# Molecular Rotations of $N^{\alpha}$-Acyl-L-lysines at Various pH Values

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**Molecular rotations of $N^{\alpha}$-acyl-L-lysines were determined in water and in water containing various amounts of HCl or NaOH. The acyl groups were formyl, acetyl, propionyl, and butyryl. Each $N^{\alpha}$-acyl-L-lysine exhibited more negative rotation in HCl or NaOH solution than in water. The plot of molecular rotation against amount of HCl or NaOH resembled that of a D-α-amino acid even though $N^{\alpha}$-acyl-lysine was of L-form. The reason for this is discussed from the standpoint of steric factors. $N^{\varepsilon}$-Acyl-L-lysines corresponding to the $N^{\alpha}$-acyl-L-lysines were synthesized as reference compounds. It was found that water-soluble $N^{\varepsilon}$-acyl-L-lysines can be easily prepared by acylation of the Cu complex solution of L-lysine hydrochloride in the presence of triethylamine. The molecular rotation plots for $N^{\varepsilon}$-acyl-L-lysines were typical of L-α-amino acids.**

**Keywords** $N^{\alpha}$-acryl-L-lysine; $N^{\varepsilon}$-acyl-L-lysine; molecular rotation; Lutz's rule

Kang *et al.* reported the hydrolytic rates of various *N*-acyl-L-phenylalanines by mold acylase.[1] In order to clarify further the substrate specificity of the acylase, we synthesized $N^{\alpha}$-acyl-L-lysines (acyl-L-Lys), in which the acyl groups were formyl (For), acetyl (Ac), propionyl (EtCO), and butyryl (*n*-PrCO). We were also interested in determining the molecular rotations ($[M]_D$) of acyl-L-Lys in solutions of various pH values. Thus, solutions of each compound were prepared in water and in water containing various amounts of HCl or NaOH. Unexpectedly, each acyl-L-Lys afforded more negative rotation in HCl or NaOH than in water, and the plot of $[M]_D$ against amount of HCl or NaOH resembled that of a D-α-amino acid.

## Results and Discussion

Acyl-L-Lys (acyl: Ac, EtCO, and *n*-PrCO) were easily synthesized by the following procedure. $N^{\alpha}$-Acyl-$N^{\varepsilon}$-benzyloxycarbonyl-L-lysines (acyl-L-Lys(Z)) derived from L-Lys(Z) and $(RCO)_2O$ were extracted with ethyl acetate (AcOEt). Then, acyl-L-Lys(Z) were converted to the desired acyl-L-Lys by hydrogenation.

Water-insoluble L-Lys(Z) was synthesized as follows.[2] A Cu complex solution of L-Lys·HCl was treated with Z-Cl and NaOH, and the insoluble L-Lys(Z)·Cu complex was collected. $H_2S$ was bubbled into L-Lys(Z)·Cu solution in HCl, CuS was filtered off, and L-Lys(Z) was collected after neutralization. For synthesizing water-soluble L-Lys(acyl), Hofmann *et al.* treated a Cu complex solution of L-Lys·HCl with HCOOEt and NaOH, collected L-Lys(For)·Cu which partially precipitated, and washed it with cold water to remove NaCl. Then, $H_2S$ was bubbled into a suspension of L-Lys(For)·Cu in water, and L-Lys(For) was isolated in 28% yield from L-Lys·HCl.[3] We used triethylamine (TEA) instead of NaOH, bubbled $H_2S$ into the reaction mixture directly, evaporated the filtrate, and collected L-Lys(For) with the aid of ethanol (EtOH) in 66% yield (TEA·HCl is soluble in EtOH).

Benoiton and Leclerc treated a mixture of L-Lys(Ac) and NaCl with Dowex 50 resin, and isolated L-Lys(Ac).[4] We reacted the Cu complex of L-Lys·HCl with $Ac_2O$ and TEA, and isolated L-Lys(Ac) in 68% yield without the use of an ion-exchange resin. Other L-Lys(acyl) (acyl: EtCO and *n*-PrCO) were similarly synthesized.

