Kinetics and mechanism of penicillin aminolysis involved in penicillin allergy

It is generally thought that the principal antigenic determinant of penicillin allergy is the penicilloyl group (see, *e.g.*, Schwartz, 1969a; Schneider, 1970). Such penicilloyl conjugates may be formed *in vivo* by direct reaction of penicillins with ε-amino groups of protein (Schneider, 1970) and/or through the penicillenic acid intermediate which reacts rapidly with amine (Levine, 1960; de Weck & Eisen, 1960). Although other possibilities for the penicilloylation have been proposed (see, *e.g.*, Schneider, 1970), the decisive pathway has not yet been established. A knowledge of penicillin aminolysis and other related reactions is fundamental to the understanding of the mechanism for penicillin allergy. Despite of the large amount of works on such reactions (Bundgaard, 1971, 1972a, b, c; Schwartz, 1968, 1969b; Schwartz & Wu, 1966; Schneider & de Weck, 1968; Wagner, Davis & Gorman, 1969), the systematic kinetic studies are still lacking. We now report results of more extensive study of the reactions of benzylpenicillin (BPC) with various amines. This is the first report of quantitative structure-reactivity relationship for penicillin aminolysis.

The observed first-order rate constants, k_{obs}, for amine-catalysed hydrolysis of BPC were found to follow the general relationship:

$$k_{obs} = k_{pH} + k_1[\text{amine}] + k_2[\text{amine}][\text{amine H}^+] + k_3[\text{amine}]^2 + k_4[\text{amine}][\text{OH}^-] \quad (1)$$

where [amine] and [amine H^+] refer to the concentrations of free amine and its conjugate acid, respectively. The pseudo-first-order rate constants, k_{obs} and k_{pH} (in the absence of amine), were determined at 35° and 60° and constant ionic strength of 0·5 by iodometric titration method and/or hydroxamic acid assay. The initial concentration of BPC was 5×10^{-3}M.

The bimolecular rate constant, k_1, was obtained for all amines except for triethylamine. The relative significance of the other kinetic terms depends on the basicity and chemical structure of amines. For triethylamine, the values of k_{obs} were in agreement with the k_{pH}'s at the pH values. Hydrazine was more reactive by factors of 40 (k_1)–600 (k_2 and k_3) than glycylglycine of the same basicity due to α-effect. The result of the product analysis by penamaldate assay (Schwartz & Delduce, 1969) showed that all the reaction terms catalysed by amine gave the corresponding penicilloylamides. These results together with deuterium solvent isotope effects on the kinetic terms, k_1–k_4, indicate strongly that amines catalyse the penicillin hydrolysis as

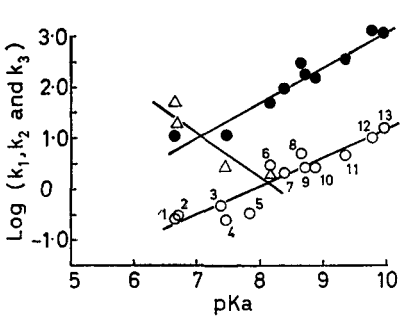

FIG. 1. Brønsted plots of the catalytic rate constants, k_1 (○), k_2 (△) and k_3 (●), for the aminolysis of benzylpenicillin at 60° and $\mu = 0{\cdot}5$. No statistical corrections have been made. Numbers refer to amines: 1, imidazole; 2, *N*-methylimidazole; 3, 2-methylimidazole; 4, glycylglycine; 5, 2,4-dimethylimidazole; 6, morpholine; 7, ammonia; 8, benzylamine; 9, 2-methoxyethylamine; 10, glycine; 11, β-alanine; 12, *n*-butylamine; 13, ε-aminocaproic acid.

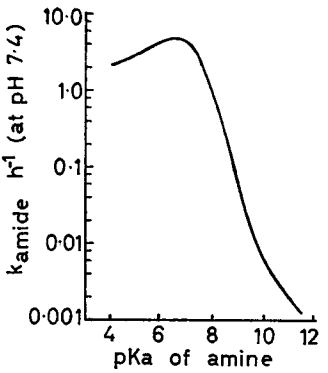

FIG. 2. Plot of the penicilloylamide formation rate constant, k_{amide}, at 35° against the pKa of amine. Calculations are for pH 7·4 based on the total concentration of 1·0M.

nucleophile with or without assistance of a second molecule of amine or hydroxide ion to produce the corresponding penicilloylamides.

