Conclusions

The HMO theory applied to the effect of methyl substitution on polarographic oxidation potential accounts reasonably well for the observed decrease in oxidation potential with increased methyl substitution. However, it fails in explaining the finer details, namely, the apparent lack of inductive effect when methyl groups are substituted *ortho* or *meta* in xylene and the constant 0.15-v. change in oxidation potential per group substituted *para*.

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A Nuclear Magnetic Resonance Study of the Protolysis Kinetics of Glycine[1]

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The exchange of protons between the amino group of glycine and aqueous solvent has been investigated by means of its effect upon the proton high resolution n.m.r. spectrum of the methylene group. The mean lifetime, τ, between exchanges of protons in the NH_3^+ group was measured at room temperature as a function of glycine concentration and of hydrogen ion concentration. Similar studies were made of the glycine methyl ester. Several mechanisms contributing to the exchange proved to be separable by means of the changes in τ with pH and glycine or ester concentration, and rate constants were obtained for the reactions. A brief comparison of these results with those obtained for other amino acids and for simple amines indicates that the proton-exchange mechanism for positively charged amino groups in strongly acidic solutions are the same for the several types of such systems studied so far.

Introduction

This paper deals primarily with the exchange of protons between the amino group of glycine and the aqueous solvent, as measured by nuclear magnetic resonance techniques. For glycine, it proved possible to measure the exchange rates over an extended range of concentrations. Thereby, we have obtained a better picture of the exchange reactions than was possible in the case of the closely related amino acid sarcosine.[3] The general approach and results of the present work, including the interpretation, follow those for sarcosine and provide confirmation of the previously proposed exchange mechanism. The study of glycine covers the exchange of amino protons of the hydrochloride at pH < 1.8 and of the zwitterion at pH > 2.7, as well as the exchange at intermediate pH where both of these ionic species are present at an appreciable concentration in the solution. A similar study was made in strongly acidic solutions, with pH < 2.2, for the methyl ester of glycine hydrochloride, and comparison of the two sets of results is useful in their analysis.

Experimental Details and Data Reduction

The high-resolution proton magnetic resonance spectra used in the rate studies were obtained with a Varian Associates HR-60 spectrometer operating at 60 Mc./sec. The temperature of the probe and sample was 23 ± 1°. The pH measurements were made at the same temperature with either a Beckman or a Radiometer pH meter, both with an expanded scale. The glycine and the glycine ester used in preparing the aqueous solutions were obtained commercially from Nutritional Biochemical Corporation. HCl was added to the solutions as needed to adjust the pH to the desired values.

In principle, the rate of proton exchange between the solvent H_2O and the NH_3^+ group in, *e.g.*, $NH_3^+CH_2COO^-$ could be determined most directly from the effects of the exchange upon the proton absorption of the NH_3^+ group itself. However, the amino proton absorption could not be detected with the usual operating conditions of the spectrometer, most probably because of a large line width due to the large N^{14} and H^1 splitting and its partial averaging by the proton-exchange reactions and by the short N^{14} T_1, the short T_1 being a result of the N^{14} nuclear quadrupole interactions.[4] Nevertheless, the proton exchange produces major resolvable changes in the CH_2 group proton resonance, as shown in Fig. 1.

In the most acidic solutions, the amino group exists as other than NH_3^+ to a negligible extent, the proton exchange is slow, and the spectrum of the methylene group consists of a quartet because of the splittings produced by the three protons of the NH_3^+ group (Fig. 1A). In *less acidic* solutions, the exchange rate increases and the methylene quartet broadens and collapses into a single line which narrows further with increasing exchange rate (Fig. 1B). It should be noted that at these low pH values the exchange is *inhibited* by the acid.

The mean lifetime, τ, between events which exchange a proton from the NH_3^+ group was calculated from the observed line shape of the methylene group, using theoretical curves based upon the "classical" treatment of chemical and spin-exchange effects in high-resolution n.m.r. spectra.[5-7] The "classical" treatment was devised and applied initially[5] to cases such that $\tau^2\delta^2 >> 1$, where δ is the frequency difference in radians/sec. between two sets of electron-coupled nuclei, one of which is undergoing exchange. However, in our glycine studies δ is a relatively small frequency difference, namely the chemical shift of ~200 c.p.s. (at 60 Mc./sec.) between the NH_3^+ and CH_2 group protons.[8] The coupling constant J between these two groups of protons is ~6 c.p.s. The coalescence of the CH_2 quartet occurs as $J\tau$ becomes smaller than unity. So there is a range of τ's which can be obtained from the exchange averaging of the quartet and which satisfy the condition $\tau^2\delta^2 >> 1$. However, for shorter τ's which do not meet this condition a quantum correction should be made.[9] This correction, as calculated by Alexander,[10] is $[1 + (1 + 9\tau^2\delta^2)^{-1}]$ for a quartet. Therefore, the short τ's obtained "classically" were corrected by setting their numerical values equal to $\tau[1 + (1 + 9\tau^2\delta^2)^{-1}]$ and solving for τ, which is now the true value.

The proton-exchange processes also affect τ_{H_2O}, the mean lifetime of protons in water molecules. Values for τ_{H_2O} were obtained from the observed exchange-broadening of the water line, by means of the relation shown in eq. 1.

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(2) On leave of absence from the Weizmann Institute of Science, Rehovoth, Israel. Some preliminary experiments on this problem were performed at the Institute in association with Dr. S. Meiboom.

(3) M. Sheinblatt, *J. Chem. Phys.*, **36**, 3103 (1962); **39**, 2005 (1963).

(4) W. B. Moniz and H. S. Gutowsky, *ibid.*, **38**, 1153 (1963).

(5) H. S. Gutowsky, D. W. McCall, and C. P. Slichter, *ibid.*, **21**, 279 (1953).

(6) N. S. Gutowsky and A. Saika, *ibid.*, **21**, 1688 (1953).

(7) E. Grunwald, A. Loewenstein, and S. Meiboom, *ibid.*, **27**, 630 (1957).

(8) This value was measured by decoupling the N^{14} from the protons in a double resonance experiment.

(9) I. Solomon and N. Bloembergen, *J. Chem. Phys.*, **25**, 261 (1956); J. Kaplan, *ibid.*, **28**, 278 (1958).

