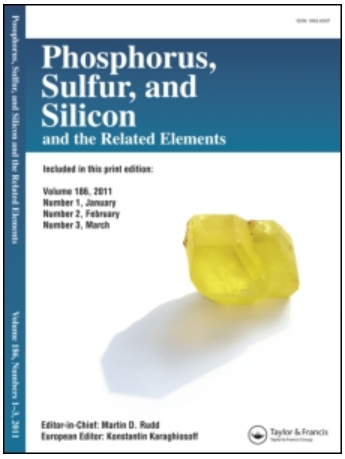

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THE DIFFERENT CHEMICAL PROPERTIES OF TWO PHOSPHORYL GROUPS IN Nα, Nε-BIS(O,O-DIISOPROPYL) PHOSPHOLYSINE

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THE DIFFERENT CHEMICAL PROPERTIES OF TWO PHOSPHORYL GROUPS IN Nα, Nε-BIS(O,O-DIISOPROPYL) PHOSPHOLYSINE

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The reactivities of Nα, Nε-bis(O,O-diisopropyl) phospholysine ($(DIPP)_2$lys) **1** in Tris-HCl buffer and n-butanol were investigated. The Nα phosphoryl group was liable to be cleaved from the lysine in pH 7.5 Tris-HCl buffer, which was observed to be a first-order kinetic reaction with $k = 2.95 \times 10^{-5}$ sec^{-1}; however, the Nε phosphoryl group was very stable. The ester groups of the Nα phosphoryl group could be exchanged by the n-butanol, which was also a first-order kinetics reaction with $k = 2.3 \times 10^{-5}$ sec^{-1}; but to Nε phosphoryl group, no ester exchange reaction occurred. The neighboring carboxyl group participation leading to the formation of penta-coordinate phosphorus transition state was suggested.

Keywords: Nα; Nε-bis(O,O-diisopropyl)phospholysine; hydrolysis; ester exchange; carboxyl group participation; penta-coordinate transition state

INTRODUCTION

More data shows that the N-phospholysine and N-phosphohistidine as the transient intermediates played a very important role in the protein phosphorylation for cell signals transduction[1–3]. It is of interest to study the phosphorylated lysine for more knowledge of the protein phosphorylation process. Up to now, there was some information about the N-phosphoamino acids[4–7]. Their reactivity such as peptide formation, ester exchange on phosphorus was presumed to be the result of the intramolecular mutual activation between the carboxyl group and N-phosphoryl group[6,7]. However, the reactivity of Nα, Nε-bis(O,O-diisopropyl)phospholysine **1** which had two phosphoryl groups in the molecule

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was not clear. Do two phosphoryl groups show the same reactivity? In this paper, the reactivities of $(DIPP)_2$lys in Tris-HCl buffer and n-butanol were investigated respectively. The results demonstrated that only Nα phosphoryl group was reactive to alkaline buffer and alcohol.

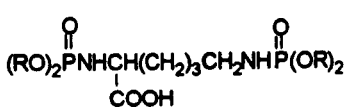
RESULTS AND DISCUSSION

1: Assignment of Two Phosphoryl Groups

Based on the ^{31}P NMR, ^{1}H NMR and ^{13}C NMR, the structure of $(DIPP)_2$lys was confirmed to be compound **1** (Scheme I), but it was difficult to assign the two ^{31}P NMR signals exactly to the Nα and Nε phosphoryl groups in the molecule. An attempt to just phosphorylate one amino group was made, but it failed due to the minor pK difference of the two amino groups (pK of α-NH_2: 9.2, ε-NH_2:10.8). In our previous paper, the relationship between the N-phosphoamino acids structure and the ^{31}P NMR chemical shift were theoretically elucidated[8]. The ^{31}P NMR chemical shift of the phosphoryl group far away from the α-carboxyl group came close to that of phosphoramidate and appeared in the higher field. The Nε phosphoryl group was about 2 ppm higher than the Nα phosphoryl group. Hence, the δ = 6.53 ppm represented the Nα phosphoryl group, δ = 8.62 ppm represented the Nε phosphoryl group.

