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The Reaction of *p*-Nitrophenyl Acetate with Lysine and Lysine Derivatives

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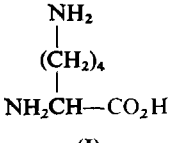
The kinetics of the reaction of *p*-nitrophenyl acetate with lysine and its derivatives has been investigated in water–dioxane solutions over the pH range 8.1–10.1. The reaction was found to be second order, and the unprotonated amino group was shown to be the reactive species. Intrinsic second-order rate constants were calculated.

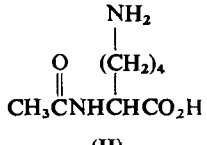
INTRODUCTION

Although the reactions of acetate esters with amines have been studied extensively (*1*), less work has been done on the reaction of acetate esters with amino acids and corresponding polyamino acids. Sacher and Laidler studied the hydrolysis of *p*-nitrophenyl acetate (NPA) catalyzed by serine, histidine, and histidylhistidine (*2*). Noguchi and Yamamoto studied the hydrolysis of NPA catalyzed by polyamino acids (*3*). They found that several synthetic polypeptides exhibited an optimum pH in the range 5.5–7.2, several displayed increasing activity with a rise in pH, whereas others showed no catalytic activity. Among the latter was an 8:1 copolymer of lysine and tryptophan.

Because the active sites in some enzymes, e.g., ribonuclease (*4*), acetoacetate decarboxylase (*5*), and glutamate dehydrogenase (*6*), involve a lysine residue, a study of the rates of reaction of lysine and polylysine should contribute to the understanding of enzyme reactions. Lysine has been variously reported to react with phenyl acetate in an aminolysis reaction (*7*), catalyze the hydrolysis of tributyrin (*8*), and not react with triacetin (*7*). We have investigated the reaction of lysine (**I**), α-acetyl lysine (**II**), ε-acetyl lysine (**III**) and polylysine (**IV**) with NPA over the pH range 8.1–10.1.



(I)



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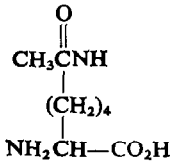

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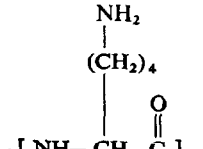

(IV)

EXPERIMENTAL

Materials

L-Lysine monohydrochloride, 99 + % Gold Label, mp 263–264°C, α-acetyl lysine hydrochloride, mp 256–258°C, and ε-acetyl lysine hydrochloride, 99%, mp 265°C, were obtained from Aldrich Chemical Co. and were used without further purification.

Poly-L-lysine hydrobromide, average molecular weight 125 000, was obtained from Pilot Chemicals, Lot Number L-71.

Dioxane, reagent grade, was freshly distilled from $LiAlH_4$ so as to decompose any peroxides which are known to react rapidly with NPA (*2*).

Trishydroxymethylaminomethane, used as buffer, was obtained from Fisher Scientific Co.

Para-nitrophenol was recrystallized from toluene.

Para-nitrophenyl acetate was washed with water and recrystallized from ethyl ether.

Procedure

In a typical experiment 2 ml of the buffered lysine (3.0 m*M*), α-acetyl lysine (3.0 m*M*), ε-acetyl lysine (3.0 m*M*) or polylysine (3.0 m*M* with respect to lysyl residues) solution at the desired pH was placed in a cuvette in a Beckman DU spectrophotometer equipped with circulating coolant that maintained the temperature at 20°C. This cooled solution was treated with 1 ml of NPA (1.66×10^{-1} m*M* in dioxane). The rates of the reactions that were measured by observing the increase of optical density at 400 nm due to the *p*-nitrophenolate ion were followed to about 5% completion and were always observed to be linear. The rates were obtained by a least squares fit of the data. Figure 1 presents typical examples of the data over a range of pH's for the aminolysis of NPA for α-acetyl lysine.