(10) S. Alexander, *ibid.*, **37**, 924, 962 (1963); **38**, 1787 (1963); **40**, 2741 (1964), erratum.

$$\frac{1}{\tau_{H_2O}} = \frac{1}{T_2} - \frac{1}{T_2'} \quad (1)$$

where $1/T_2$ is the half-line width, in radians sec.$^{-1}$, at half-maximum intensity observed in the solution, and $1/T_2'$ is that in the absence of exchange. The T_2 values were obtained from the widths of the water line in the solutions and T_2' from an external water sample. Equation 1 applies only to the initial broadening case, that is, when the exchange does not cause any appreciable collapse of well resolved lines corresponding to the different groups between which the exchange occurs, in this case the proton resonance of NH_3^+ and water. Therefore, the results for τ_{H_2O} were limited to circumstances in which the water line was broadened and in which it was confirmed by N^{14} decoupling experiments that the NH_3^+ and water lines were indeed well separated.

The exchange processes occur under conditions of thermodynamic equilibrium, so each of the exchange reactions is pseudo first order in the concentration of the species for which τ is determined by the nuclear magnetic labeling, in this case by the spin states of the protons in the NH_3^+ group. Thus, for a given process i which exchanges protons from group A with any other group, τ_{iA} is related to the corresponding reaction rate by

$$\frac{1}{\tau_{iA}} = -\frac{1}{[A]}(d[A]/dt)_i \quad (2)$$

In turn, the rate depends upon the concentrations of the species involved and the specific rate constant k_i for the given process, as, *eg.*, a bimolecular exchange of a single proton between A and B

$$(d[A]/dt)_i = -k_i[A][B] \quad (3)$$

Equation 2 may be derived from eq. 3 by noting that [B] is constant but that [A] represents the concentration of the "label," which is the thermodynamic concentration of A at $t = 0$ and which decays exponentially thereafter. A comparison of eq. 2 and 3 gives $1/\tau_{iA} = k_i[B]$. Extension to cases such as glycine where several processes occur is direct. The total exchange rate is the sum over all exchange processes, $\sum_i (d[A]/dt)_i$, and therefore an observed lifetime, τ, is determined by the summation

$$1/\tau = \sum_i 1/\tau_i \quad (4)$$

In order to unravel the kinetics of the exchange, it is necessary to determine the various contributions to τ in eq. 4, and their concentration dependences, as in eq. 3. For this, it is necessary to know the concentrations of the various species which may participate in the exchange. These can be calculated from the stoichiometric concentration of glycine, and the pH, by means of the known ionization constants, K_i. The latter are given by the equations

$$K_1 = \frac{[NH_3^+CH_2COO^-][H^+]}{[NH_3^+CH_2COOH]} = \frac{[R^\pm][H^+]}{[R^+]} \quad (5)$$

$$K_2 = \frac{[NH_2CH_2COO^-][H^+]}{[NH_3^+CH_2COO^-]} = \frac{[R^-][H^+]}{[R^\pm]} \quad (6)$$

$$K_3 = \frac{[NH_2CH_2COOH][H^+]}{[NH_3^+CH_2COOH]} = \frac{[R][H^+]}{[R^+]} \quad (7)$$

The pK values of the ionization constants are[11,12]

$$pK_1 = 2.34, \quad pK_2 = 9.60, \quad pK_3 = 7.73 \quad (8)$$

Another useful constant is defined as

$$K_4 = \frac{[NH_3^+CH_2COO^-]}{[NH_2CH_2COOH]} = \frac{K_1}{K_3} = 2.46 \times 10^5 \quad (9)$$

For the methyl ester of glycine, none of these equilibria occur except that in eq. 7.

(11) E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids, and Peptides," Reinhold Publishing Corp., New York, N. Y., 1943, p. 99.

(12) The value of pK_3 is obtained by assuming that it is the same as that for the corresponding equilibrium of the ester; see ref. 11, p. 28.

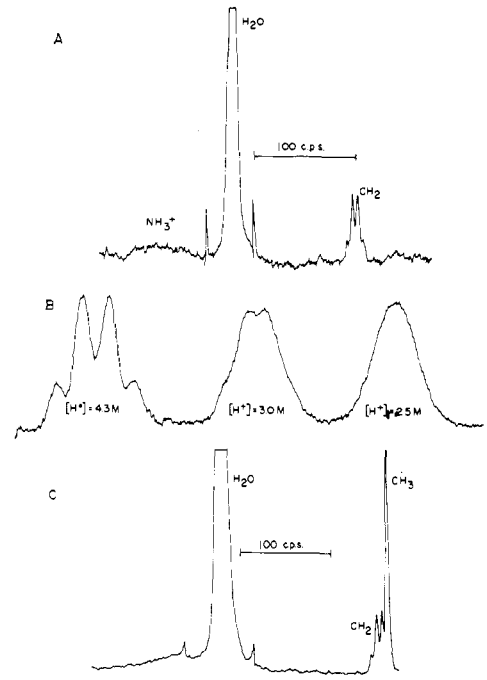


Fig. 1.—Typical proton high resolution spectra of glycine and glycine methyl ester in aqueous solution. These particular spectra were recorded at a fixed frequency of 56.4 Mc./sec. with the magnetic field increasing from left to right. (A) The spectrum of glycine at $[H^+] = 4.5\ M$. The two small peaks near the water line are spinning side bands. (B) The dependence upon $[H^+]$ of the methylene spectrum in glycine. Note that the exchange rate increases with decreasing acidity. (C) The spectrum of glycine methyl ester at $[H^+] = 3.85\ M$.

Results and Their Interpretation

Possible Mechanisms for NH_3^+ Proton Exchange at Low pH.—Inspection of eq. 5–8 reveals that in strongly acid solutions, glycine and its methyl ester exist primarily as the cation, $NH_3^+CH_2COOH(CH_3)$. For glycine, the next most important species is the zwitterion which is at most a few per cent of the stoichiometric concentration over the pH range in question here. The existence of very rapid transfer of the carboxylic acid proton from $NH_3^+CH_2COOH$ to the zwitterion is shown by the appearance of only one CH_2 group resonance even in less acid solutions where the zwitterion concentration is large. However, there are two strong arguments against the zwitterion playing any important role in the NH_3^+ exchange for glycine at low pH. The strongest is that the exchange rates are nearly the same for glycine as for its ester, and the latter forms no zwitterion. Also, the analysis presented in a subsequent section of NH_3^+ exchange in the zwitterion indicates that it occurs by mechanisms which would not be important in the strongly acid solutions. Therefore, we will limit our initial considerations to proton-exchange processes involving the $NH_3^+CH_2COOH(CH_3)$ cation.