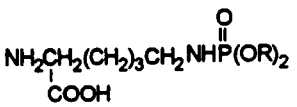
2: The Reactivity in Tris-HCl Buffer

Generally speaking, the P—N bond in the phosphoramidate was labile to the acids but stable to the alkaline[9,10]. However, the cleavage of P—N bond in the N-phosphoamino acids had been observed under alkaline conditions[11]. At 40°C,



1

Nα, Nε- bis(O,O-diisopropyl) phospholysine



2

Nε-(O,O-diisopropyl) phospholysine

SCHEME I The structure of N-(O,O-diisopropyl)phospholysine.

when the $(DIPP)_2$lys was dissolved in the pH 4.0 HCl solution, the hydrolysis reaction occurred simultaneously to both P—N bonds as in the phosphoramidate. When compound **1** was dissolved in the pH 7.5 Tris-HCl buffer, an interesting phenomenon was found in the ^{31}P NMR spectra. Figure 1 shows the ^{31}P NMR stack spectra. Only the Nα phosphoryl group varied with the reaction time, no observable change on the Nε phosphoryl group was detected. Because the integral of the ^{31}P NMR signals was proportional to the concentration of the corresponding phosphorus compound, it is rational to calculate the concentrations of all phosphorus compounds appearing in the reaction system with their ^{31}P NMR integrals. During the reaction course, it was found that the concentration of the Nε phosphoryl group was unchanged, although the Nα phosphoryl group kept decreasing (Figure 1). The relationship between the concentration of the Nα phosphoryl group (A) characterized by its ^{31}P NMR integral and the reaction time (t) was processed according to $-\ln A \sim t$, a straight line was obtained (Figure 2). It suggested that due to the excess of Tris-buffer, a pseudo-first-order kinetic reaction of $k = 2.95 \times 10^{-5}$ sec^{-1} occurred at the Nα phosphoryl group. Whereas the Nε phosphoryl group was very stable to the alkaline buffer. The compounds derived from the Nα phosphoryl group were at about 0 ppm and confirmed to be phosphate, isopropylphosphate and diisopropylphosphate with authentic samples in our previous paper[11]. The total reaction can be demonstrated in Scheme II.

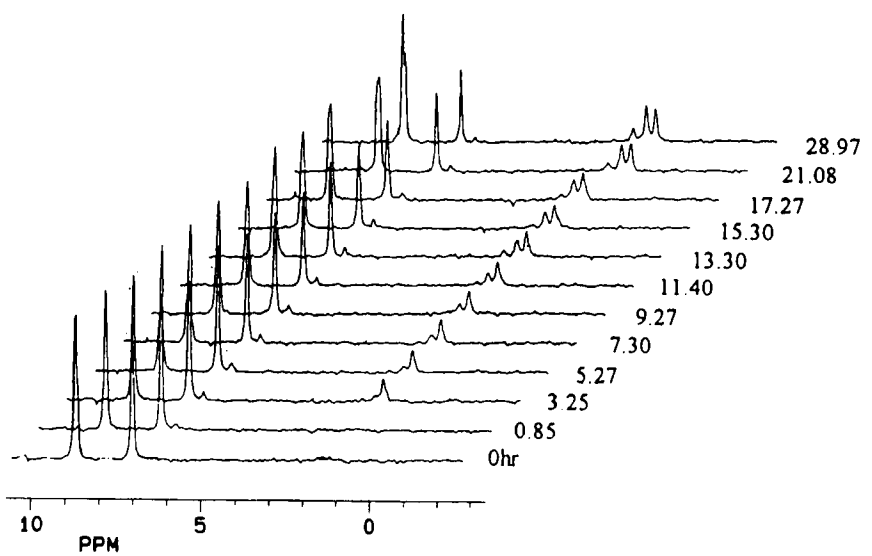


FIGURE 1 The ^{31}P NMR stack spectra for reaction of the $(DIPP)_2$lys in the pH 7.5 Tris buffer. The $\delta = 7.21$ ppm was the Nα phosphoryl group. The compounds at about 0 ppm represented phosphates.