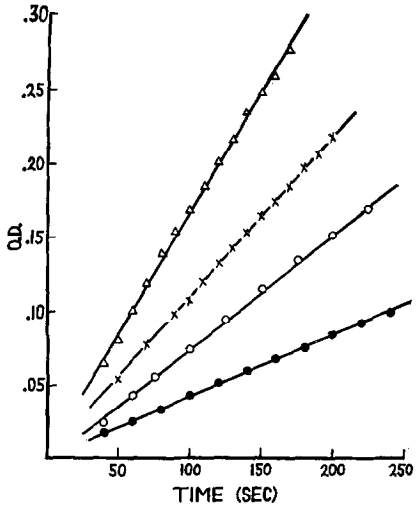

FIG. 1. Plot of *OD* vs time at pH 8.59 (●), 9.51 (○), 9.09 (×), and 9.96 (△) for α-acetyl lysine. The NPA concentration was 0.0033 mg/ml and the α-acetyl lysine concentration was 1.96 m*M*.

The rates were then corrected for by subtracting the spontaneous hydrolysis of NPA as determined by using only buffer and NPA at the various pH's studied and then converted to moles of product by using the experimentally determined molar extinction coefficients of *p*-nitrophenol in 33% (*v*/*v*) dioxane:water mixtures.

DISCUSSION

The reactions were found to be first order with respect to both amine and NPA concentrations by varying the concentrations of each species.

The rates of reaction of lysine, α-acetyl lysine, ε-acetyl lysine, and polylysine with NPA over the pH range 8.1–10.1 are given in Fig. 2. Although Noguchi and Yamamoto (*3*) found no hydrolytic activity for their predominantly lysine-containing polymer at pH 7.2, we found that polylysine reacts with NPA at pH 8.1 and above. Perhaps the rate of nucleophilic reaction was too slow to observe at pH 7.2.

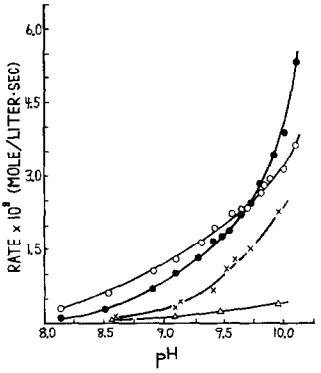

FIG. 2. Rates of aminolysis as a function of pH for lysine (●), polylysine (○), α-acetyl lysine (×), and ε-acetyl lysine (△).

The rates of aminolysis by all four nucleophiles increased with increasing pH in agreement with previous work utilizing other amino acids (*9*). This is reasonable in view of the fact that in the pH region studied significantly more free amine would be present at higher pH's. The pK_a values of the α-amino group of lysine, the ε-amino group of lysine, α-acetyl lysine, ε-acetyl lysine and polylysine are 8.95, 10.53, 10.5, 9.63 and 11.1, respectively, in aqueous solution (*10–13*). It has been demonstrated that pK_a values of both carboxyl and amino groups of amino acids are altered very little by the addition of dioxane (*12*).

Since all the nucleophiles are of the same kind (primary aliphatic amines) their nucleophilicities should correspond fairly well with their basicities. The ε-acetyl lysine has the lowest reactivity toward NPA as expected from the fact that it is least basic (pK_a 9.63). The α-acetyl lysine, pK_a 10.5, is more reactive than the isomeric ε-acetyl lysine. Lysine with two primary amines, pK_a 8.95 and 10.53, was found to react faster than either of the acetyl lysines over the entire pH range studied. Polylysine, with the most basic amino group (pK_a 11.1) exhibited unusual behavior. It reacted faster than any other species at pH values below 9.7. However, above this pH the reactivity

of the polylysine fell below that of the lysine in spite of the fact that above pH 9.7 the fraction of free amine in polylysine continues to increase significantly. A possible explanation for this relatively decreased reactivity might involve 1) the fact that lysine has two amino groups and 2) a conformational effect, since polylysine undergoes a helix-coil transition in the pH range studied here. Below pH 8.1 polylysine exists in the fully protonated, random coil form; as the pH increases, the net charge decreases with a concomitant formation of helical structures (*13*). By plotting the rates of reaction per unit concentration of NPA vs the concentration of free amine for each species, controlled by changing the pH (Fig. 3), we find that the rate of reaction of polylysine

