56[e] | $\mathrm{Zn^{2+} + sal^- + \alpha{-}ala^- \rightleftarrows Zn(sal{-}\alpha{-}ala) + H_2O}$ | 9.56 |
| $\mathrm{Zn^{2+} + 2\alpha{-}ala^- \rightleftarrows Zn(\alpha{-}ala)_2}$ | 8.56[e] | $\mathrm{Zn^{2+} + 2sal^- + 2\alpha{-}ala^- \rightleftarrows Zn(sal{-}\alpha{-}ala)_2{}^- + 2H_2O}$ | 16.23 |
| $\mathrm{Zn^{2+} + 3\alpha{-}ala^- \rightleftarrows Zn(\alpha{-}ala)_3{}^-}$ | 10.57[e] | | |
| $\mathrm{Zn^{2+} + sal^- + \alpha{-}ala^- \rightleftarrows Zn(sal)(\alpha{-}ala)}$ | 7.8[f] | | |
| | | β-Alanine | |
| $\mathrm{H^+ + \beta{-}ala^- \rightleftarrows H(\beta{-}ala)}$ | 10.21 | $\mathrm{sal^- + \beta{-}ala^- \rightleftarrows (sal{-}\beta{-}ala)^{2-} + H_2O}$ | 0.82 |
| $\mathrm{2H^+ + \beta{-}ala^- \rightleftarrows H_2(\beta{-}ala)^+}$ | 13.83 | $\mathrm{H^+ + sal^- + \beta{-}ala^- \rightleftarrows H(sal{-}\beta{-}ala)^- + H_2O}$ | 12.23 |
| $\mathrm{Zn^{2+} + \beta{-}ala^- \rightleftarrows Zn(\beta{-}ala)^+}$ | 3.90 | $\mathrm{Zn^{2+} + sal^- + \beta{-}ala^- \rightleftarrows Zn(sal{-}\beta{-}ala^-) + H_2O}$ | 8.40 |
| $\mathrm{Zn^{2+} + 2\beta{-}ala^- \rightleftarrows Zn(\beta{-}ala)_2}$ | 7.20 | $\mathrm{Zn^{2+} + 2sal^- + 2\beta{-}ala^- \rightleftarrows Zn(sal{-}\beta{-}ala)_2 + 2H_2O}$ | 16.20 |
| $\mathrm{Zn^{2+} + 3\beta{-}ala^- \rightleftarrows Zn(\beta{-}ala)_3{}^-}$ | 10.40 | | |
| $\mathrm{Zn^{2+} + sal^- + \beta{-}ala^- \rightleftarrows Zn(sal)(\beta{-}ala)}$ | 6.0[b] | | |
| | | Glycinate[g] | |
| $\mathrm{H^+ + gly^- \rightleftarrows H(gly)}$ | 9.70 | $\mathrm{sal^- + gly^- \rightleftarrows (sal{-}gly)^{2-} + H_2O}$ | 0.48 |
| $\mathrm{2H^+ + gly^- \rightleftarrows H_2(gly)^+}$ | 12.18 | $\mathrm{H^+ + sal^- + gly^- \rightleftarrows H(sal{-}gly)^- + H_2O}$ | 11.69 |
| $\mathrm{Zn^{2+} + gly^- \rightleftarrows Zn(gly)^+}$ | 4.88 | $\mathrm{Zn^{2+} + sal^- + gly^- \rightleftarrows Zn(sal{-}gly) + H_2O}$ | 9.65 |
| $\mathrm{Zn^{2+} + 2gly^- \rightleftarrows Zn(gly)_2}$ | 9.11 | $\mathrm{Zn^{2+} + sal^- + 2gly^- \rightleftarrows Zn(sal{-}gly)(gly)^- + H_2O}$ | 13.42 |
| $\mathrm{Zn^{2+} + 3gly^- \rightleftarrows Zn(gly)_3{}^-}$ | 11.56 | $\mathrm{Zn^{2+} + 2sal^- + 2gly^- \rightleftarrows Zn(sal{-}gly)_2{}^{2-} + 2H_2O}$ | 16.73 |
| $\mathrm{Zn^{2+} + sal^- + gly^- \rightleftarrows Zn(sal)(gly)}$ | 7.36[b] | | |
| | | Salicylaldehyde[g] | |
| $\mathrm{H^+ + sal^- \rightleftarrows H(sal)}$ | 8.22 | | |
| $\mathrm{Zn^{2+} + sal^- \rightleftarrows Zn(sal)^+}$ | 2.87 | | |
| $\mathrm{Zn^{2+} + 2sal^- \rightleftarrows Zn(sal)_2}$ | 5.00 | | |

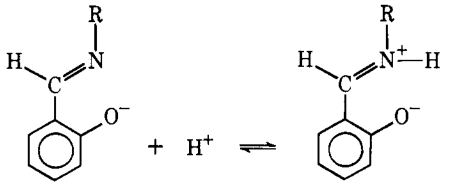
[a] The abbreviations correspond to those defined in footnote 17. [b] Estimated value; see ref 4. [c] The values of the Schiff-base formation constants do not include the $H_2O$ concentration. [d] $\mathrm{H_2(sal{-}eta)^+ \rightleftarrows H^+ + H(sal{-}eta)}$; ref 8. [e] From ref 16. [f] From fit of zero-time volumes of pH-Stat curves. [g] From ref 3.

equilibrium constants for the formation of the unprotonated Schiff bases (eq 1) correlate with the basicity