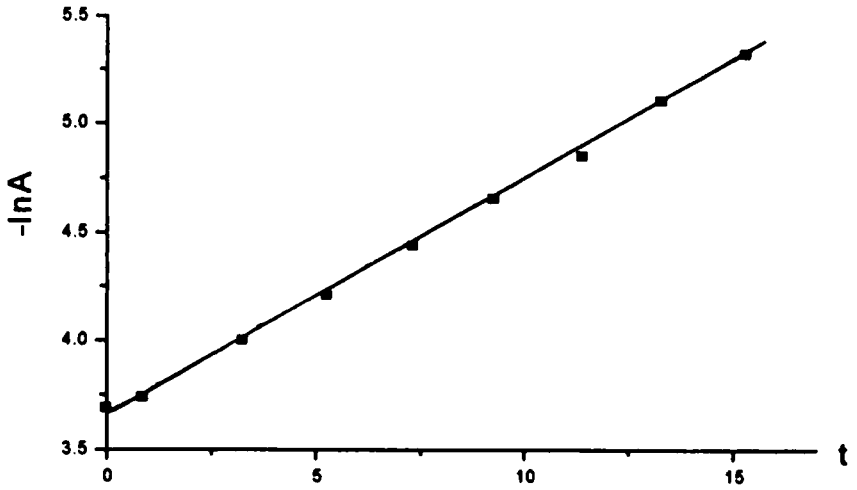
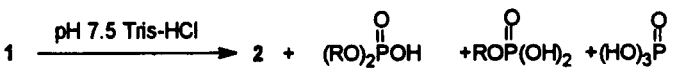


FIGURE 2 The -InA ~ t (hr) diagram for the Nα phosphoryl group in the Tris buffer.

3: The Interaction with n-BuOH

Contrary to the phosphoramidate, the N-phosphoamino acid could exchange its ester groups with other alcohols under mild conditions[12]. The interaction of $(DIPP)_2$lys with n-butanol was also investigated. To accelerate the reaction, the imidazole of catalysis-quantity was added to the reaction system[7]. This was first monitored by the ^{31}P NMR. The findings indicated that only the Nα phosphoryl group changed with time (Figure 3). In contrast with the Nα phosphoryl group, two upfield signals appeared on the ^{31}P NMR spectra which suggested the ester exchange products. The integrals of all peaks were measured, which indicated that the new peaks were equal to the diminished Nα phosphoryl group while the Nε phosphoryl group remained unchanged. The -In A ~ t diagram for the Nα phosphoryl group showed a straight line (Figure 4). After the 15 hrs' incubation, the reaction system was analyzed with negative ion FAB-MS. Besides the molecular ion of $(DIPP)_2$ lys 473[M − 1](40%), there were two new molecular ions 487[M + 14 − 1](85%) and 501[M + 14 × 2 − 1](100%) which confirmed that the isopropoxyl group was exchanged with one butoxyl group and two butoxyl groups respectively to produce compounds **3**, **4** in the reaction system; but more high molecular wt. ions which suggested tri-, even tetra-ester exchange



SCHEME II The total reaction of the $(DIPP)_2$lys in the pH 7.5 Tris buffer.