In Brønsted plots for k_1, k_2 and k_3 at 35° and 60° against pKa of amines, the rate constants for primary, secondary amines and imidazole compounds fall close to each of lines (see Fig. 1) except for specific types of amines such as diamines, ethanolamines and hydrazines. The following relationships were obtained at 35° from the least squares treatment:

$$\log k_1\ (M^{-1}\ h^{-1}) = 0{\cdot}64\ pKa - 6{\cdot}17 \quad (2)$$

$$\log k_2\ (M^{-2}\ h^{-1}) = -0{\cdot}87\ pKa + 7{\cdot}21 \quad (3)$$

$$\log k_3\ (M^{-2}\ h^{-1}) = 0{\cdot}68\ pKa - 4{\cdot}23 \quad (4)$$

In connection with penicillin allergy, one of the major problems is the rates of penicilloylation under physiological conditions. The penicilloylamide formation rate constant, k_{amide}, at pH 7·4 and 35° can be calculated from eqns (1)–(4). In Fig. 2 is plotted k_{amide} thus derived for 1M amine concentration against the pKa of amine. At pH 7·4, amines with a pKa around 7 are kinetically the most reactive toward penicillin with the half-life of only 9 min. On the other hand, the direct reaction with amines having a pKa around 10–11 is extremely slow in the neutral pH region. The half-life of the aminolysis reaction is expected to be *ca* 100–400 h. Therefore, the rapid formation of penicilloyl conjugate *in vivo* is difficult to account for by the direct aminolysis of penicillin with ε-amino group of proteins.

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Faculty of Pharmaceutical Sciences,
Kanazawa University,
13–1 Takara-machi,
Kanazawa 920, Japan.
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TSUKINAKA YAMANA
AKIRA TSUJI
ETSUKO MIYAMOTO
EMI KIYA

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Lithium and ethanol preference

Recently lithium has been reported to reduce ethanol consumption in chronic alcoholics (Wren, Kline & others, 1974) and it has also been used in the treatment of ethanol withdrawal (Sellers, Copper & Zilm, 1974) but little is known about the interactions between lithium and ethanol. We now present some preliminary data to show that treatment with a dose of lithium, comparable to that used in man reduced significantly the volitional consumption of ethanol in rats. In addition, the concentrations of brain acetylcholine showed good correlation with the behavioral observations.

Adult male Sprague-Dawley rats, 200 to 250 g, were kept in individual cages at a constant 70°F. Two graduated glass drinking tubes (Kimax Instrument Co.) were fitted onto the outside of each cage, one filled with water and the other with varying concentrations of ethanol. The positions of the tubes were changed daily and also different tubes were used so as to prevent the development of a position habit. The ethanol used was diluted from 95% ethanol with tap water (v/v) to the required concentration. The preference-aversion cut off concentrations and the base-line consumption for each rat was determined as described by Amit, Stern & Wise (1970). Food, water and ethanol were freely available, consumption and body weight were recorded at 10 a.m. each day. Lithium (0·3 m equiv kg^{-1}) was given intraperitoneally at 12 hourly intervals as lithium chloride adjusted to an isotonic solution with distilled water. The control animals were treated similarly with normal saline. The concentrations of lithium in the blood and brain were well below the toxic levels, similar doses having previously yielded mean plasma concentrations of less than 0·2 m equiv $litre^{-1}$ and brain concentrations of 0·05 m equiv kg^{-1} approximately. At the concentrations of lithium used, there was no significant effect on water consumption and diuresis was minimal compared with the controls. At the end of the treatment, the rats were killed and the brains were dissected on ice. One side of the brains was used for choline transferase determination; the other side was extracted and assayed for acetylcholine. Choline transferase activity was determined using 1-[^{14}C]acetylcoenzyme A as substrate according to Ho, Singer & Gershon (1971). Acetylcholine was extracted from the brains, after homogenization in a glass homogenizer, with at least 4 volumes of 10% trichloroacetic acid, and then extracted with ether as described by Hebb (1963). The extracts were assayed biologically using the guinea-pig isolated ileum preparation as described by Bentley & Shaw (1952).

Within the same strain of rats, there were significant individual variations in the preference-aversion cut off concentrations; among 30 rats, these ranged from 3 to 11%. Rats given lithium showed a marked reduction in their daily consumptions of ethanol irrespective of the cut-off concentrations used (Fig. 1). The total fluid intake appeared not to have been altered significantly as the decrease in ethanol intake was compensated by an increase in water consumption. The reduction of ethanol consumption lasted throughout the whole treatment period, but returned to the base-line 2 days after the discontinuation of lithium.