For glycine, these processes are

$$NH_3^+CH_2COOH + H_2O \underset{}{\overset{k_I'}{\rightleftharpoons}} NH_2CH_2COOH + H_3^+O \quad \text{I}$$

$$NH_3^+CH_2COOH + OH^- \underset{}{\overset{k_{II}}{\rightleftharpoons}} NH_2CH_2COOH + H_2O \quad \text{II}$$

$$NH_3^+CH_2COOH + NH_3^+CH_2COO^- \underset{}{\overset{k_{III}}{\rightleftharpoons}} NH_2CH_2COOH + NH_3^+CH_2COOH \quad \text{III}$$

$$NH_3^+CH_2COOH + NH_2CH_2COOH \underset{}{\overset{k_{IV}}{\rightleftharpoons}} NH_2CH_2COOH + NH_3^+CH_2COOH \quad \text{IV}$$

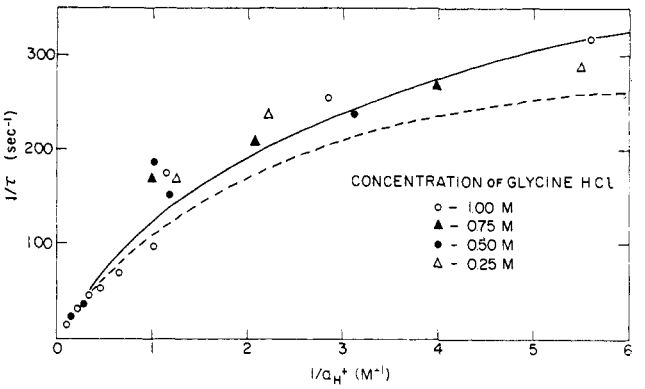


Fig. 2.—The mean lifetime τ between exchanges of the protons in an NH_3^+ group in very highly acidic aqueous solutions of glycine at 23°, plotted as $1/\tau$ *vs.* $1/a_{H^+}$ for various concentrations of glycine. The dashed line is that obtained by fitting eq. 11 for reactions Ia and Ib to the data for the most acidic solutions; the solid line is eq. 17 which includes exchange reactions III and IV as well.

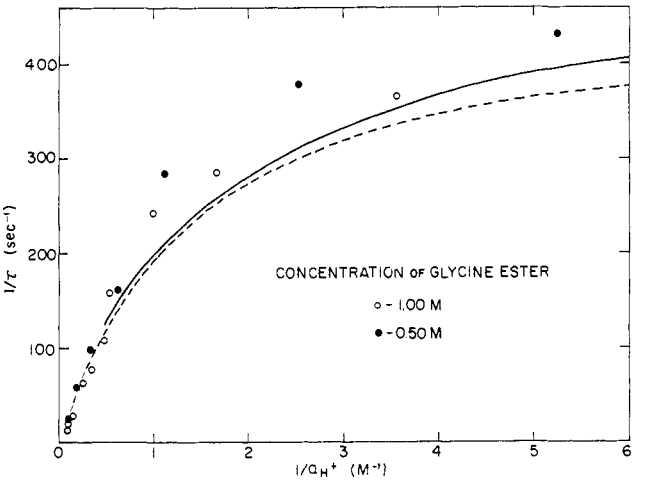


Fig. 3.—The mean lifetime τ between exchanges of the protons in an NH_3^+ group in very highly acidic solutions of glycine methyl ester at 23°, plotted as $1/\tau$ *vs.* $1/a_{H^+}$ for various concentrations of the ester. The dashed line is that obtained by fitting eq. 11 for reactions Ia and Ib to the data for the most acidic solutions; the solid line is eq. 18 which includes exchange reaction IV as well.

For the glycine methyl ester, the reaction equivalent to III is blocked by the methyl group. Furthermore, in the acidic solutions studied, the OH^- concentration is very small so we will neglect reaction II. Therefore, τ for the glycine solutions can be expressed by means of eq. 2-9 as

$$\frac{1}{\tau} = k_I'[H_2O] + k_{III}\frac{K_1[R^+]}{[H^+]} + k_{IV}\frac{K_3[R^+]}{[H^+]} \quad (10a)$$

and for the ester

$$\frac{1}{\tau} = k_I'[H_2O] + k_{IV}\frac{K_3[R^+]}{[H^+]} \quad (10b)$$

General Aspects of NH_3^+ Proton Exchange at Very Low pH.—Experimentally, it was found that the amino protons exchange with the aqueous solvent in two different ways depending upon the hydrogen ion concentration, as shown by the data in Fig. 2–5. In the very acidic range, pH <0.6, the lifetime τ between exchanges of any protons of the NH_3^+ group is virtually independent of the stoichiometric concentration of either glycine or of its methyl ester. However, τ does depend upon the pH. This dependence, given as plots of $1/\tau$ *vs.* $1/a_{H^+}$ in Fig. 2 and 3, is nonlinear, with $1/\tau$ increasing very rapidly with $1/a_{H^+}$ at large a_{H^+} and less rapidly at smaller a_{H^+}. On the other hand in less acidic solutions, with pH >1.0, $1/\tau$ for both substances increases linearly with $1/a_{H^+}$ and also with the concentration of either glycine or its methyl ester according to the data in Fig. 4 and 5, respectively.