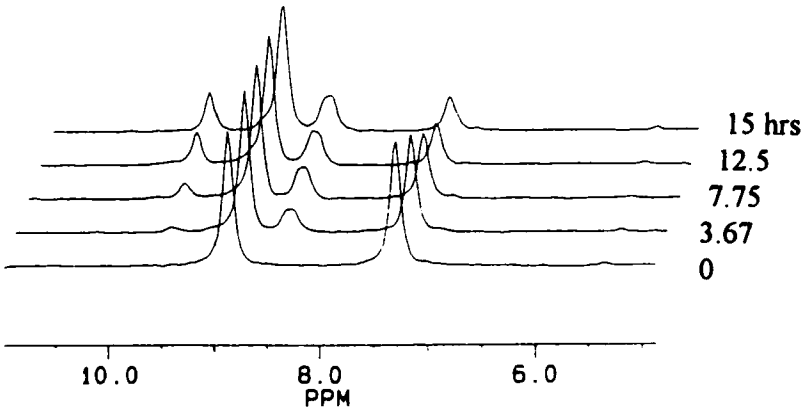


FIGURE 3 The ^{31}P NMR stack spectra for interaction of the $(DIPP)_2$lys with the n-butanol. The δ = 7.5 ppm was the Nα phosphoryl group.

products failed to be checked out. Based on ^{31}P NMR and FAB-MS analysis, it could be concluded that only the Nα phosphoryl group was reactive and could be exchanged by n-butanol through a pseudo-first-order kinetic reaction of k = 2.3×10^{-5} sec^{-1}. The ester exchange reaction can be demonstrated in Scheme III.

There was significant discrepancy in the reactivities between N-phospho-amino acids and phosphoramidates. The N-phosphoramidates were stable to the alkaline, but N-phosphoamino acids could be cleaved at the P--N bond[10,11]. The

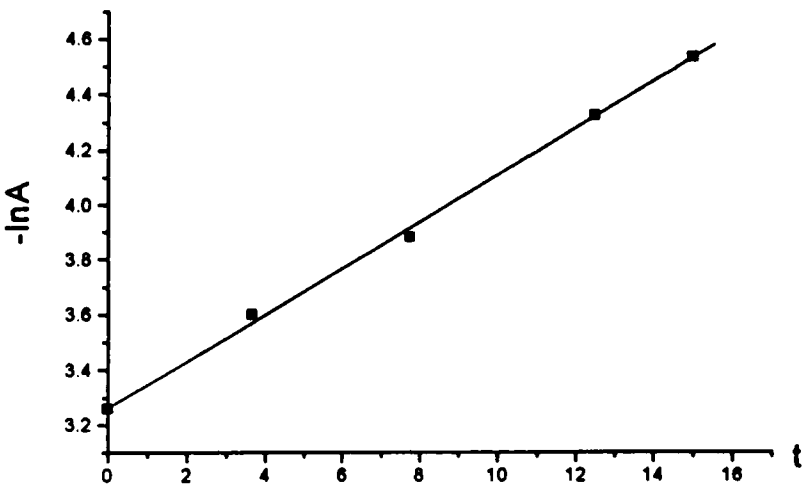
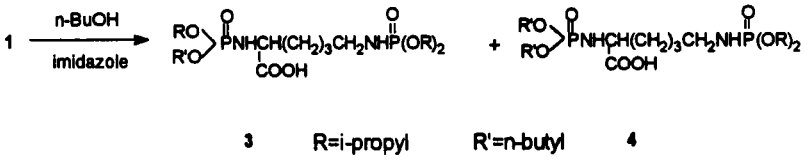
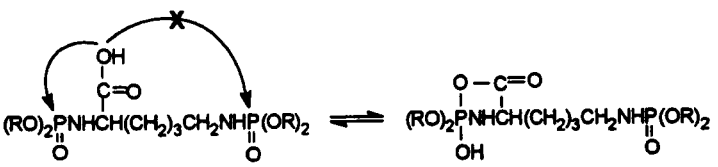


FIGURE 4 The -InA ~ t (hr) diagram for the Nα phosphoryl group in the n-butanol.



SCHEME III The reaction occurred to the $(DIPP)_2$ lys in the n-butanol.