(1)

of the parent amine, increasing in the order $gly^- < \alpha\text{-}ala^- < \beta\text{-}ala^- <$ eta. Correlating to a somewhat lesser extent with the basicity of the amine are the constants for the protonation of the Schiff base (eq 2),



the site of which is the imine group.[13-15] The Schiff-base p$K_a$ values which can be calculated from the data of Table I are 11.55 (eta), 11.41 ($\beta$-ala$^-$), 11.21 (gly$^-$), and 10.97 ($\alpha$-ala$^-$). Green and Sleet[18] report a p$K_a$ of 11.8 for H(sal–eta) at lower ionic strength, and a kinetically determined value of 11.81 is given in ref 8 for conditions similar to those of this work. These latter authors also give log $K$ = 4.96 for H(sal–eta) + $\mathrm{H^+ \rightleftarrows H_2(sal{-}eta).^+}$

The formation constants for the Schiff base–Zn(II) complexes in Table I have been calculated for the reactions which involve the fundamental unassociated species

$$\mathrm{sal^- + xyz + Zn^{2+} \rightleftarrows Zn(sal{-}xyz) + H_2O}$$

The values depend primarily on the strength of the C=N bond, the basicity of the imine group, the denticity of the Schiff base, and steric factors. Within a closely related series of complexes, it is possible to minimize variations in the first two factors and focus attention on the last two by comparing metal ion–proton exchange constants, $\mathrm{H(sal{-}zyx) + Zn^{2+} \rightleftarrows Zn(sal{-}xyz) + H^+}$. Values of log $K_{\text{exchange}}$ calculated from the data of Table I are −2.0 for gly$^-$, −2.2 for $\alpha$-ala$^-$, −3.8 for $\beta$-ala$^-$, and −4.8 for eta. The relatively low value for *N*-salicylideneethylamine reflects bidentate binding compared to tridentate binding for the other Schiff bases. *N*-Salicylidene-$\beta$-alaninatezinc(II), which possesses two fused six-membered chelate rings, is seen to have a much lower exchange constant than gly$^-$ and $\alpha$-ala$^-$, each of which forms five- and six-membered fused ring systems. The presence of the methyl group of $\alpha$-ala$^-$ is seen to cause a small, but measurable, de-

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(18) R. W. Green and R. J. Sleet, *Aust. J. Chem.*, **19**, 2101 (1966).

crease in the stability of its Schiff-base complex. This effect does not appear to arise from interaction of the methyl group with other coordinated ligands, since the presence of bulkier ligands has little effect on the relative stability; the logarithms of the constants for the reaction Zn(sal–xyz) + H(sal–xyz)$^-$ $\rightleftarrows$ Zn(sal–xyz)$_2^{2-}$ + H$^+$ are −4.6 for glycinate and −5.1 for $\alpha$-alaninate. Probably the influence of the methyl groups arises from its effects on the solvation of the complex and on the $=$NCH(R)CO$_2^-$ bond angle.

**Rates of Formation of Schiff Bases and Schiff-Base Complexes.** The rates of formation of the Schiff bases and their Zn(II) complexes were found in this work to conform to the equation

$$\frac{d(\text{sal–xyz})_\Sigma}{dt} = (k_a[\text{H}^+]^2 + k_b[\text{H}^+] + k_c + k_d[\text{M}^{2+}] + k_e[\text{M}^{2+}][\text{H}^+])(\text{sal}^-)(\text{xyz})$$

where (sal–xyz)$_\Sigma$ is the sum of all forms of the Schiff base. The values of the constants are given in Table II.

**Table II.** Rate Constants for *N*-Salicylideneamine Formation at 25°, $\mu$ = 0.5 (KCl)[a]

| | xyz | | | |
|---|---|---|---|---|
| | Ethylamine | $\alpha$-Alaninate | $\beta$-Alaninate | Glycinate[b] |
| $k_a$, $M^{-3}$ sec$^{-1}$, × $10^{-15}$ [c] | 1.6 | 1.4 | 6.0 | 6.9 |
| $k_b$, $M^{-2}$ sec$^{-1}$, × $10^{-9}$ [c] | 2.5 | 0.15 | 1.8 | 0.67 |
| $k_c$, $M^{-1}$ sec$^{-1}$ | 1.0 | 0.037 | 0.48 | 0.18 |
| $k_d$, $M^{-2}$ sec$^{-1}$, × $10^{-4}$ | 8.0 | 0.72 | 6.5 | 5.9 |
| $k_e$, $M^{-3}$ sec$^{-1}$, × $10^{-10}$ [c] | ~0 | 0.45 | 10.4 | 3.85 |
| $k_f$, $M^{-3}$ sec$^{-1}$, × $10^{-9}$ | *d* | *d* | *d* | 1.2 |

[a] sal$_\Sigma$ + xyz$_\Sigma$ → (sal–xyz)$_\Sigma$ + H$_2$O; d(sal–xyz)$_{\text{tot}}$/d$t$ = ($k_a$[H$^+$]$^2$ + $k_b$[H$^+$] + $k_c$ + $k_d$[Zn$^{2+}$] + $k_e$[Zn][H$^+$] + $k_f$ [Zn$^{2+}$][xyz])(sal$^-$)(xyz). [b] Reference 4. [c] In evaluating the rate constants, the hydrogen ion activity has been used in place of concentration. The other terms are in concentration units. [d] The pH range in which this path becomes important was not examined.

This rate law is identical with the rate laws found earlier for formation of the glycinate Schiff-base complexes of pyruvate in the presence and absence of Zn(II)[7] and of salicylaldehyde in the presence and absence of Co(II), Ni(II), Cu(II), Zn(II), Mg(II), Mn(II), Pb(II), and Cd(II).[4,19] A common reaction mechanism for all of the amines studied is indicated not only by these identical rate laws but also by the fact that the ratios of the rate constants $k_a$:$k_b$ and $k_b$:$k_c$ have roughly the same values. The slightly lower values of these ratios found for the ethylamine reactions may possibly arise from simple electrostatics: the negatively charged aminoacidate anion reactions would be influenced more by an increase in the degree of protonation of the activated complex than the reactions involving neutral ethylamine.