The fact that the $1/\tau$ data given in Fig. 2 and 3 for the very acidic range, $0 < 1/a_{H^+} < 5$, are independent of the stoichiometric concentration of either glycine or of its ester implies that the exchange is governed by reaction(s) involving only one of the solute species and that to first order. Of the three mechanisms still under consideration, reactions III and IV do not meet this criterion; reaction I does, so it is the dominant exchange mechanism in the very acid range. However, the sharply nonlinear dependence of $1/\tau$ upon $1/a_{H^+}$ is not predicted by reaction I, as given above. To be sure, the already rejected reactions III and IV would give contributions to $1/\tau$ which are proportional to $1/a_{H^+}$. But for the pH range in question, eq. 9 and 10 predict a *linear* dependence of $1/\tau$ upon $1/a_{H^+}$ because R^+ is virtually the stoichiometric concentration of glycine and does not change appreciably with pH. So reactions III and IV are eliminated again, this time by the observed *nonlinear* dependence of $1/\tau$ upon $1/a_{H^+}$.

Proton Exchange Mechanism for pH <0.6.—For the reasons just outlined, it appears necessary, as in the case of sarcosine, to adopt for reaction I a two-step kinetic scheme of the type used by Grunwald and co-workers[13] for proton exchange between an amino group and solvent water. The first step, Ia, recognizes the hydrogen bonding of an H_2O molecule to the amino

$$(CH_3)HOOCCH_2\overset{H}{\underset{H}{N}}{}^+H^*\cdots OH_2 \underset{k_{-I}}{\overset{k_I}{\rightleftharpoons}} (CH_3)HOOCCH_2\overset{H}{\underset{H}{N}}\cdots H^*OH + H^+ \quad (Ia)$$

$$(CH_3)HOOCCH_2\overset{H}{\underset{H}{N}}\cdots H^*OH \xrightarrow{k_H} (CH_3)HOOCCH_2\overset{H}{\underset{H}{N}} + H^*OH \quad (Ib)$$

group for both the cation R^+ and the parent species R. Furthermore, Ia provides the possibility that ionization of the protonated, hydrated species $R^+\cdots OH_2$ is not an exchange process because the proton comes from the original H_2O molecule rather than from the NH_3^+ group. Moreover, the reverse reaction, which is very fast, will restore the original conditions. Only the second step, Ib, the breaking of the hydrogen bond between H_2O and the amino group, results in a net exchange of the NH_3^+ protons. Application of the steady-state approximation to the concentration of $R\cdots H_2O$ leads to the expression

$$\frac{1}{\tau} = \frac{k_I k_H}{k_H + k_{-I}[H^+]} \quad (11)$$

Implicit in this model are the assumptions: (a) the hydrogen bonding of R^+ molecules to H_2O molecules is

(13) M. T. Emerson, E. Grunwald, M. L. Kaplan, and R. A. Kromhout, *J. Am. Chem. Soc.*, **82**, 6307 (1960).

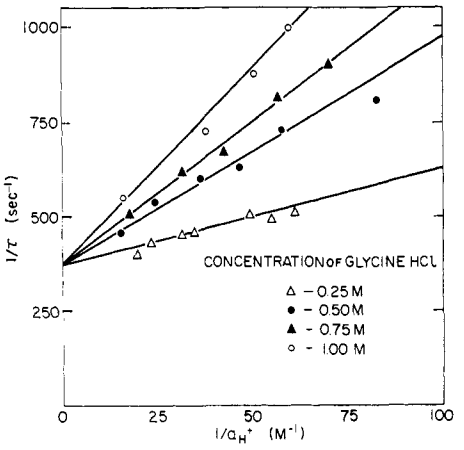


Fig. 4.—The mean lifetime τ between exchanges of protons in an NH_3^+ group in very acidic aqueous solutions of glycine at 23°, plotted as $1/\tau$ *vs.* $1/a_{H^+}$ for various concentrations of glycine. The data are fitted by eq. 14.

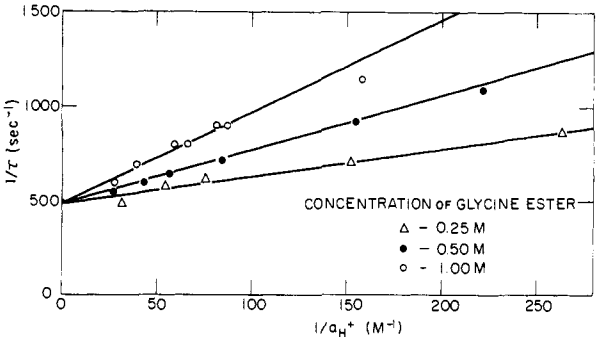


Fig. 5.—The mean lifetime τ between exchanges of protons in an NH_3^+ group in very acidic aqueous solutions of glycine methyl ester at 23°, plotted as $1/\tau$ *vs.* $1/a_{H^+}$ for various concentrations of ester. The data are fitted by eq. 15.

virtually complete; (b) the reaction $R^+ + H_2O \rightarrow R^+\cdots OH_2$ is too fast to exert any apparent influence upon the over-all rate of proton exchange; (c) the proton exchange among H_2O molecules is too fast for the back reaction of Ib to affect the NH_3^+ proton-exchange rate.

Equation 11 represents two limiting situations depending upon the relative magnitudes of k_H and $k_{-I}\cdot[H^+]$ and accounts for the nonlinear dependence, in Fig. 2 and 3, of $1/\tau$ upon $1/a_{H^+}$. In very acid solutions, $k_H < k_{-I}[H^+]$ and eq. 11 reduces to

$$\frac{1}{\tau} = \frac{k_I k_H}{k_{-I}[H^+]} = \frac{K_3 k_H}{[H^+]} \qquad (12)$$

The substitution in eq. 12 of K_3 for k_I/k_{-I} is valid because reaction Ia is the actual equilibrium represented by K_3 in eq. 7. According to eq. 12, $1/\tau$ is directly proportional to $1/a_{H^+}$; hence the limiting slopes of the curves in the very acidic range of Fig. 2 and 3 are K_3k_H and can be used with the known value of K_3 to obtain k_H. This procedure yields 0.85×10^{10} sec.$^{-1}$ and 1.52×10^{10} sec.$^{-1}$ for k_H in the glycine and glycine ester solutions, respectively.

