Aminolysis of Benzylpenicillin by Aliphatic Diamines

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The kinetics of reaction of penicillin with a series of aliphatic diamines, $NH_2(CH_2)_nNH_2$ (n = 2–6), show terms both first order and second order in diamine. The second-order terms are basicity dependent and account for general base-catalyzed nucleophilic attack by the monoprotonated species. The first-order term includes a contribution by both monoprotonated and unprotonated diamine. An argument in favor of intramolecular general base catalysis by the latter is given and the possible relationship between this mechanism and penicillin antigen formation is discussed.

DIRECT AMINOLYSIS of penicillins by ε-amino groups of lysine residues on proteins has been shown to occur (1, 2) and may be involved in the *in vivo* formation of the penicilloyl-protein conjugate which is considered to be the principal antigenic determinant in penicillin allergy (3). Aminolysis of penicillin by primary amines is however a relatively slow reaction at neutral pH because there is usually only a small fraction of the amine present in the nucleophilic unprotonated form. Investigation of the mechanism of reaction of glycine with penicillins (4) revealed participation by two molecules of glycine anion demonstrating a requirement for general base catalysis. This result indicated that reaction of an amine with penicillin might be more rapid where a second amino group was present on the same molecule and intramolecular general base catalysis could occur. To test this hypothesis studies were conducted of the reaction of benzylpenicillin with a series of aliphatic diamines [$NH_2(CH_2)_nNH_2$ with n = 2 through 6] and the results are reported herein.

EXPERIMENTAL

Materials—Benzylpenicillin was kindly supplied by Bristol Laboratories as the potassium salt. The aliphatic diamines were obtained as, or converted to, the dihydrochloride salts and recrystallized from aqueous methanol. All other materials were reagent grade.

Rate Studies—All measurements were made at 35°. Generally the diamine solution, adjusted to the desired pH with sodium hydroxide, acted as both buffer and reactant. In some cases where the buffer capacity was too low constant pH was maintained by a radiometer TTT-1 pH-stat. Ionic strength was kept constant throughout the study by addition of KCl to the reaction mixture. Initial penicillin concentration was 1.0×10^{-3} M and the diamine concentration was always in excess (above 0.04 M).

The rate of penicillin loss was followed by one of two methods: (*a*) Samples of reaction mixture were removed at appropriate intervals and assayed for residual penicillin by the method of Brandriss *et al.* (5). (*b*) The change in optical rotation of the solution was followed at 313 mμ with the Perkin-Elmer model 141 photoelectric polarimeter with Sargent SR recorder (2.5 mv.). Opening of the β-lactam of penicillins is accompanied by a large decrease in optical rotation. In some instances where both methods were utilized excellent agreement in the results was observed.

The acid dissociation constants (pKa′) of the diamines were determined at 35° and ionic strength 0.5 by potentiometric titration, and the overlapping constants separated by the method of Noyes (6). The values are presented in Table I (see results).

RESULTS

Since the diamine concentration was in excess of penicillin first-order kinetics was observed at constant pH. The rate constants (k_{obs}) were determined either from plots of log penicillin concentration *versus* time when the assay method was employed, or Guggenheim treatment of the optical rotation data. In either case excellent linearity was observed through at least two half-lives and generally through 3–4 half-lives.

Plots of $k_{obs.}$ at constant pH as a function of diamine concentration showed an upward curvature with increasing diamine, indicating an order greater than unity in base. Linearity was observed however when $k_{obs.}/B_T$ was plotted against B_T, where B_T represents the total concentration of all species of diamine present in solution. Figure 1 shows such a plot for 1,3-propanediamine which is typical of those observed with the other diamines. It can be seen from this plot that both the slopes and intercepts vary with pH.

The intercepts, representing terms in the rate law which are first order in diamine, express the sum of rate constants for nucleophilic attack by each of the basic species in solution:

$$\text{Int.} = k_1 f_B + k_2 f_{BH^+} \qquad \text{(Eq. 1)}$$

where f_B is the fraction of the total diamine unprotonated and f_{BH^+} the fraction of monoprotonated species.

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TABLE I—SPECIFIC RATE CONSTANTS[a] FOR REACTION OF BENZYLPENICILLIN WITH $H_2N(CH_2)_nNH_2$

n	pK_1'	pK_2'	k_1[b]	k_2[b]	k_3[c]	k_4[c]
2	7.07	9.91	5.3	0.1	0.32	14
3	8.70	10.60	47	0.3	4	—
4	9.45	10.77	25	0.1	9	—
5	9.88	10.85	15	—	21	—
6	10.06	10.80	18	—	39	—

[a] $k_{obs} = k_1[B] + k_2[BH^+] + k_3[BH^+]^2 + k_4[B][BH^+]$. [b] Units are M^{-1} min.$^{-1}$. [c] Units are M^{-2} min.$^{-1}$.