The paths giving rise to the $k_b$ terms probably involve the attack of amine on neutral salicylaldehyde.[4] Since electrostatic effects are thereby minimized, it is of interest to note that the $k_b$ values correlate with the basicity of the amine: glycinate < $\beta$-alaninate < eta. The low value for $\alpha$-ala$^-$ indicates appreciable steric interference by the methyl side group.

The rates of hydrolysis of *N*-salicylideneethylamine as a function of pH have been reported in ref 8. It is possible then to compare the observed rates with those which can be calculated from the forward rates given in Table II and the equilibrium constants given in Table I. Our data predict a plateau for the effective first-order metal-independent rate of hydrolysis of *N*-salicylideneethylamine at pH 13 and above of about 0.09 sec$^{-1}$, compared to values of 0.10—0.12 sec$^{-1}$ given in Figure 1 of ref 8. Similarly, the results of Tables I and II predict a metal-independent rate plateau in the pH range 7–8 of 6.5 × $10^{-4}$ sec$^{-1}$ compared to 7.0 × $10^{-4}$ sec$^{-1}$ measured directly. Below pH 4 the observed rates of hydrolysis become much slower than those predicted from the forward rates owing to a change in the rate-determining step. The very good agreement between the two sets of data above pH 5 shows that in slightly acidic to alkaline media the same rate-determining step is observed whether initial rates of formation or of hydrolysis are studied.

The methyl group of $\alpha$-ala$^-$ also causes relatively low values for the Zn(II)-dependent paths described by $k_d$. On the other hand, the corresponding rates for eta, $\beta$-ala$^-$, and gly$^-$ are seen to lie very close to each other. This leveling effect is consistent with the promnastic model which involves mediation *via* a mixed complex.[4]

$$\text{M}^{2+} + \text{sal}^- + \text{xyz} \underset{\beta_{11}^*}{\overset{\text{fast}}{\rightleftharpoons}} \text{M(sal)(xyz)} \underset{k_p}{\xrightarrow{\text{slow}}} \text{M(sal–xyz)} + \text{H}_2\text{O}$$

The value of $k_d$ is equal to the product $\beta_{11}^*k_p$, and those factors which will tend to increase $\beta_{11}^*$ will produce the opposite effect on $k_p$ and *vice versa*. The product of these two terms will show less variation than either one.

A proton-catalyzed Zn(II) path ($k_e$) was observed for all the amines studied except ethylamine. It would seem that it is the protonation of the carboxylate group which leads to Schiff-base formation *via* this path. This conclusion is obscured, however, owing to the electrostatic effects which appear to be present in the aminoacidate Schiff bases.

The reaction systems involved in the amine-exchange experiments are presented schematically in Figure 1. Paths III and −III represent direct amine exchange, paths I and −I represent the formation and hydrolysis of *N*-salicylideneaminoacidate *via* reactions involving salicylaldehyde and aminoacidate, and paths II and −II represent the corresponding reactions for *N*-salicylideneethylamine.

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(19) Owing to the lack of good equilibrium constants for the respective Schiff-base complexes, it was unfortunately not possible to analyze the pH-Stat data obtained from the Co(II)–, Ni(II)–, or Cu(II)–(sal–eta) systems. It is necessary to known these constants because the proton affinity of *N*-salicylideneethylamine is relatively high compared to its affinity for Zn(II). Consequently, the Schiff base is distributed appreciably over the protonated and complexed forms in the pH range which was used experimentally. The rate of uptake of NaOH which is necessary to maintain constant pH in the reacting solution is in turn influenced considerably by the product distribution. As an example of the importance of this effect, the rates of NaOH uptake with Co(II) and Ni(II) were found to actually increase as the metal-ion concentrations were increased, indicating apparent catalysis by these metal ions. However, by assuming reasonable values for the metal ion–Schiff base formation constants this effect was shown simply to have its origins in the conversion of the principle form of the product from H(sal–eta) and H$_2$(sal–eta)$^-$ to M(sal–eta)$^+$ as the concentration of M$^{2+}$ increased. Thus, even though the reaction rate remained essentially unchanged, the rate of NaOH uptake from the pH-Stat increased. Qualitatively, Co$^{2+}$ and Ni$^{2+}$ do not appear to be kinetically active in the (sal–eta) system either. The results obtained with Cu$^{2+}$ showed additional complications and were not amenable to analysis. The tendency for Cu$^{2+}$–Schiff base solutions to undergo side reactions in acid media[20] seems also to apply here.

(20) L. Davis, F. Roddy, and D. E. Metzler, *J. Amer. Chem. Soc.*, 83, 127 (1961).

**Table III.** Stopped-Flow Determination of Amine-Exchange Rates, $(\text{sal–eta})_\Sigma + \text{xyz}' \rightleftarrows (\text{sal–xyz}')_\Sigma + \text{eta}$, $\mu = 0.5$ (KCl), 25°