NH_3^+ Proton Exchange for pH >0.6.—For less acidic solutions, with $k_H > k_{-I}[H^+]$, eq. 11 reduces to the constant value

$$1/\tau = k_I \qquad (13)$$

Such a limiting situation is not apparent from the data plotted in Fig. 2 and 3, although $1/\tau$ changes much less rapidly with $1/a_{H^+}$ for small a_{H^+} than for large. Instead, the behavior becomes that shown in Fig. 4 and 5 for $1/a_{H^+} > 20$, in which $1/\tau$ increases linearly with $1/a_{H^+}$ for a given stoichiometric concentration of amine, and also with the amine concentration at constant pH. Extrapolation to $1/a_{H^+} = 0$ of the linear $1/\tau$ *vs.* $1/a_{H^+}$ plots for each of several amine concentrations does lead to a common intercept, 370 sec.$^{-1}$ for glycine and 470 sec.$^{-1}$ for the glycine ester.

The simplest interpretation of these results is that for the pH range covered in Fig. 4 and 5 the contribution of reactions Ia and Ib to $1/\tau$ is given by eq. 13, while the pH and amine concentration dependences result from the terms for reactions III and IV in eq. 10a and 10b. Accordingly, the straight lines in Fig. 4 and 5 correspond to

$$1/\tau = k_I + (k_{III}K_1 + k_{IV}K_3)([R^+]/a_{H^+}) \qquad (14)$$

and

$$1/\tau = k_I + k_{IV}K_3([R^+]/a_{H^+}) \qquad (15)$$

respectively, for glycine and its methyl ester. Thus, the intercepts give k_I directly, namely 370 sec.$^{-1}$ for glycine and 470 sec.$^{-1}$ for the ester. And by using these values for k_I with the numerical value of 1.86×10^{-8} for $K_3 = k_I/k_{-I}$, we obtain k_{-I} values of 2.0×10^{10} and 2.5×10^{10} (M sec.)$^{-1}$ for glycine and ester.

It is not possible to obtain separate numerical values for k_H and k_{-I} from our kinetics data without introducing the independently known value for the equilibrium constant K_3. This may be seen from the rearranged form of eq. 11

$$1/\tau = \frac{k_I}{1 + (k_{-I}/k_H)[H^+]} \qquad (16)$$

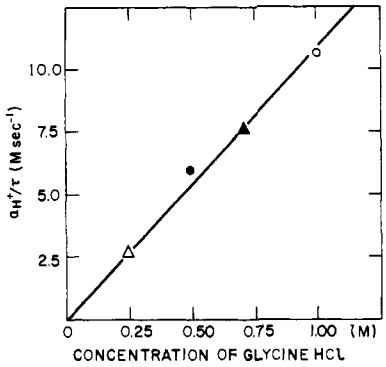
This equation involves only two independent parameters, namely k_I and the *ratio* k_{-I}/k_H. Nevertheless, eq. 16 (or eq. 11) can be used to calculate $1/\tau$ as a function of $1/a_{H^+}$ to show how well the model and the numerical values given above for the rate constants do fit experiment. The results of such calculations are shown by the dashed curves in Fig. 2 and 3. In these figures, the contributions to $1/\tau$ by reactions III and IV become appreciable for the larger values of $1/a_{H^+}$. Thus, to cover the pH range of the figures, the appropriate terms must be added to eq. 11, giving the following "complete" equations for glycine and the ester, respectively.

$$\frac{1}{\tau} = \frac{k_I k_H}{k_H + k_{-I}[H^+]} + (k_{III}K_1 + k_{IV}K_3)\frac{[R^+]}{a_{H^+}} \qquad (17)$$

$$\frac{1}{\tau} = \frac{k_I k_H}{k_H + k_{-I}[H^+]} + k_{IV}K_3\frac{[R^+]}{a_{H^+}} \qquad (18)$$

Numerical values were determined for k_{III} and k_{IV} as discussed below. By means of them and the other constants, eq. 17 and 18 were used to calculate the solid lines in Fig. 2 and 3, respectively. The agreement with the data appears to be within experimental error.

The straight lines of $1/\tau$ *vs.* $1/a_{H^+}$ in Fig. 4 and 5 have slopes which depend upon k_{III} and k_{IV} as indicated by eq. 14 and 15. For the simple case of the ester, the



. Fig. 6.—The slopes, in units of a_{H^+}/τ, of the straight lines in Fig. 4 plotted as a function of cation (glycine) concentration, $[R^+]$. These data are consistent with eq. 17 which predicts that $(a_{H^+}/\tau) = (k_{III}K_1 + k_{IV}K_3)[R^+]$.

slopes give $k_{IV}K_3[R^+]$ for each concentration of the ester. From $[R^+]$ and the slopes, k_{IV} was calculated to be $k_{IV} = 2.8 \times 10^8$ $(M$ sec.$)^{-1}$. Similarly, the slopes in Fig. 4 give $(k_{III}K_1 + k_{IV}K_3)[R^+]$ for glycine. These slopes are plotted *vs.* $[R^+]$ in Fig. 6 to show the linear dependence of $1/\tau$ upon $[R^+]$. The linearity of the plot and its extrapolation to the origin indicate that contributions from other reactions are negligible compared with the experimental error, thereby supporting the validity of the analysis. The slope of the line in Fig. 6 gives only the sum $(k_{III}K_1 + k_{IV}K_3)$. However, reaction IV is similar for both glycine and its ester, so it should be a reasonable, first approximation to assume that k_{IV} for glycine is the same as that found for the ester. With this assumption, k_{III} was calculated to be 1.2×10^3 $(M$ sec.$)^{-1}$ from the slope in Fig. 6.

Exchange of R^+ Protons with Water Molecules.—For the high acidity range just discussed, the two-step process Ia and Ib is the only explicit reaction which exchanges water protons directly between nonequivalent sites. If no other exchange affects τ for protons in H_2O, then τ_{H_2O} should be given by the first term in eq. 17 and 18 for the exchange of NH_3^+ protons, multiplied by twice the concentration ratio $[H_2O]/[R^+]$. The factor of two arises, although Ia and Ib involve the exchange of a single proton between NH_3^+ and H_2O, because $1/\tau_{NH_3^+}$ is the "total" rate constant for exchange of all three protons in the NH_3 group but $1/\tau_{H_2O}$ is the rate constant for exchange of each proton in the H_2O molecule. As a basis for comparison of the H_2O proton exchange with that of NH_3^+, we define f to be the fraction of the NH_3^+ proton exchange events which involve a proton in a water molecule, where

$$f = (2[H_2O]/[NH_3^+])\tau_{NH_3^+}/\tau_{H_2O} \qquad (19)$$

Unfortunately $\tau_{H_2O} >> \tau_{NH_3^+}$ because the factor $2[H_2O]/[NH_3^+]$ is large and, therefore, measurements of τ_{H_2O} from the H_2O line width, *via* eq. 1, were not accurate enough for any very detailed analysis.