The $[M]_D^{20}$ values of acyl-L-Lys and L-Lys(acyl) were determined. Solutions of each compound were prepared with water and water containing various amounts of HCl and NaOH. The rotations were measured, and $[M]_D^{20}$ values were calculated (Table I). Plots of $[M]_D$ against amount of HCl or NaOH for Ac-L-Lys and Z-L-Lys are given in Figs. 1 and 2, respectively. Those of other acyl-L-Lys (acyl: For, EtCO, and *n*-PrCO) were similar in shape to that of Ac-L-Lys.

Lutz and Jirgensons reported that an L-α-amino acid exhibits more positive rotations in HCl up to a constant and limiting value than in water solution (Lutz's rule).[5] All L-Lys(acyl) in this study followed Lutz's rule (Figs. 1 and 2, and Table I). On the other hand, the curves of acyl-L-Lys resembled that of a D-α-amino acid. The $[M]_D$ values shifted to the negative side with increase of HCl up to *ca.* 5 eq compared to that in water only. The curves, however, tended to shift to the positive side, like that of an L-α-amino acid, from *ca.* 5 to 100 eq. The curves of Ac-L-Lys and Z-L-Lys in NaOH also resembled in shape that of a D-α-amino acid (Figs. 1 and 2).

TABLE I. Molecular Rotations of Lysine Derivatives in Different Solvents

| Lys derivative | $[M]_D^{20}$ (°) ($c$=0.05 M)[a] | | | |
|---|---|---|---|---|
| | NaOH (3 eq) | $H_2O$ | HCl (5 eq) | HCl (100 eq) |
| For-L-Lys | −5.0 | +7.6 | −15.0 | —[b] |
| Ac-L-Lys | +3.8 | +9.0 | −31.8 | −15.2 |
| EtCO-L-Lys | +0.6 | +8.6 | −40.0 | −32.8 |
| *n*-PrCO-L-Lys | −5.8 | −0.4 | −47.2 | −38.8 |
| Z-L-Lys | −21.2 | —[c] | −36.2 | −23.0 |
| L-Lys(For) | +16.8 | +6.4 | +34.4 | —[b] |
| L-Lys(Ac) | +19.2 | +7.6 | +37.8 | +40.8 |
| D-Lys(Ac) | −19.0 | −7.6 | −38.2 | −41.0 |
| L-Lys(EtCO) | +17.5 | +6.4 | +38.8 | +40.4 |
| L-Lys(*n*-PrCO) | +14.0 | +5.8 | +40.0 | +43.8 |
| L-Lys(Z) | +9.4 | —[c] | +36.0 | +39.8 |
| D-Lys(Z) | −9.4 | —[c] | −35.8 | −39.6 |

*a*) $[M]_D^{20}=\alpha\times(10\,\mathrm{M}/k\,\mathrm{M})$; temperature 20 °C, α observed rotation, $k$ concentration in mol dm$^{-3}$ of a derivative, cell pathlength 10 cm. *b*) $[M]_D^{20}$ of For-L-Lys and L-Lys(For) was not determined because the For group was liberated gradually after 10 min in 5 M HCl. *c*) Z-L-Lys and L-Lys(Z) were insoluble in water.

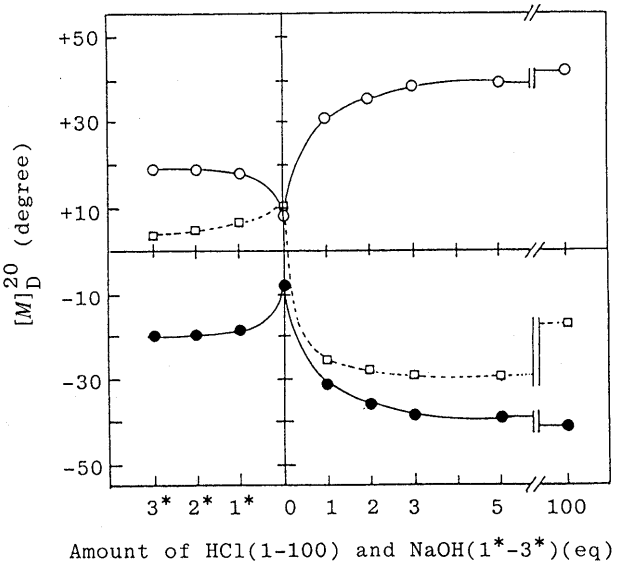


Fig. 1. Plots of Molecular Rotation $[M]_D^{20}$ against Amount of HCl and NaOH for Ac-L-Lys and L(and D)-Lys(Ac)

□---□, α-Ac-L-Lys; ○—○, L-Lys(Ac); ●—●, D-Lys(Ac).