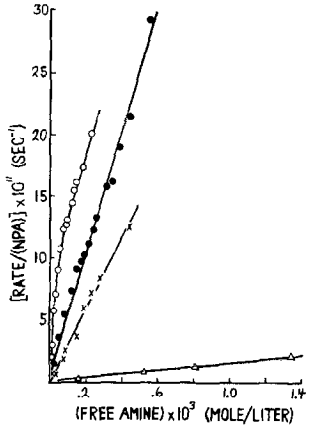

FIG. 3. Rates of aminolysis per unit concentration of NPA as a function of free amine concentration. Polylysine (○), α-acetyl lysine (×), ε-acetyl lysine (△), ε-amine of lysine (●).

decreases per unit concentration of free amine and then approaches a linear behavior. This change in reactivity occurs at a free amine concentration of approximately 0.14×10^{-3} *M*. Since the polylysine concentration in the reaction mixture was 2.00×10^{-3} *M*, the percent free amine in this region is approximately 7, a value that corresponds to a pH of 9.9 where significant (30%) amounts of helical conformation of polylysine begin to appear (*13*). An increase in the helical content of polylysine would reduce the number of neighboring protonated amino groups which could catalyze the aminolysis reaction because the helical conformation requires that adjacent lysine residues have their amino groups directed outward from the center of the helix.[1] The regions of significant free amine concentration (where nucleophilic attack occurs) would be those very areas that are helical. The reactivity of polylysine per unit of free amine above pH 10 should be constant but greater than α-acetyl lysine because of its higher pK_a, 11.1 vs 10.5.

In Fig. 3, the rate of aminolysis per unit of NPA by lysine is plotted against the concentration of free ε-amine, the group which appears to account for the majority of the lysine reaction. Freedman and Radda (*12*) estimated that the α-amine accounts for only 2.9% of the lysine aminolysis of 2,4,6-trinitrobenzene sulfonic acid at pH 7.4. In order to establish whether the ε-amine made the greater contribution in the NPA

[1] For an example of the catalysis of the aminolysis of esters by protonated amino groups, see Ref. (*9b*).

reaction, the rate of lysine aminolysis was plotted against free ε-, free α-, and free (α + ε)-amine concentrations (Fig. 4). A nearly straight-line relationship exists for the free ε-amino concentration, but not for the free α-. This linearity of the rate of reaction with free ε-amino concentration signifies that it is the ε-amino group which is the reactive portion of the lysine. Thus the intrinsic nucleophilicity of the ε-group remains constant. In order for the α-amino group to be the reactive site of lysine it would be necessary

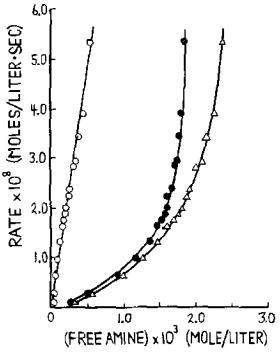

FIG. 4. Rate of aminolysis by lysine as a function of ε- (○), α- (●), and (ε + α)-free amine (△) concentrations.

TABLE 1

INTRINSIC SECOND-ORDER RATE CONSTANTS FOR THE AMINOLYSIS OF *p*-NITROPHENYLACETATE

	k, (1/mol-sec)	pK_a
α-Acetyl lysine	1.60×10^{-8}	9.63
ε-Acetyl lysine	28.6×10^{-8}	10.5
Lysine	49.0×10^{-8}	10.53, 8.95
Polylysine	90×10^{-8}	11.1

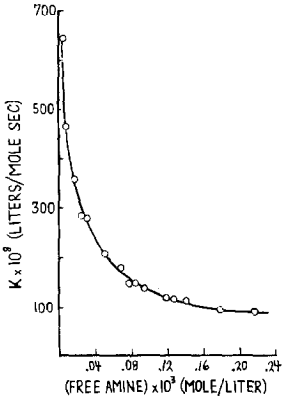

FIG. 5. Intrinsic second-order rate constant of polylysine as a function of free amine concentration

to develop a concept of varying intrinsic nucleophilicity. Thus, we conclude that lysine does indeed react primarliy at the ε-amino position.