However, the experimental values obtained for f and its pH dependence in very acidic solutions are of interest. For glycine, τ_{H_2O} was measured in 1 M solutions over the pH range 0.07 to 1.7 and for the methyl ester, from 0.2 to 2.2. For glycine f decreased from $\sim$1 in the most acidic solutions to $\sim$0.9 for pH 1.7, and for the methyl ester from $\sim$1 for pH 0.2 to $\sim$0.5 for pH 2.2. These results support several important features of the kinetic scheme, namely that R^+ is hydrated, that the

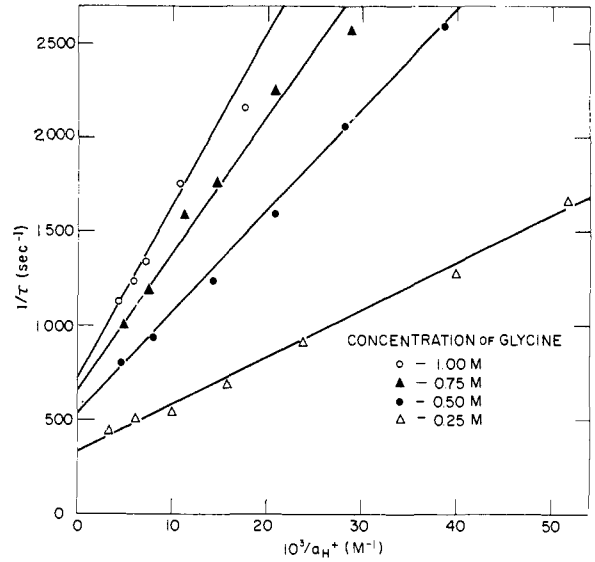


Fig. 7.—The mean lifetime τ between exchanges of the protons in an NH_3^+ group in the zwitterion of glycine at 23°, plotted as $1/\tau$ *vs.* $1/a_{H^+}$ for various concentrations of glycine, which practically equal the zwitterion concentrations. The data are fitted by eq. 20.

exchange in the very acidic range is primarily between H_2O and R^+, and that in less acidic solutions R^+ exchanges protons directly with $R^\pm$ and/or R.

NH_3^+ Proton Exchanges of the Zwitterion.—At pH values between 2.7 and 4.7 glycine exists in aqueous solution primarily as the zwitterion $R^\pm$, with small amounts of the anion R^- and of the undissociated glycine present. The experimental values obtained for $\tau_{NH_3^+}$ in this pH range are given in Fig. 7 as a function of $1/a_{H^+}$ for several concentrations of glycine. The main feature of these data is the linear dependence of $1/\tau$ upon $1/a_{H^+}$ for a given glycine concentration. Furthermore, the slopes of the $1/\tau$ *vs.* $1/a_{H^+}$ lines in Fig. 7 and their intercepts for $1/a_{H^+} = 0$ are themselves linear functions of the glycine concentration, as shown in Fig. 8 and 9, respectively.

As to the exchange mechanism, the zwitterion can participate in reactions analogous to those considered for the cation R^+, namely

$$NH_3^+CH_2COO^- + H_2O \underset{}{\overset{k_I'}{\rightleftharpoons}} NH_2CH_2COO^- + H_3^+O \qquad I$$

$$NH_3^+CH_2COO^- + OH^- \underset{}{\overset{k_{II}}{\rightleftharpoons}} NH_2CH_2COO^- + H_2O \qquad II$$

$$NH_3^+CH_2COO^- + NH_3^+CH_2COO^- \underset{}{\overset{k_{III}}{\rightleftharpoons}} NH_2CH_2COO^- + NH_3^+CH_2COOH \qquad III$$

$$NH_3^+CH_2COO^- + NH_2CH_2COOH \underset{}{\overset{k_{IV}}{\rightleftharpoons}} NH_2CH_2COO^- + NH_3^+CH_2COOH \qquad IV$$

In addition, the anion R^- is no longer present to a negligible extent and the following may occur.

$$NH_3^+CH_2COO^- + NH_2CH_2COO^- \underset{}{\overset{k_V}{\rightleftharpoons}} NH_2CH_2COO^- + NH_3^+CH_2COO^- \qquad V$$

Also, intramolecular exchange is possible.

$$NH_3^+CH_2COO^- \underset{}{\overset{k_{VI}}{\rightleftharpoons}} NH_2CH_2COOH \qquad VI$$

Thus, the over-all expression for the average exchange lifetime of NH_3^+ groups is shown in eq. 20.

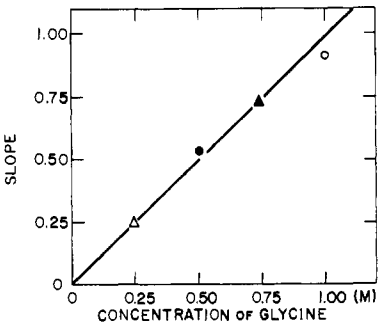


Fig. 8.—The slopes, in units of a_{H^+}/τ, of the straight lines in Fig. 7 plotted as a function of zwitterion (glycine) concentration ($R^{\pm}$). These data are consistent with eq. 20 which predicts that $(a_{H^+}/\tau) = k_{II}K_w + k_VK_2[R^{\pm}]$.