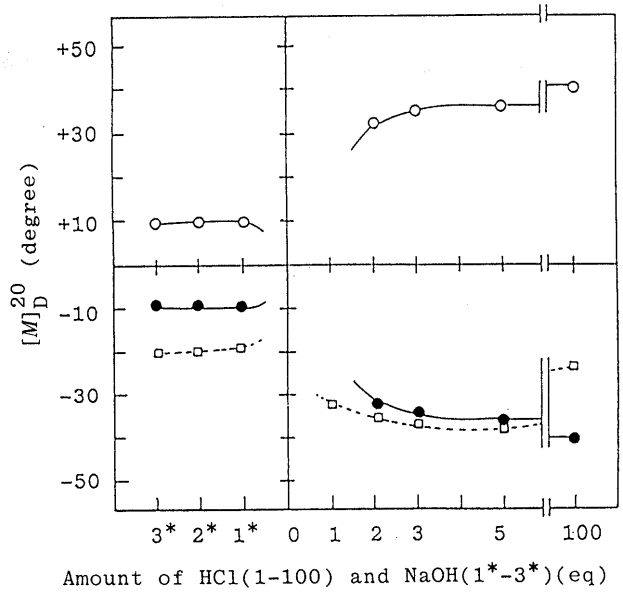


Fig. 2. Plots of Molecular Rotation $[M]_D^{20}$ against Amount of HCl and NaOH for Z-L-Lys and L(and D)-Lys(Z)

□---□, Z-L-Lys; ○—○, L-Lys(Z); ●—●, D-Lys(Z).

These unexpected results can be interpreted in steric terms (Fig. 3). L-Lys(Ac) can be drawn as **1** in the zwitterion form, and Ac-L-Lys as **3a**. The structure **3a** is equivalent to **3b** on rotation of the **3a** molecule, and **3b** resembles D-Lys(Ac) (**2**).[6]

The CD curve of Ac-L-Lys also resembled that of a D-α-amino acid, except at 228—240 nm (Fig. 4). The CD curves of other acyl-L-Lys (acyl: For, EtCO, and *n*-PrCO) were similar to Ac-L-Lys.

## Experimental

TLC was carried out on Silica gel G (Merck) with the following solvent systems (v/v): $Rf^1$ = *n*-BuOH–AcOH–pyridine–$H_2O$ (15:3:10:12), $Rf^2$ = $CHCl_3$–MeOH–AcOH (95:5:1). Rotations were measured on a Horiba SEPA 200 polarimeter under the conditions indicated in Table I. Circular dichroism (CD) measurements were performed on a JASCO J-500C spectropolarimeter under the following conditions; cell path-length 0.1 cm, concentration (*c*) in $H_2O$ 0.002 M, temperature 15°C. The following compounds were synthesized by the cited procedures: For-L-Lys,[3] Z-L-Lys,[7] and L (and D)-Lys(Z).[2]

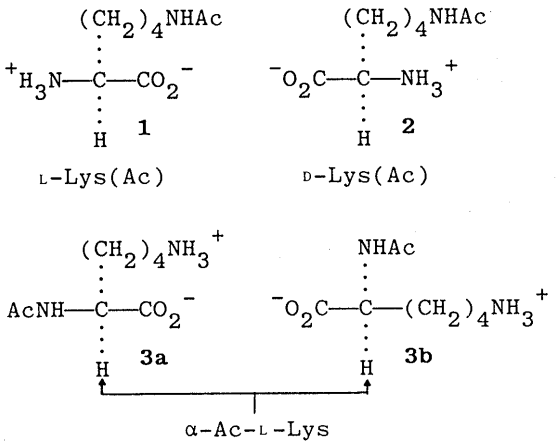


Fig. 3. Zwitterionic Structures of L(and D)-Lys(Ac) and Ac-L-Lys

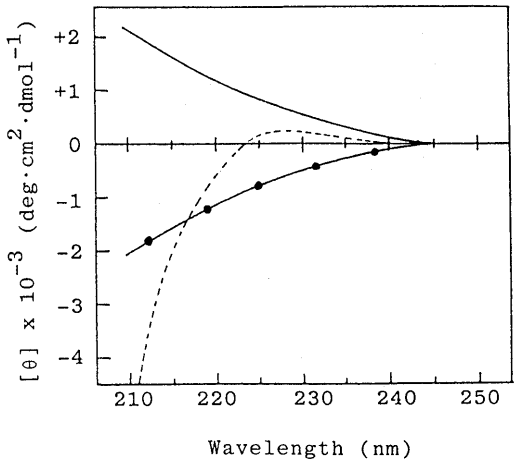


Fig. 4. CD Spectra of Ac-L-Lys and L(and D)-Lys(Ac)