| Solution I, initial comp [xyz'], $M$ | Solution I, initial comp pH | Mixed solution pH | Mixed solution $[\text{Zn}_{aq}{}^{2+}]$, $M \times 10^3$ | Mixed solution [xyz'], $M \times 10^5$ | Fraction of Schiff base present as $H_2$-(sal–eta)$^+$ | Fraction of Schiff base present as H-(sal–eta) | Fraction of Schiff base present as Zn-(sal–eta)$^+$ | Fraction of Schiff base present as H-(sal–eta) | $k_{obsd} = \dfrac{1}{A_0}\dfrac{dA_0}{dt}$, sec$^{-1}$ | $k_{-II}$,[c] sec$^{-1}$ | $k_{III}$,[d] sec$^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| xyz' = gly$^-$[a] | | | | | | | | | | | |
| 0.100 | 5.50 | 5.91 | 6.3 | 0.74 | 0.092 | 0.81 | 0.063 | 0.022 | 0.0034 | 0.0010 | 0.0025 |
| 0.200 | 5.70 | 6.05 | 3.3 | 2.1 | 0.066 | 0.82 | 0.046 | 0.029 | 0.0084 | 0.0009 | 0.0080 |
| 0.400 | 5.70 | 6.05 | 1.6 | 4.2 | 0.068 | 0.84 | 0.022 | 0.058 | 0.018 | 0.0009 | 0.019 |
| 0.100 | 6.10 | 6.44 | 3.0 | 2.2 | 0.029 | 0.72 | 0.092 | 0.013 | 0.0080 | 0.0007 | 0.0075 |
| 0.200 | 6.10 | 6.44 | 1.3 | 4.8 | 0.026 | 0.77 | 0.043 | 0.029 | 0.019 | 0.0007 | 0.018 |
| xyz' = $\alpha$-ala$^-$[b] | | | | | | | | | | | |
| 0.100 | 5.60 | 5.98 | 6.9 | 0.60 | 0.077 | 0.81 | 0.081 | 0.024 | 0.0024 | 0.0011 | 0.0015 |
| 0.200 | 5.60 | 5.96 | 5.2 | 1.15 | 0.082 | 0.81 | 0.058 | 0.032 | 0.0041 | 0.0011 | 0.0033 |
| 0.400 | 5.60 | 5.94 | 3.3 | 2.3 | 0.085 | 0.82 | 0.036 | 0.051 | 0.0058 | 0.0011 | 0.0050 |
| 0.100 | 6.00 | 6.36 | 4.8 | 1.3 | 0.029 | 0.73 | 0.12 | 0.014 | 0.0027 | 0.0008 | 0.0020 |
| 0.200 | 6.00 | 6.35 | 2.8 | 2.7 | 0.031 | 0.75 | 0.072 | 0.024 | 0.0042 | 0.0008 | 0.0035 |
| 0.400 | 6.00 | 6.34 | 1.4 | 5.6 | 0.032 | 0.78 | 0.036 | 0.048 | 0.0083 | 0.0008 | 0.0082 |
| 0.100 | 6.30 | 6.65 | 3.1 | 2.5 | 0.013 | 0.62 | 0.13 | 0.011 | 0.0027 | 0.0007 | 0.0022 |
| 0.200 | 6.30 | 6.64 | 1.5 | 5.25 | 0.014 | 0.66 | 0.066 | 0.023 | 0.0043 | 0.0006 | 0.0040 |
| 0.400 | 6.30 | 6.64 | 0.57 | 11.0 | 0.015 | 0.72 | 0.028 | 0.057 | 0.0085 | 0.0006 | 0.0086 |

[a] Solution II $1.46 \times 10^{-4}$ $M$ $\text{sal}_{tot}$; pH 8.0; 1.00, 0.80, 0.80, 0.60, 0.60 $M$ eta·HCl (in decreasing order). [b] Solution II $1.43 \times 10^{-4}$ $M$ $\text{sal}_{tot}$; pH 8.0; 0.6 $M$ eta·HCl for all experiments. [c] $k_{-II} = (k_a[H^+] + k_b)f_{H(\text{sal-eta})}/\beta_{H(\text{sal-ata})} + (k_d + k_e[H^+])f_{Zn(\text{sal-eta})}/\beta_{Zn(\text{sal-eta})}$; $k_{-II}$ is the effective first-order rate of hydrolysis of the Schiff base sal–eta. [d] Pseudo-first-order rate constant for the excess rate of disappearance of $(\text{sal–eta})_\Sigma$.

In the stopped-flow experiments an attempt was made to measure rates along path III directly from the rate of disappearance of the absorbance when solutions of H-(sal–eta)$^+$ are mixed with those containing Zn(II) and a partially neutralized amino acid. Aside from interest in its kinetic properties, it was necessary to have Zn(II) present to provide both favorable free energies and observable spectral changes. To achieve this latter condition, conditions were arranged so that the principle overall reactions were $\text{H(sal–eta)} + \text{Zn}^{2+} + \text{xyz}^- \rightarrow \text{Zn(sal–xyz)} + \text{Heta}^+$. (A reaction mechanism is not implied by this equation.) Examples of the experimental conditions employed and the distributions of reactant and product Schiff bases are given in Table III for glycinate and $\alpha$-alaninate exchange with $N$-salicylideneethylamine. The pH range over which the spectral changes could be observed was restricted somewhat by the tendency for both reactant and product Schiff bases to be converted to the Zn(II) complex at higher pH and by the suppression of the concentration of the exchanging amine through protonation at lower pH.

It was also possible to examine ethylamine exchange by $\beta$-alaninate and exchange between $N$-salicylidene-$\beta$-alanine and glycinate or $\alpha$-alaninate. Exchange of glycinate by $\alpha$-alaninate was not experimentally observable owing to the similar stabilities of corresponding species. This precluded obtaining suitable spectral changes.

Psuedo-first-order rate constants were calculated from the initial values of absorbance and initial slopes using the equation

$$k_{obsd} = -(1/A)_{t=0}\left(\frac{dA}{dt}\right)_{t=0}$$

where $A$ = absorbance.

Hydrolysis of the parent Schiff base causes a decrease in absorbance, as also do those proton–Zn(II) exchange reactions which occur on mixing and involve the parent Schiff base.

$$\text{H(sal–eta)}^+ + \text{Zn}^{2+} \rightleftharpoons \text{Zn(sal–eta)}^{2+} + \text{H}^+$$

Separate experiments showed that the latter reactions are very fast and equilibrium is reached within 10 msec after mixing. Therefore, on the time scale of the amine exchange and hydrolysis reactions (seconds), it is valid to apply equilibrium theory to calculate the fractions of the Schiff bases distributed among the complexed and protonated forms.