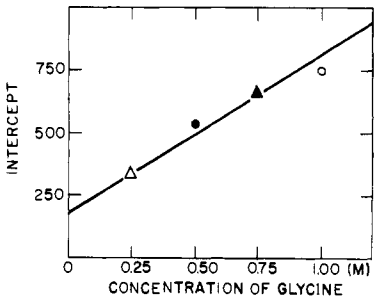


Fig. 9.—The $1/\tau$ intercepts at $1/a_{H^+} = 0$ of the straight lines in Fig. 7 plotted as a function of zwitterion (glycine) concentration, $[R^{\pm}]$. These data are consistent with eq. 20 which predicts that $(1/\tau)_0 = k_I'[H_2O] + k_{IV} + \{k_{III} + (k_{IV}/K_4)\}[R^{\pm}]$.

$$1/\tau = k_I'[H_2O] + k_{II}\frac{K_w}{[H^+]} + k_{III}[R^{\pm}] + k_{IV}\frac{[R^{\pm}]}{K_4} + k_VK_2\frac{[R^{\pm}]}{[H^+]} + k_{VI} \quad (20)$$

According to eq. 20, the slopes of the straight line $1/\tau$ *vs.* $1/a_{H^+}$ plots in Fig. 7 are numerically equal to $k_{II}K_w + k_VK_2[R^{\pm}]$ and the intercepts to $k_I'[H_2O] + k_{III}[R^{\pm}] + (k_{IV}/K_4)[R^{\pm}] + k_{VI}$. In turn, the linear plot in Fig. 8 of the slopes *vs.* $[R^{\pm}]$ corresponds to $k_{II}K_w + k_VK_2[R^{\pm}]$; *its slope* is k_VK_2, and its intercept is $k_{II}K_w$. Similarly, the linear plot in Fig. 9 of the intercepts *vs.* $[R^{\pm}]$ is $k_I'[H_2O] + k_{VI} + \{K_{III} + (k_{IV}/K_4)\}\cdot[R^{\pm}]$; *its intercept* is $k_I'[H_2O] + k_{VI}$, and its slope is $k_{III} + (k_{IV}/K_4)$. From these relations, the following numerical values were obtained from Fig. 8 and 9 for the various rate constants.

$$k_V = 3.8 \times 10^8\ (M\ \text{sec.})^{-1} \qquad k_{II} \lesssim 2 \times 10^{11}\ (M\ \text{sec.})^{-1}$$

$$k_I'\ [H_2O] + k_{VI} = 180\ \text{sec.}^{-1} \qquad k_{III} + \frac{k_{IV}}{K_4} = 640\ (M\ \text{sec.})^{-1} \quad (21)$$

In this analysis, the direct proton exchange between the zwitterion and water has been written for simplicity as the bimolecular reaction I rather than as the two-step, steady-state mechanism Ia and Ib involving the hydrated form even though the latter is probably what occurs. If this is indeed the case, the $k_I'[H_2O]$ should be replaced by k_I in eq. 20 and the preceding paragraph. Experimental determination of the correct version by measuring $1/\tau$ for large a_{H^+}, as in the study of the R^+ cation, is prevented by the accompanying conversion of the zwitterion $R^{\pm}$ to R^+. It is possible, nonetheless, to make an independent estimate of the rate constant, whether it be $k_I'[H_2O]$ or k_I, and thereby of k_{VI}. The reason is that reaction I or Ia for $R^{\pm}$ corresponds to the equilibium constant K_2 in eq. 6, and Ia for R^+ corresponds to K_3 in eq. 7. Furthermore, whatever the mechanism of the forward reaction, the rate of the reverse reaction must be controlled primarily by diffusion of the hydrogen ion and thus be nearly the same for $R^{\pm}$ as for R^+. Therefore, we can state that

$$\frac{k_I(R^{\pm})}{k_I(R^+)} \text{ or } \frac{k_I'[H_2O](R^{\pm})}{k_I(R^+)} \cong \frac{K_2}{K_3} \quad (22)$$

where the symbols $(R^{\pm})$ and (R^+) indicate that the preceding rate constant k_I is for $R^{\pm}$ and R^+, respectively. Upon introduction of the numerical values for $k_I(R^+)$, K_2, and K_3, eq. 22 leads to k_I or $k_I'[H_2O] \cong 5.1$ sec.$^{-1}$ for the zwitterion. In turn, this result combined with eq. 21 yields $k_{VI} \cong 175$ sec.$^{-1}$.

None of the zwitterion-exchange reactions as written involve H_2O directly, except I. However, it is possible that some of the exchange reactions involve H_2O molecules at least indirectly, for $R^{\pm}$ as well as R^+, even though the experimental results provide no direct answer to the question. Reaction VI is written as a purely intramolecular proton transfer, $R^{\pm} \rightarrow R$, and such a process may well occur, but for $\tau_{NH_3^+}$ to be affected, a water molecule must be involved at some stage or another. Otherwise, the reverse process $R \rightarrow R^{\pm}$ would return the original proton, with unchanged spin state, and there would be no net effect upon the spectrum of the CH_2 group. It seems most likely that an NH_3^+ proton "jumps" to the carboxylic acid group and then changes rapidly with a water molecule. Alternately, the exchange would have to occur *via* a water molecule, so that the proton appearing on the COOH would not be the same as that leaving the $R^{\pm}$.

NH_3^+ Proton Exchange at Intermediate pH 1.8 to 2.7. —In this pH range the relative concentrations of the glycine cation and of the zwitterion are both high and change rapidly with pH. Measurements of τ for NH_3^+ proton exchange in such solutions gave results consistent with those described above for R^+ and $R^{\pm}$ "separately." A detailed analysis was not attempted, in part because of its complexity and the improbability of anything new developing, and in part because of the point noted earlier. Only one methylene resonance was apparent, which means that there is "fast" exchange of the COOH proton from R^+ to $R^{\pm}$. This exchange could contribute appreciably to the width(s) of the methylene line(s), but by an amount depending upon its unknown rate and upon the unknown NH_3^+ and CH_2 proton shifts between R^+ and $R^{\pm}$.

In general, it was found that τ for these glycine solutions was less dependent upon $1/a_{H^+}$ than was τ for the ester or τ for glycine in the more acidic solutions. In fact, for pH between 2.4 and 2.7, $1/\tau$ decreases with increasing $1/a_{H^+}$, the reverse of the data in Fig. 2-5. However, this behavior is expected because the concentration of $R^{\pm}$ increases at the expense of R^+, and the NH_3^+ exchange rate of the $R^{\pm}$ in this pH range is much slower than that of R^+.