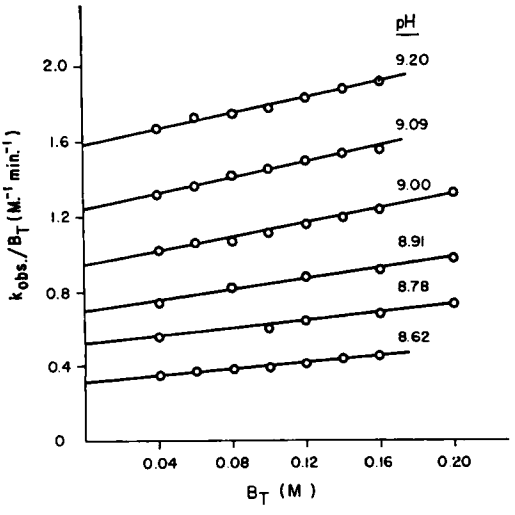

Fig. 1—Plot of k_{obs}/B_T vs. B_T for 1,3-propanediamine as a function of pH.

Dividing by f_{BH^+}:

$$\frac{\text{Int.}}{f_{BH^+}} = k_1 \frac{f_B}{f_{BH^+}} + k_2 \qquad \text{(Eq. 2)}$$

A plot of the left-hand side of Eq. 2 against f_B/f_{BH^+} should be linear with slope k_1 and intercept k_2. A plot of Eq. 2 for 1,3-propanediamine is shown in Fig. 2 and values of k_1 and k_2 were obtained from such plots for all of the diamines. These are given in Table I.

By a similar treatment it was found that the slopes (S) of the lines in Fig. 1 could be fitted by Eq. 3.

$$S = k_3(f_{BH^+})^2 + k_4(f_{BH^+})(f_B) \qquad \text{(Eq. 3)}$$

The values for k_3 and k_4 were determined from plots of $S/(f_{BH^+})^2$ *versus* f_B/f_{BH^+} All of these had zero slope (k_4 = zero) except the line for ethylenediamine where a significant value for k_4 could be determined. The values for k_3 and k_4 are also given in Table I.

Using the constants in Table I slopes and intercepts for lines of the type shown in Fig. 1 were calculated. Table II presents a comparison of these calculated values with those obtained experimentally showing generally good agreement.

DISCUSSION

The rate law for reaction of aliphatic diamines with benzylpenicillin is given in Eq. 4:

$$\text{Rate} = \{k_1[B] + k_2[BH^+] + k_3[BH^+]^2 + k_4[B][BH^+]\}(P) \qquad \text{(Eq. 4)}$$

where P denotes penicillin concentration. With the exception of the term k_2 $[BH^+]$ each of the terms may represent a general base-catalyzed nucleophilic attack by an amine. The k_2 term may denote intramolecular general acid catalysis but makes such a small contribution to the overall rate it is almost negligible. In addition there is considerable error involved in the determination of k_2 from the intercepts of plots of secondary data. The k_4 term is significant only for ethylenediamine and represents general base catalysis of nucleophilic attack by one of the species involved.

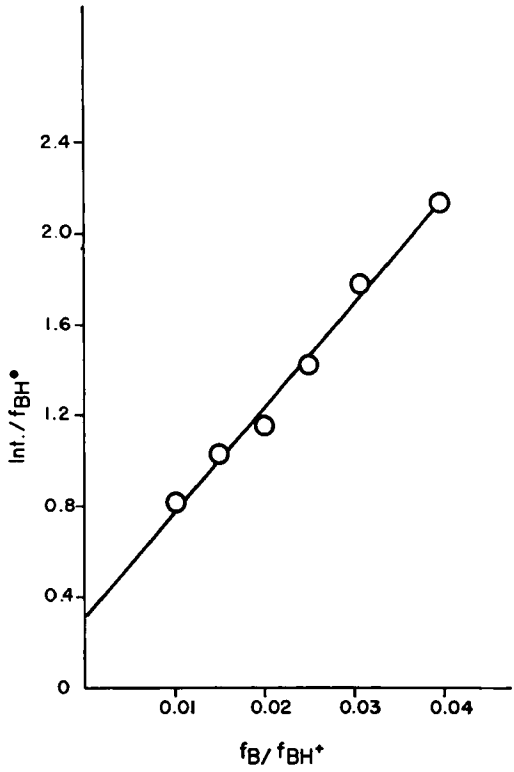

Fig. 2—Plot of equation for 1,3-propanediamine.

The values for k_3 are a function of the basicity of the amine as seen from the typical Bronsted plot in Fig. 3. Comparing the value previously obtained for glycine anion (k = 2.3, pKa = 9.10 at 50°) (4) with this data it may be seen that glycine would fall well below the line. The greater reactivity of the monoprotonated diamines probably reflects the difference in electrostatic charge of the attacking species relative to substrate. The penicillin, which exists as anion in the pH range studied, should attract the amine but tend to repel glycine anion.