(O,O-dialkyl)phosphoramidate did not exchange its ester groups at all, but N-(O,O-dialkyl)phosphoamino acids easily exchanged their ester groups[12]. For these, an intramolecular carboxyl group participation leading to the penta-coordinate phosphorus transition state mechanism was proposed by Zhao et al[4]. In fact, the penta-coordinate phosphorus derived from N-phosphoamino acids could be formed by the participation of intramolecular carboxyl group[13]. The results presented here indicate that the reactivity of the phosphoryl group has a connection with the relative position of the carboxyl group. In $(DIPP)_2$lys, the Nα phosphoryl group flanked closely by the carboxyl group was reactive; however, the Nε phosphoryl group, which was spatially far away from the carboxyl group, behaved like the stable phosphoramidate. Therefore, the high reactivity of the Nα phosphoryl group was the result of the carboxyl group participation. Owing to the five-membered ring structure and apical entering, apical departure rule for it[14,15], the penta-coordinate phosphorus transition state at the Nα position which was formed first by the carboxyl group participation was followed immediately by the nucleophilic attack of H_2O and R'OH to cause hydrolysis and ester exchange reaction. Since the Nε phosphoryl group was too far away from the α-carboxyl group, it was almost impossible for it to form penta-coordinate phosphorus transition state (Scheme III). Therefore, it was very stable to the alkaline buffer and alcohol. The kinetics datum indicated that the reaction rate of the Nα phosphoryl group was mainly decided by its concentration rather than the media also suggested that two reaction systems share the same transition state formation from the $(DIPP)_2$lys.



SCHEME IV The carboxyl group participation leading to the formation of the penta-coordinate phosphorous transition state only at the N_2 position.

CONCLUSION

The reactivity of $(DIPP)_2$ lys in pH7.5 Tris buffer and n-butanol were investigated. The results indicated that there was obviously a difference between the two phosphoryl groups. Only the Nα phosphoryl group showed reactive. The main cause could be attributed to the participation of the carboxyl group.

EXPERIMENTAL

Methods

The ^1H NMR(TMS as internal standard), ^{13}C NMR($CDCl_3$ internal standard) and ^{31}P NMR (85% H_3PO_4 as external standard) spectra were taken on a BRUKER AC200P spectrometer. The negative FAB-MS data were obtained on a Finnigan MAT 90 double-focusing magnetic mass spectrometer.

Preparation of Nα, Nε-Bis(O,O-diisopropyl) Phosphoryl Lysine

To an ice-water cooled solution of 20mmol L-lysine in H_2O(30ml)/$(Et)_3N$(8ml)/ C_2H_5OH(10ml), the mixture of 40mmol diisopropylphosphite and 10ml CCl_4 was added dropwise. Having been stirred at 0°C for 30 minutes and 1 hr at R.T., the mixture was evaporated in vacuum and washed with ether (2*20ml). The water layer was adjusted to pH 3 ~ 4 with 3N HCl and extracted with 2:3 terbutyl alcohol-ethyl acetic ester (5*30ml). The combined extracts were washed with saturated aqueous NaCl, dried with $MgSO_4$ and concentrated in vacuum. A colorless product **1** was obtained, yield 70%. ^{31}P NMR: 6.53ppm, 8.62ppm(E-tOAc), ^1H MR($CDCl_3$): 1.0 ~ 1.5(m, 30H), 2.8 ~ 2.9(br, 2H, NH), 3.0 ~ 3.3(2H, ε-CH_2), 3.6 ~ 3.8(1H, α-CH), 4.3 ~ 4.6 (m, 4H, OCH), 11.5(br, 1H, COOH).

^{13}C NMR ($CDCl_3$): 23.79 (CH_3), 70.36(-CH-O), 173.09(-COOH), 54.34(α-C), 41.24(β-C), 31.4, 34.2, (other 2*CH_2).

The Reactivity in Tris-HCl Buffer

30mg $(DIPP)_2$lys was dissolved in the 0.8 ml 0.05M pH7.5 Tris-HCl buffer. The system was incubated in a 5mm NMR tube at 40°C and monitored with ^{31}P NMR.

Ester Exchange Reaction:

About 30mg $(DIPP)_2$lys and 20mg imidazole were incubated with 0.8ml n-BuOH in 5mm NMR tube at 40°C, ^{31}P NMR monitored the reaction. After the 15 hrs' incubation, a drop of reaction system was sended for FAB-MS data (glycerol as matrix).

Acknowledgment

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