------, α-Ac-L-Lys; ——, L-Lys(Ac); ●—●, D-Lys(Ac).

**Determination of $[\alpha]_D^{20}$ and $[M]_D^{20}$** Preparation of Ac-L-Lys solution containing 5 eq of HCl is described as an example. Into a 5-ml flask containing 47.1 mg (0.25 mmol) of Ac-L-Lys was added 1 M HCl (1.25 ml). The flask was made up to 5.0 ml with $H_2O$, so that the concentration of the compound was 0.05 M. Rotation of the solution was observed as −0.159°, and $[\alpha]_D^{20}$ and $[M]_D^{20}$ were calculated as −16.9° and −31.8°, respectively. When a flask containing 0.25 mmol of the compound is made up to 5.0 ml with 5 M HCl, the amount of HCl becomes 100 eq.

**Ac-L-Lys** L-Lys(Z) (5.61 g, 20 mmol) dissolved in 0.5 M NaOH (40 ml) was cooled on ice, and $Ac_2O$ (2.64 ml, 28 mmol) and TEA (5.39 ml, 36 mmol) were added in two portions (0 h and 30 min). The mixture was stirred for a further 1.5 h at 0 °C and 1 h at room temperature, then washed with ether. After addition of 5 M HCl (12 ml), Ac-L-Lys(Z) was extracted with EtOAc (80 ml), and the extract was dried ($Na_2SO_4$) and evaporated *in vacuo*. Yield 5.6 g (87%), $Rf^1$ 0.67, $Rf^2$ 0.13. The resultant oil was dissolved in 40 ml of AcOH–MeOH–$H_2O$ (8:2:1) and hydrogenolyzed with Pd charcoal (0.3 g). The filtrate was evaporated and evaporation was repeated several times after addition of water. The residual solid was collected after addition of EtOH, and recrystallized from hot water–EtOH. Yield 2.03 g (54% from L-Lys(Z)), mp 264—266 °C (dec.), $Rf^1$ 0.29, $Rf^2$ 0.02, $[\alpha]_D^{20}$ +4.8° (*c* = 0.94, $H_2O$). Neuberger and Sanger synthesized Ac-L-Lys(Z) from L-Lys(Z) (1 eq), $Ac_2O$ (2 eq) and NaOH (4 eq), and obtained Ac-L-Lys by hydrogenation.[8] Reported values of Ac-L-Lys: mp 250 °C (dec.), $[\alpha]_D$ +4.7° ($H_2O$).[8] We reduced the amounts of $Ac_2O$ (1.4 eq) and alkali (2.8 eq) to avoid possible racemization of Ac-L-Lys(Z).

**EtCO-L-Lys** L-Lys(Z) (20 mmol) was treated with $(EtCO)_2O$ (3.57 g, 28 mmol) as described above. Yield of oily EtCO-L-Lys(Z) 5.72 g (85%), *Rf* 0.71. The oil was hydrogenated, and the resultant solid was recrystallized from water–EtOH. Yield 1.94 g (48% from L-Lys(Z)), mp 231—233 °C (dec.), $Rf^1$ 0.32, $[\alpha]_D^{20}$ +4.3° ($c$=1.01, $H_2O$). *Anal.* Calcd for $C_9H_{18}N_2O_3$: C, 53.44; H, 8.97; N, 13.85. Found: C, 53.40; H, 9.06; N, 13.93.