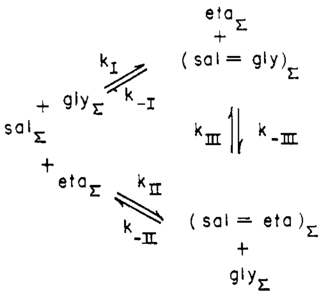


Figure 1. Reaction scheme for amine-exchange system: $\text{sal}_\Sigma = \text{Hsal} + \text{sal}^- + \text{Zn(sal)}^+ + 2\text{Zn(sal)}_2 + \text{Zn(sal)(gly)}$; $\text{gly}_\Sigma = \text{H}_2\text{gly}^+ + \text{Hgly} + \text{gly}^- + \text{Zn(gly)}^+ + 2\text{Zn(gly)}_2 + 3\text{Zn(gly)}_3{}^- + \text{Zn(sal)(gly)} + \text{Zn(sal–eta)(gly)}$; $\text{eta}_\Sigma = \text{Heta}^+ + \text{eta} + \text{Zn(eta)}^{2+} + 2\text{Zn(eta)}_2{}^{2+} + 3\text{Zn(eta)}_3{}^{2+}$; $(\text{sal–gly})_\Sigma = \text{H(sal–gly)}^- + \text{sal–gly}^{2-} + \text{Zn(sal–gly)} + \text{Zn(sal–gly)(gly)}^- + 2\text{Zn(sal–gly)}_2{}^{2-}$; $(\text{sal–eta})_\Sigma = \text{H}_2\text{(sal–eta)}^+ + \text{H(sal–eta)} + \text{sal–eta}^- + \text{Zn(sal–eta)}^+ + \text{Zn(sal–eta)(gly)} + \text{Zn(sal–eta)}_2$.

Values of the constants for the rate of amine exchange were then obtained using the equation

$$k_{III} = \frac{f_{H(\text{sal-xyz})}}{f_{H(\text{sal-xyz})} - f_{H(\text{sal-xyz}')}}(k_{obsd} - k_{-II})$$

where $k_{-II}$ is the effective rate constant for the hydrolysis of the parent Schiff base (sal–xyz) in the medium employed, xyz' is the exchanging amine, and $f_{HSB}$ represents the fraction of each Schiff base which is in the protonated form. This equation embodies the fact that at 390 m$\mu$ the reactant and product species H(sal–xyz) absorb with almost identical extinction coefficients regardless of the nature of the amines investigated here.

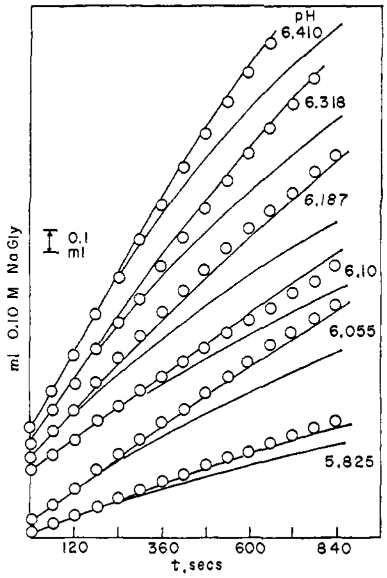


Figure 2. pH-Stat volume–time curves for the rate of formation of *N*-salicylideneglycinate in the presence of Zn(II) and ethylamine hydrochloride: ○, points taken from the experimental curves; ———, theoretical curves; the lower branch of each set is calculated for $k_{\text{III}}$ and $k_{-\text{III}} = 0$; the upper branch is calculated using values of $k_{\text{III}}$ and $k_{-\text{III}}$ obtained from the constants given in Table IV. The scale of the ordinate is the same for all curves, but the curves have been placed so as to avoid overlap. Initial volumes are (pH, obsd vol, ml, calcd vol, ml): 5.825, 0.81, 0.80; 6.055, 0.69, 0.69; 6.10, 1.41, 1.41; 6.187, 0.86, 0.87; 6.318, 1.13, 1.13; 6.410, 1.32, 1.35. The initial compositions (concentrations, *M*) of the probe solutions are (pH, $\text{Zn}_{\text{tot}}$, $\text{sal}_{\text{tot}}$, eta·$\text{HCl}_{\text{tot}}$, $\text{HCl}_{\text{tot}}$): 6.055, 0.01644, 0.0219, 0.300, 0.0005; 6.187, 0.01644, 0.0219, 0.300, 0.0005; 6.318, 0.01644, 0.0219, 0.300, 0.0005; 6.410, 0.01644, 0.0219, 0.300, 0.0005; 6.808, 0.01644, 0.0155, 0.150, 0.0005; 5.825, 0.0329, 0.216, 0.150, 0.0006; 6.100, 0.0329, 0.0216, 0.150, 0.0006. The initial volume was 25.00 ml and the titrant was 0.1014 *M* sodium glycinate.

The quotient involving the fractions on the right-hand side of the above equation corrects for the effect on the absorbance of incomplete protonation of the reactant Schiff base and incomplete Zn(II) complexation of the product Schiff base. Back reactions were assumed to be negligible during the initial phases of the reaction. Values of $k_{-\text{II}}$ were obtained using the forward rate constants given in Table II and the equilibrium constants given in Table I. The results of these calculations and values of $k_{\text{III}}$ are given in Table III.