There may be some controversy as to whether or not intramolecular general base catalysis does indeed occur in the reaction with the unprotonated diamine.

TABLE II—COMPARISON OF CALCULATED AND EXPERIMENTAL DATA

pH	Intercept Calcd.	Intercept Found	Slope Calcd.	Slope Found	pH	Intercept Calcd.	Intercept Found	Slope Calcd.	Slope Found
	Ethylenediamine					**1,4-Butanediamine**			
7.79	0.12	0.13	0.30	0.27	9.10	0.20	0.20	0.85	0.67
8.09	0.16	0.16	0.43	0.50	9.20	0.29	0.41	1.14	1.26
8.56	0.31	0.41	0.82	0.78	9.43	0.59	0.62	2.06	2.51
9.10	0.79	0.80	1.83	1.63	9.60	1.01	1.03	3.25	3.90
9.27	1.06	1.06	2.31	2.40			**1,5-Pentanediamine**		
9.43	1.40	1.30	2.80	2.80	9.60	0.28	0.25	2.4	2.4
	1,3-Propanediamine				9.80	0.58	0.57	4.0	3.0
8.62	0.36	0.33	0.82	0.75	9.90	0.81	1.06	4.9	5.0
8.78	0.55	0.53	1.18	1.30	10.10	1.5	1.53	6.7	7.5
8.91	0.76	0.70	1.46	1.45			**1,6-Hexanediamine**		
9.00	0.97	0.94	1.72	1.95	9.43	0.14	0.14	1.38	1.30
9.09	1.22	1.24	1.92	2.10	9.60	0.29	0.34	2.50	2.1
9.20	1.59	1.58	2.16	2.15	9.80	0.63	0.59	4.6	4.8
					9.90	0.89	0.85	6.0	6.9

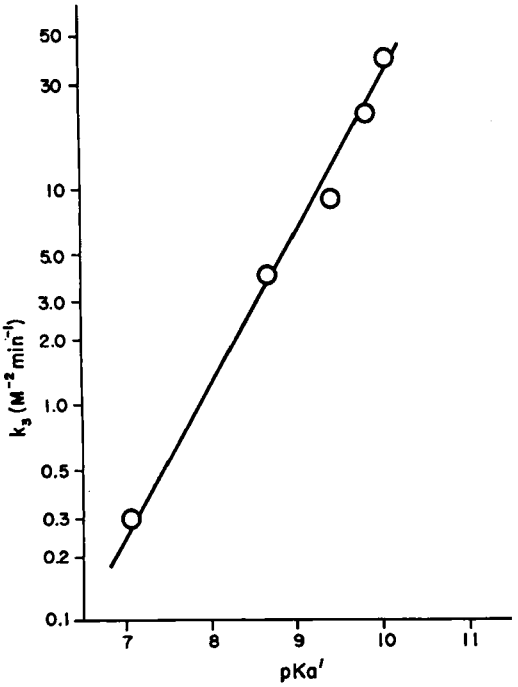

Fig. 3—Bronsted plot of k_3 *(log scale) as a function of* pK_1.

The rate law for reaction of these same diamines with phenyl acetate (7) showed only terms first order in diamine and a conclusion could not be drawn from the data as to whether or not intramolecular catalysis was taking place. On the other hand the kinetics of reactions of glycine with phenyl acetate (8) revealed terms both first and second order in amine while with penicillin as substrate only a second-order term was observed. It appears then that aminolysis of benzylpenicillin requires general base catalysis. These differences between penicillin and phenyl acetate are probably a reflection of the relative susceptibility of these two compounds to nucleophilic attack. Using rates of alkaline hydrolysis as a basis, the ester is much more susceptible, *i.e.*, k_{OH} for penicillin is 12.5 M^{-1}min.$^{-1}$ at 31.5° (9) and for phenyl acetate is 223 M^{-1}min.$^{-1}$ at 30° (10). It has been concluded from studies of aminolysis of phenyl esters that general catalysis becomes of greater significance as the leaving tendency of the phenol decreases (11). The contribution of general base catalysis is probably of more importance with those substrates less susceptible to nucleophilic attack.

It should be noted that it has previously been shown that benzyl penicillin reacts with 1,6-hexanediamine to form a penicilloamide derivative (12).

Intramolecular general base catalysis of aminolysis of penicillins by amino groups on proteins may be one route by which relatively rapid conjugation of the drug to protein may occur, forming antigen through which individuals are sensitized to the drug.

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Keyphrases

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Optical rotation—reaction rate
Ionic strength—potentiometric titration
Dissociation constants, acid—diamines