***n*-PrCO-L-Lys** L-Lys(Z) (20 mmol) was treated with $(n\text{-PrCO})_2O$ (4.59 ml, 28 mmol). Yield of oily *n*-PrCO-L-Lys(Z) 6.3 g (90%), $Rf^1$ 0.76. Yield of recrystallized *n*-PrCO-L-Lys 2.21 g (51% from L-Lys(Z)), mp 216—221 °C (dec.), $Rf^1$ 0.41, $[\alpha]_D^{20}$ −0.2° ($c$=1.08, $H_2O$). *Anal.* Calcd for $C_{10}H_{20}N_2O_3$: C, 55.53; H, 9.32; N, 12.79. Found: C, 55.36; H, 9.27; N, 12.95.

**L-Lys(For)** A mixture of L-Lys·HCl (20 mmol) and $CuCO_3 \cdot Cu(OH)_2 \cdot H_2O$ (3.66 g, 60 mmol) in water (100 ml) was heated in a boiling bath for 30 min, and insoluble cupric carbonate was filtered off. MeOH (50 ml) was added to the filtrate, and HCOOEt (9.6 ml, 120 mmol) and TEA (18 ml, 120 mmol) were added in three portions (0, 1.5, and 3 h) at room temperature. Stirring was continued for a further 1 d, and the reaction mixture was evaporated to *ca.* 100 ml. $H_2S$ was bubbled into the suspension, the filtrate was evaporated, and the solid collected after addition of EtOH was recrystallized from water–EtOH. Yield 2.31 g (66%), mp 213—215 °C (dec.), $Rf^1$ 0.31, $[\alpha]_D^{20}$ +3.7° ($c$=0.87, $H_2O$). Hofmann *et al.* reacted Cu complex solution of L-Lys·HCl with HCOOEt and NaOH at 5 °C, and obtained a product of mp 214—215 °C (dec.), $[\alpha]_D^{26}$ +4.1° ($H_2O$).[3]

**L-Lys(Ac)** $Ac_2O$ (28 mmol) and TEA (6.0 ml, 40 mmol) were added in two portions (0 h and 30 min) to a Cu complex of L-Lys·HCl (20 mmol) in water (100 ml) at 0 °C. Stirring was continued for 1.5 h at 0 °C and 1 h at room temperature, then $H_2S$ was bubbled into the suspension, and the filtrate was evaporated. The solid collected after addition of EtOH was recrystallized from water–EtOH. Yield 2.56 g (68%), mp 250—253 °C (dec.), $Rf^1$ 0.42, $[\alpha]_D^{20}$ +21.7° ($c$=0.94, 5 M HCl). Reported value: $[\alpha]_D^{22}$ +22.1° (5 M HCl).[4]

**D-Lys(Ac)** Yield from D-Lys·HCl 65%, mp 251—253 °C (dec.), $Rf^1$ 0.42, $[\alpha]_D^{20}$ −21.8° ($c$=0.94, 5 M HCl).

**L-Lys(EtCO)** L-Lys·HCl (20 mmol) was treated with $(EtCO)_2O$ (28 mmol) as described for L-Lys(Ac). Yield 2.86 g (71%), mp 255—257 °C (dec.), $Rf^1$ 0.46, $[\alpha]_D^{20}$ +20.0° ($c$=1.01, 5 M HCl). *Anal.* Calcd for $C_9H_{18}N_2O_3$: C, 53.44; H, 8.97; N, 13.85. Found: C, 53.61; H, 8.85; N, 13.90.

**L-Lys(*n*-PrCO)** Yield 64%, mp 275–278 °C (dec.), $Rf^1$ 0.52, $[\alpha]_D^{20}$ +20.3° ($c$=1.08, 5 M HCl). Chibata *et al.* treated the Cu complex of L-Lys·HCl with *n*-PrCO–Cl and NaOH to obtain a product of mp 260—261 °C (dec.), $[\alpha]_D^{29}$ +23.0° (5 M HCl).[9]

## References and Notes


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6) Similar studies were presented at the Joint Meeting of the Chugoku–Shikoku and Kyushu Branches of the Chemical Society of Japan, Kochi, Nov. 1993, Abstract of Papers, pp. 363, 364, 365; the plots of $[M]_D$ against amount of HCl or NaOH for $N^{\alpha}$-acyl-L-Orn, $N^{\alpha}$-acyl-L-Arg, $N^{\alpha}$-acyl-L-His, and H-L-Asp–Gly–OMe resembled that of Ac-L-Lys, and the ionic structures of these compounds would be similar to that of Ac-L-Lys (**3b**).
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