The separation of the exchange mechanisms for R^+ and R= by choosing appropriate ranges of low and high pH values is in principle not complete. For example,

TABLE I

SUMMARY OF PROTON-EXCHANGE RATE CONSTANTS FOUND FOR THE GLYCINE CATION (G^+), FOR THE CATION OF THE GLYCINE METHYL ESTER (GME^+), FOR THE GLYCINE ZWITTERION ($G^\pm$), FOR THE SARCOSINE CATION (S^+),[a] FOR THE CATION OF THE SARCOSINE METHYL ESTER (SME^+),[a] AND FOR THE SARCOSINE ZWITTERION ($S^\pm$)[b,c]

Species	k_I, sec.$^{-1}$	k_H, sec.$^{-1}$	k_{-I}, (M sec.)$^{-1}$	k_{III}, (M sec.)$^{-1}$	k_{IV}, (M sec.)$^{-1}$	k_V, (M sec.)$^{-1}$	k_{VI} sec.$^{-1}$
G^-	370	0.85×10^{10}	2.0×10^{10}	$\sim 1.2 \times 10^3$	$\sim 2.8 \times 10^8$	...	...
GME^+	470	1.52×10^{10}	2.5×10^{10}	...	2.8×10^8	...	...
$G^\pm$	~ 5.1	...	...	$< 0.64 \times 10^3$	$< 1.6 \times 10^8$	3.8×10^8	175
S^-	110	1.15×10^{10}	4.4×10^{10}	...	...	...	...
SME^-	120	2.9×10^{10}	4.8×10^{10}	...	...	...	...
$S^\pm$	~ 4.5	...	...	$< 0.09 \times 10^3$	$< 2.0 \times 10^8$	1.5×10^8	80

[a] See ref. 3a. [b] See ref. 3b. [c] The previously reported results for S^+, SME^- and $S^\pm$ have been converted to the somewhat different notation introduced here for glycine; also, a few of the rate constant values differ slightly because of their recomputation by the methods applied in our glycine work.

the k_I value of 370 sec.$^{-1}$ found for the glyine cation includes a small contribution from the finite concentration of the zwitterion, mainly *via* reactions I and/or VI. But such effects appear to be negligible in comparison with the experimental errors.

Discussion

In the "very acidic" range, the kinetics of the amino proton exchange described above for glycine and its ester are similar to those reported earlier for sarcosine and its ester. There is the added feature that the mechanism[14] Ia and Ib, proposed for the exchange in all four compounds, is consistent with the data obtained for glycine and its ester in the less acidic range (Fig. 4 and 5), from which a more accurate value can be obtained for k_I than from the results for more acidic solutions (Fig. 2 and 3). Also, similar results have been obtained in experiments not yet reported[15] on the amino proton exchange of N-dimethylglycine as well as of the NH_3^+ group in glycylglycine. All of these results, together with those of Grunwald and co-workers[13] for simple amines, indicate that the proton-exchange mechanism for a positively charged amino group in very acid solution is the same for the considerable variety of such systems studied thus far.

A summary is given in Table I of the rate constants found for the seven exchange reactions which are important for the glycine cation, the zwitterion and/or the cation of the methyl ester. The less extensive results reported previously for sarcosine[3] are included for comparison. In making comparisons, it should be remembered that, even though they are given the same index number, the reactions for the zwitterions $R^\pm$ differ from those for the cations R^+ and RME^+ because of the negative charge on the zwitterion.

In the case of k_I, k_{III}, k_V, and k_{VI}, the values are smaller by a severalfold factor for the sarcosine species than for the glycine, while for k_H, k_{-I}, and k_{IV}, the reverse is true. These differences appear reasonable in terms of the inductive and steric effects of the N–H group in glycine compared with the $N–CH_3$ group in sarcosine. The dependence of the rate constant for a given reaction upon the particular species involved is the same for sarcosine as for glycine. For example, we see that $k_I(GME^+) > k_I(G^+) >> k_I(G^\pm)$ and that the same inequality holds for sarcosine. The second part of this inequality, $k_I(R)^+ >> k_I(R^\pm)$, is related to our using eq. 22 to estimate $k_I(R^\pm)$ from $k_I(R^+)$ and the ratio of equilibrium constants K_2/K_3. In the latter, $K_3 >> K_2$ because the cation R^+ is more acidic than the zwitterion $R^\pm$, due most likely to the difference in their net charges. The validity of this approach, with its implicit relation between acidity and exchange rate, is supported by the fact that not only are $k_I(R^+)$ and $k_I(R^\pm)$ larger for glycine than for sarcosine but also G^+ and $G^\pm$ are more acidic than S^+ and $S^\pm$, as shown by the larger values of K_2 and K_3 for glycine. Similar interpretations can be made of the observations that $k_V(G^\pm) > k_V(S^\pm)$ and $k_{VI}(G^\pm) > k_{VI}(S^\pm)$.

Also, reasons for the relative magnitudes of the several rate constants for a particular species are readily apparent. The large magnitudes ($\sim 10^{10}$) of k_H and k_{-I} are compatible with the first involving a reaction which breaks a hydrogen bond and the second, proton transfer between the solvent water and a hydrated NH_2 group. Reactions III and IV also involve proton transfer, but while k_{IV} is $\sim 10^8$, k_{III} is only about 10^3. In fact k_{IV}/k_{III} is of the same order as K_4. For the cation R^+, reaction III is of the form $R^+ + R^\pm \rightarrow R + R^+$ and IV is $R^+ + R \rightarrow R + R^+$. Thus ΔF° for IV is zero, but for III it is greater than zero. This ΔF° adds to the activation energy for reaction III and would make $k_{III} < k_{IV}$, as observed. The analysis is more complicated for the zwitterion $R^\pm$ because its negative charge makes $\Delta F^\circ \neq 0$ for reaction IV. However, similar arguments lead one to expect that $K_{III}(R^\pm) < k_{IV}(R^\pm)$, as observed.

In conclusion, it appears that the kinetic mechanism proposed for the proton exchange not only provides an internally consistent interpretation of the exchange data but also is in at least qualitative agreement with the magnitudes found for the various rate constants for the several species studied.

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(14) C. G. Swain, J. T. McKnight, and V. P. Kreiter, *J. Am. Chem. Soc.*, **79**, 1088 (1957).

(15) M. Sheinblatt, unpublished results.