Comparison of $k_{\text{obsd}}$ with $k_{-\text{II}}$ in Table III shows that the rate of disappearance of H(sal–eta) is considerably faster than can be accounted for by hydrolysis. Furthermore, inspection of the values of $k_{\text{III}}$ show that this pseudo-first-order rate constant for the excess rate of disappearance is approximately first order with respect to the concentration of the exchanging amine. These observations are consistent with the following reaction sequence for the excess rate.

$$\text{H(sal–eta)} + \text{xyz}^- \overset{\text{slow}}{\rightleftharpoons} \text{H(sal–xyz)}^- + \text{eta}$$

$$\text{H(sal–xyz)}^- + \text{Zn}^{2+} \overset{\text{fast}}{\rightleftharpoons} \text{Zn(sal–xyz)} + \text{H}^+$$

where $\text{xyz}^- = \text{gly}^-$ or α-$\text{ala}^-$. Further evidence for this reaction sequence is the parallel *increase* in absorbance at 350 nm, an absorption maximum of Zn(sal–xyz).[15]

In the pH-Stat experiments, no Schiff base is present at the beginning of the reaction and the reactants are present in the test solution principally as $\text{Zn}_{\text{aq}}^{2+}$, H(sal), and $\text{H(eta)}^+$. The reaction pH of this originally acidic solution is established by the rapid addition of Na–gly (or Na–α-ala) solution from the buret of the pH-Stat when the apparatus is activated. Depending on conditions, more or less titrant is initially added in order to establish preequilibria which involve partial neutralization of H(sal) and H(eta) and the formation of species such as $\text{Zn(sal)}^+$, $\text{Zn(sal)}_2$, $\text{Zn(eta)}^{2+}$, $\text{Zn(gly)}^+$, $\text{Zn(gly)}_2$, and $\text{Zn(eta)(gly)}^+$. Zero time for the slower subsequent rate of Schiff-base formation is taken when a sufficient volume of titrant has been added to establish the reaction pH. Typical volume–time curves are shown in Figure 2. The open circles represent points from the observed curves.

After the solution pH has been attained, the glycinate (or α-alaninate) and ethylamine Schiff bases form along paths I and II according to the rate laws found for the individual systems. The effect of transimination is not manifested in the volume–time curves until the concentrations of the Schiff bases have built up to appreciable values. Under the conditions of these pH-Stat runs, the concentrations of $\text{Zn}_{\text{aq}}^{2+}$ were considerably greater, up to an order of magnitude, than their concentrations in the stopped-flow experiments. The levels of free $\text{gly}^-$ or α-$\text{ala}^-$ were correspondingly lower since other concentrations were comparable. The first of these differences emphasized the Zn(II) paths, whereas the latter slowed the rates sufficiently to make them amenable for study on the pH-Stat apparatus.

In order to interpret the volume–time curves, a numerical integration procedure was used because pseudo-first-order conditions did not prevail. Therefore, it was necessary to correct for changes in the species distribution among the reactants and products as the reactions proceeded. Rates along paths I and II were taken according to the rate laws found above, and a trial rate law for transimination was assumed. The resulting rate equations were then integrated numerically. Owing to the large number of species which are taken into account in computing the distribution (see the legend of Figure 1), the second-order Runge–Kutta method was found to be considerably faster than the fourth-order method, even though a slightly smaller integration step was required for stability. Shown with each experimental curve for a given pH in Figure 2 are two theoretical solid curves; the lower branch of each set was calculated assuming that no direct transimination occurs along path III and the upper branch was calculated using the rate law described below for path III and the values of the rate constants given in Table IV.

In addition to the good agreement obtween the observed and calculated initial volumes, the initial slopes of the observed and calculated curves are seen to be the same. These initial slopes depend essentially only on the formation reactions along paths I and II, and the excellent agreement between the observed and calculated values shows that the rate laws found previously in each of the simpler ternary systems apply to the more complicated quaternary systems, and, therefore, cross acid–base catalysis can be ruled out.

Analysis of the data for the glycinate stopped-flow experiments showed a reaction which was predominantly first order in $\text{gly}^-$ and first order in H(sal–gly) indicating the pathway

Table IV. Rate Constants for Amine Exchange at 25°, $\mu$ = 0.5 (KCl)

| Reaction | $k_{\text{forward}}$, $M^{-1}$ sec$^{-1}$ or $M^{-2}$ sec$^{-1}$ | $k_{\text{backward}}$, $M^{-1}$ sec$^{-1}$ or $M^{-2}$ sec$^{-1}$ |
|---|---|---|
| H(sal–eta) + gly$^-$ $\rightleftarrows$ H(sal–gly)$^-$ + eta | $4 \times 10^2$ | $3 \times 10^3$ |
| Zn(sal–eta)$^+$ + gly$^-$ + H$^+$ $\rightleftarrows$ Zn(sal–gly) + eta + H$^+$ | $1 \times 10^{10}$ | $1 \times 10^8$ |
| H(sal–eta) + $\alpha$-ala$^-$ $\rightleftarrows$ H(sal–$\alpha$-ala)$^-$ + eta | 50–100 | $3\text{–}7 \times 10^2$ |
| Zn(sal-eta) + $\alpha$-ala$^-$ + H$^+$ $\rightleftarrows$ Zn(sal–$\alpha$-ala) + H$^+$ + eta | $2\text{–}4 \times 10^9$ | $3\text{–}6 \times 10^7$ |
| H(sal–eta) + $\beta$-ala$^-$ $\rightleftarrows$ H(sal–$\beta$-ala)$^-$ + eta | 200 | 500 |
| H$^+$ + Zn(sal–eta)$^+$ + $\beta$-ala$^-$ $\rightleftarrows$ Zn(sal–$\beta$-ala) + eta + H$^+$ | ? (higher H$^+$ terms) | |
| H(sal–$\beta$-ala)$^-$ + gly$^-$ $\rightleftarrows$ H(sal–gly)$^-$ + $\beta$-ala$^-$ | 100 | 350 |
| Zn(sal–$\beta$-ala) + gly$^-$ + H$^+$ $\rightleftarrows$ Zn(sal–gly) + $\beta$-ala$^-$ + H$^+$ | $6\text{-}8 \times 10^8$ | $3\text{–}4 \times 10^7$ |
| H(sal–$\beta$-ala)$^-$ + $\alpha$-ala$^-$ $\rightleftarrows$ H(sal–$\alpha$-ala)$^-$ + $\beta$-ala$^-$ | 40–60 | $1\text{–}2 \times 10^2$ |
| Zn(sal–$\beta$-ala) + $\alpha$-ala$^-$ + H$^+$ $\rightleftarrows$ Zn(sal–$\alpha$-ala) + $\beta$-ala$^-$ + H$^+$ | $4\text{–}7 \times 10^8$ | $3\text{–}5 \times 10^7$ |

$$\text{H(sal–eta)} + \text{gly}^- \xrightarrow{k_{\text{HSE,G}}} \text{H(sal–gly)} + \text{eta}$$