Intrinsic second-order rate constants were obtained from Fig. 3. The results in Table 1 show that these reactivities correspond to the respective basicities. The variations in the rate constant for polylysine with pH, possibly due to conformational changes, asymptotically approached a value of 90×10^{-8} (Fig. 5).

What we have observed here is the sum of the effects of pK_a, nucleophilicity, and conformation of polylysine on its reactivity with NPA. Unfortunately, it is impossible to obtain the relative contributions of each of these factors in this system because the change in conformation of the polymer is here related to the pH. To disentangle these factors it will be necessary to go to a new system, one that will permit the variation of the conformation of the polymer while simultaneously keeping the fraction of free amine constant.

REFERENCES

1. T. C. Bruice and S. Benkovic, "Bioorganic Mechanisms," W. A. Benjamin, New York, 1966. For recent examples of this work see F. M. Menger and A. C. Vitale, *J. Amer. Chem. Soc.*, **95**, 4931 (1973); Chi-Wu Su and J. W. Watson, *J. Amer. Chem. Soc.*, **96**, 1854 (1974).
2. E. Sacher and K. J. Laidler, *Can. J. Chem.*, **42**, 2404 (1964).
3. J. Noguchi, S. Tokura, T. Komai, H. Yamamoto, and A. Ohnishi, *J. Biochem. (Tokyo)*, **64**, 703 (1968); J. Noguchi and H. Yamamoto, *J. Biochem. (Tokyo)*, **65**, 123 (1969); H. Yamamoto and J. Noguchi, *J. Biochem. (Tokyo)*, **67**, 103 (1970); A. Ohonishi, S. Tokura, and J. Noguchi, *J. Biochem. (Tokyo)*, **68**, 97 (1970); J. Noguchi and H. Yamamoto, *J. Biochem. (Tokyo)*, **69**, 119 (1971); J. Noguchi, S. Tokura, I. Komai, K. Kokazi, and T. Azuma, *J. Biochem. (Tokyo)*, **69**, 1033 (1971); T. Komai and J. Noguchi, *J. Biochem. (Tokyo)*, **70**, 467 (1971).
4. D. H. Meadows and O. Jardetzky, *Proc. Nat. Acad. Sci. USA*, **61**, 406 (1968).
5. D. E. Schmidt and F. H. Westheimer, *Biochemistry*, **10**, 1249 (1971).
6. F. M. Veronese, D. Piszewicz, and E. L. Smith, *J. Biol. Chem.*, **247**, 754 (1972).
7. R. W. Huffman, A. Donzel, and T. C. Bruice, *J. Org. Chem.*, **32**, 1973 (1967).
8. A. Gero and C. L. Withrow, *Nature (London)*, **180**, 1354 (1957).
9. a. W. P. Jencks and J. Carriuolo, *J. Amer. Chem. Soc.*, **82**, 675 (1960); b. T. C. Bruice and R. G. Willis, *J. Amer. Chem. Soc.*, **87**, 531 (1965).
10. "Handbook of Biochemistry and Molecular Biology," Chemical Rubber Company, Cleveland, OH, 1970.
11. R. B. Freedman and G. K. Radda, *Biochem. J.*, **108**, 383 (1968).
12. E. L. Duggan and C. L. A. Schmidt, *Arch. Biochem.*, **1**, 453 (1943).
13. R. C. Parker, L. J. Slutsky, and K. R. Applegate, *J. Phys. Chem.*, **72**, 3177 (1968).