The quotient $k_{\text{III}}/f_{\text{H(sal–eta)}}(\text{gly}^-)$ gives roughly constant values lying in the range $4\text{-}5 \times 10^2$ $M^{-1}$ sec$^{-1}$. However, this reaction path was found to be inadequate to explain the pH-Stat results where the levels of $\text{Zn}_{\text{aq}}{}^{2+}$ were higher. Inclusion of a parallel Zn(II)-dependent, proton-catalyzed path

$$\text{Zn(sal–eta)}^+ + \text{H}^+ + \text{gly}^- \xrightarrow{k_{\text{ZnSE,G,H}}} \text{Zn(sal–gly)} + \text{H}^+ + \text{eta}$$

was found to give satisfactory agreement with the observations. After correcting the stopped-flow rates for the contribution of this path, final values of $k_{\text{HSE,G}}$ equal to about $4 \times 10^2$ and $k_{\text{ZnSE,G,H}}$ to equal about $1 \times 10^{10}$ $M^{-2}$ sec$^{-1}$ were obtained. The upper solid branch in each set of curves in Figure 2 was calculated using these values.

The stopped-flow experiments with $\alpha$-ala$^-$ indicated the presence of both metal-dependent and metal-independent paths. Assuming the parallel reactions

$$\text{H(sal–eta)} + \alpha\text{-ala}^- \xrightarrow{k_{\text{HSE,A}}} \text{products}$$

and

$$\text{Zn(sal–eta)} + \alpha\text{-ala}^- + \text{H}^+ \xrightarrow{k_{\text{ZnSE,A,H}}} \text{products}$$

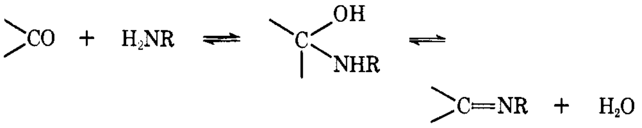
a plot of $k_{\text{III}}/[(\alpha\text{-ala}^-)f_{\text{H(sal–eta)}}]$ *vs.* $f_{\text{Zn(sal–eta)}}(\text{H}^+)/f_{\text{H(sal–eta)}}$ causes the points to fall roughly along a straight line, giving a value of $k_{\text{HSE,A}}$ lying in the range 0–100 $M^{-1}$ sec$^{-1}$ and $k_{\text{ZnSE,A,H}}$ equal to $3\text{–}4 \times 10^9$ $M^{-2}$ sec$^{-1}$. These results were found to be consistent with those obtained from the pH-Stat determinations.

The reactions of $\beta$-ala with *N*-salicylideneethylamine were found to be complicated by the presence of higher order H$^+$ terms, but extrapolation of the data to zero Zn(II) gave an apparent value of $k_{\text{HSE,B}}$ of the order of $2\text{–}4 \times 10^2$ $M^{-1}$ sec$^{-1}$. On the other hand, reaction of gly$^-$ and $\alpha$-ala$^-$ with H(sal–$\beta$-ala) seemed to be free of higher order proton terms and an analysis of the data was straightforward. The rate constants for all of these experiments are summarized in Table IV. The reactions involving exchange of one amino acid for another in a Schiff base are slightly slower than when ethylamine is a reaction partner. Nevertheless, it should be noted that the reactions of the amines with Schiff bases to yield exchange products are faster than the analogous reactions of the amines with salicylaldehyde to give Schiff bases.

The results which were obtained with the stopped-flow and pH-Stat data are generally consistent with the proposed model. Nevertheless, deviations which seem to lie outside the range of expected errors were encountered. Owing both to the complicated nature of the quaternary systems and to the experimental uncertainties, a more detailed kinetic picture is not warranted with the present data. However, modest success has been attained in demonstrating transimination and uncovering the principle reaction paths. Further studies to provide a more accurate picture and possibly to determine the reactivities of minor species, *e.g.*, $\text{H}_2$(sal–eta)$^+$, are in progress in these laboratories.

## Discussion

Schiff-base formation and hydrolysis is generally conceded[21] to take place in two distinct steps, addition of amine to carbonyl yielding an intermediate carbinolamine followed by dehydration.



The very simple relationship which exists between the rates of formation of *N*-salicylideneethylamine and its hydrolysis in aqueous media at pH 5 and above shows that in this pH range one step is rate determining and the other is fast, or the steady-state approximation applies. However, neither the hydrolysis nor the formation rate data determine which is the more rate-lmiting step. Rate laws which give equally good fits to the data are obtained by assuming either step is slow and the other is fast. Hydrolysis data are not available for the salicylaldehyde Schiff bases of the amino acids investigated here, but in view of the very close similarities of the rates and equilibria between these systems and those of ethylamine, it seems reasonable to assume that similar considerations apply to the amino acid Schiff bases.

A resolution of the ambiguity in the reaction mechanism may possibly be obtained from the observed effects of metal ions on the reaction rates. The intermediate carbinolamine which contains one phenolate and one amine group (and an additional carboxylate group with the amino acids) must interact strongly and rapidly with divalent metal ions. Furthermore, in our experiments metal-ion-independent paths for the formation of the Schiff-base complexes contributed appreciably to the overall rate of formation of the Schiff bases, even in those cases where the metal ions showed appreciable kinetic activity. Therefore, metal-ion effects on the reaction rates will furnish clues for the mechanisms

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[21] W. P. Jencks, "Catalysis and Chemistry in Enzymology," McGraw-Hill, New York, N. Y., 1969.

of the metal-independent paths as well as for the metal-dependent paths.

The observation in this and the earlier work[4] that intermediate metal ion–carbinolamine complexes do not accumulate can be cited as evidence in favor of slow addition. Calculations pertaining to the experiments of Figure 2 show that the presence of carbinolamine–Zn(II) complexes having formation constants as low as $10^6$ for eta or $10^7$ for glycinate would give rise to measurable differences between the observed and calculated volume–time curves. Since trapping of the carbinolamine by metal ions would be fast, the very good agreement between the observed and calculated initial time volumes rules out the fast formation of carbinolamine by *any* path, unless its stability is unexpectedly low.

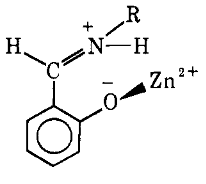
Secondly, trapping of intermediate carbinolamine by metal ions depends primarily on its proton–metal ion exchange constant. It can be shown that these constants need only be of the order of $10^{-5}$–$10^{-6}$ in order for appreciable complexing of carbinolamine to occur under the conditions described in Figure 2. Even if the stability of the Zn(II)–carbinolamine complex is not sufficiently high to give rise to an appreciable accumulation of product, metal-ion complexing of the intermediate is likely. Thus, if carbinolamine dehydration is involved in a rate-determining step, the presence of *any* complexing metal should produce a measurable effect on the kinetics. Of particular significance in this respect is the absence of kinetic effects by such good trapping agents as Co(II), Ni(II), and Cu(II).[22] Thus, assuming that carbinolamine dehydration is rate limiting for either metal-ion-independent or -dependent paths implies unusual metal ion–carbinolamine behavior. On the other hand, a mechanism which assumes that the formation rates are limited by the rate of addition followed by fast dehydration requires no unusual behavior on behalf of the intermediate and easily accomodates the observations.[23]

Rate-determining addition has also been proposed for the rates of Schiff-base formation of 3-hydroxypyridine-4-aldehyde.[24] On the other hand, rate-determining hydration–dehydration is postulated for reactions involving Schiff bases, oximes, and hydrazones of benzaldehyde.[10] The pH–rate profiles for the benzylidene hydrolysis reactions show the opposite slope in the vicinity of pH 6 from those obtained for the salicyclidenes, indicating that fundamental differences exist between the two types of systems and that their respective rate-determining steps need not be the same.

It is instructive to compare quantitatively the fast amine-exchange rates with those obtained for amine attack on salicylaldehyde. Reducing the third-order constants for $k_b$ in Table III to second-order constants by division with $\beta_{\mathrm{Hsal}}$ gives a value of 4 $M^{-1}$ sec$^{-1}$ for the rate constant of the reaction H(sal) + gly$^-$ → Schiff base and 7 $M^{-1}$ sec$^{-1}$ for H(sal) + eta → Schiff base. The corresponding amine-exchange reactions H(sal–eta) + gly$^-$ ⇄ H(sal–gly) + eta are 400 $M^{-1}$ sec$^{-1}$ and $3 \times 10^3$ $M^{-1}$ sec$^{-1}$ for the forward and reverse reactions, respectively. Thus the reactions of the amines with the iminium ions are $10^2$–$10^3$ times faster than the reactions with the corresponding carbonyl compounds.

Molecular orbital calculations[25] show that the carbon atom of the $>C{=}NH^+$ group is more susceptible to nucleophilic attack than that of the $>C{=}OH^+$ group. This, together with the considerably higher zwitterion content of the protonated salicylidenes compared to H(sal), must account to a large extent for the greater reactivities of the Schiff bases.

The Zn(II)-mediated paths also appear to be faster in transimination than in Schiff-base formation. By withdrawing electrons, coordinating metal ions would certainly serve to further increase the electrophilic character of the imine carbon atom. Models show that protonation of the imine nitrogen atom should cause the zinc(II) ion to be forced out of the plane of the aromatic ring.



In such a position the Zn(II) would be suitably located to interact with the nitrogen $\pi$ electrons and would thereby tend to induce a still greater positive charge on the 7-carbon atom. Furthermore, with coordination sites still available to accept the entering amine, the metal ion can also serve to aid the reaction through the promnastic effect, *i.e.*, rapidly forming in a preequilibrium step a mixed complex which contains both reactants. By reducing the overall reaction from second order to first order a more favorable entropy of activation would be obtained.

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(22) It was proposed in ref 8 that the absence of any observed kinetic activity by Cu(II) may be due to the repression of the sal$^-$ and gly$^-$ levels by the relatively high acidities which obtained in the Cu(II) experiments. This argument fails to take into account that even under these conditions Cu(II) forms ample stable binary and mixed-ligand complexes with sal$^-$ and gly$^-$. These complexes show no kinetic activity. Furthermore, the proton-catalyzed path, $k_e$, is less subject to repression by the lower pH. No $Cu^{2+}$ activity along this path is observed. The value of $k_e$ for Cu(II)–sal$^-$–gly$^-$ must be less than $10^{10}$. Co(II) and Ni(II) show no kinetic activity even at the same pH where Zn(II) is active.

(23) The change in the rate-determining step of the hydrolysis which occurs at low pH[8] is consistent with rate-limiting attack of $OH^-$ on $(sal{-}eta)H_2^+$ with a rate constant of about $1.5 \times 10^9$ $M^{-1}$ sec$^{-1}$. Since hydration of the C=N bond is considered to involve the addition of an $OH^-$, it is not surprising that a diffusion-controlled rate of reaction with $OH^-$ may be encountered at low pH.

(24) T. C. French, D. S. Auld, and T. C. Bruice, *Biochemistry*, **4**, 77 (1965).

(25) J. A. Anderson and P. S. Song, *Arch. Biochem. Biophys.*, **122** 224 (1967).