Heteroatom Chemistry
Volume 4, Number 4, 1993

Phosphoryl Transfer Reaction of Phospho-Histidine

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Received 1 July 1992

ABSTRACT

N-phosphorylhistidine was converted into its ester and phosphoric ester exchanged products in alcoholic media. It was demonstrated that the co-participation of the three functional groups of phosphoryl, imidazolyl, and carboxyl in the molecule is essential for these reactions to occur. Also, the neat phosphoryl transfer reaction occurs with N^{α}, N^{Im}-Bis-phospho-histidine.

INTRODUCTION

It is known that histidine is present in the active site of many enzymes, such as chymotrypsin, pancreatic ribonuclease, and carboxypeptidase, etc., and that phosphoryl histidine is present as an intermediate in reactions involving acid phosphatase, phosphofructokinase, and histamine kinase [1–6]. In addition, it is known that the phosphoryl-histidine regulates the enzyme's activity [6–8]. Because the imidazole moiety is the side chain in histidine, it was anticipated that the catalytic effect resulted from its participation in each of the enzyme's actions. In this article, the results of our study of the intrinsic relationship between the phosphoryl, imidazolyl, and carboxyl groups are reported.

RESULTS AND DISCUSSION

The N^{α}-(o,o-diisopropyl)-phospho-histidine, (N^{α}-DIPP-HIS) **1**, was synthesized by a previously reported general procedure [9,10]. It was found that the phospho-histidine, **1**, was not as stable as the N-phosphorylated derivatives of the simple alkyl side chain amino acids, such as DIPP-alanine, **2**, and DIPP-γ-hydroxyproline, **3** [11,12]. When compound **1** was incubated in the alcoholic medium at 40° for 24 hours, the results were checked by fast atom bombardment mass spectra (Table 1) and the ^{31}P NMR spectroscopy, as shown in Figure 1 and Scheme 1. An interesting finding was that the parent molecular weight peak at 320 *m/z* was reduced from 90 to 10% (relative to the 110 *m/z* peak), and new peaks at 334 (M + 14), 348 (M + 28), 376 (M + 56), 390 (M + 14 + 56), and 404 (M + 28 + 56) corresponding to monobutyl and di-butyl ester exchange and their concomitant esterification products were observed. This series of products gave ^{31}P NMR signals at δ 5.2, 6.3, and 7.3, where each *n*-butyl group substituted for an isopropyl group resulted in a 1.0 ppm lower field shift, which is consistent with the reported oxygen substitution γ-effect for N-phospho-amino acids to [13]. The DIPP-his, **1**, had a ^{31}P NMR shift very close to its *n*-butyl ester, because a slightly overlapped broad peak at δ 5.2 was observed for them. Similarly, the mono-

TABLE 1 Relative Peak Intensity of FAB-MS of Nα-DIPP-His Before and After Incubation in *n*-Butanol

m/z	*Before*	*After*	*Compounds*
320	92	10	Nα-DIPP-His, **1**
334	0	23	mono-butyl-exchanged of **1**
348	0	45	di-butyl-exchanged of **1**
376	0	13	Nα-DIPP-His-OC_4H_9
390	0	10	butyl ester of mono-butyl-exchanged of **1**
404	0	8	N-DBP-His-OC_4H_9

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1042-7163/93/$5.00 + .25

FIGURE 1 (a) ^{31}P NMR of pure Nα-DIPP-His at δ 5.45. (b) ^{31}P NMR of the reaction mixture of Nα-DIPP-His in *n*-Butanol: δ 5.2, 6.3, and 7.3.

n-butyl exchanged product and its *n*-butyl ester also had an overlapped peak at δ 6.2.

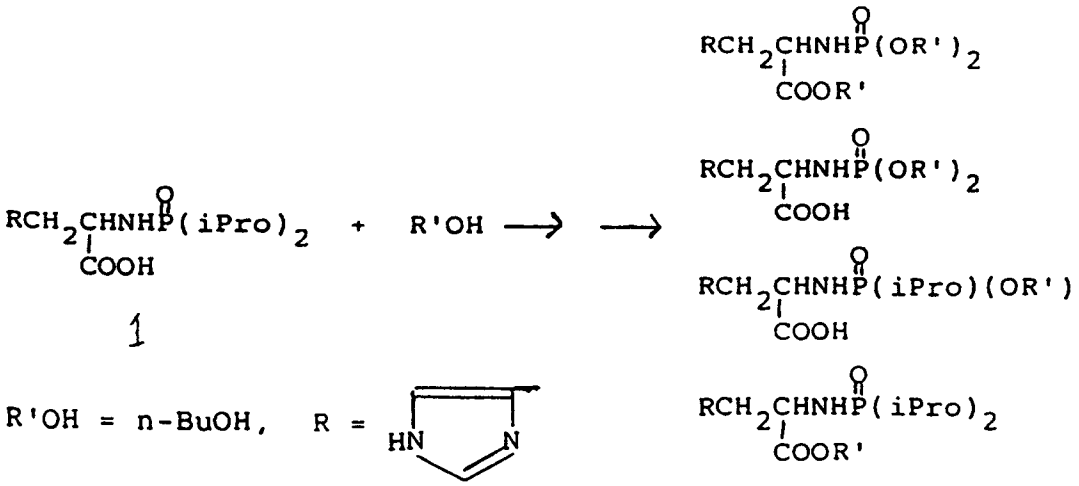
In order to study the mechanism regulating the reactions depicted in Scheme 1, the histidine-methyl ester was N-phosphorylated to DIPP-His-OMe, **4**, which was a persistent stable compound, even after prolonged incubation in 1-butanol. This indicates that the carboxylic acid group participated in the transition state. Also, the imidazole group functions as an intramolecular catalyst, since the DIPP-His, **1**, but not the simple DIPP-Ala, **2**, gave the ester exchange and esterification products under the same conditions. This indicates that the imidazole catalyzed the phosphoryl ester exchange reaction. In the usual case, the imidazole group can act as a base to promote the formation of intermediate (A) (Scheme 2) which precedes the esterification or ester exchange reaction. Since, in the proposed mechanism, the imidazole only acts as the base, it was expected that the DIPP-alanine should undergo the ester exchange reaction under acceleration by the addition of imidazole. Instead, in the presence of base, the reactivity was inhibited completely. Therefore, this implies that the catalysis must be intramolecular. Hence, a different mechanism other than one involving the base effect was considered. Indeed, the unique imidazole catalyzed alcoholysis of phosphate was not a simple base promotion effect but involved nucleophilic attack on the phosphorus [14–16].

To account for these facts, a hexa-coordinate phosphorus compound with co-participation of all three groups of phosphoryl, imidazole, and carboxyl in the DIPP-His, **1**, is proposed for the phosphoryl transfer reaction (Scheme 3).

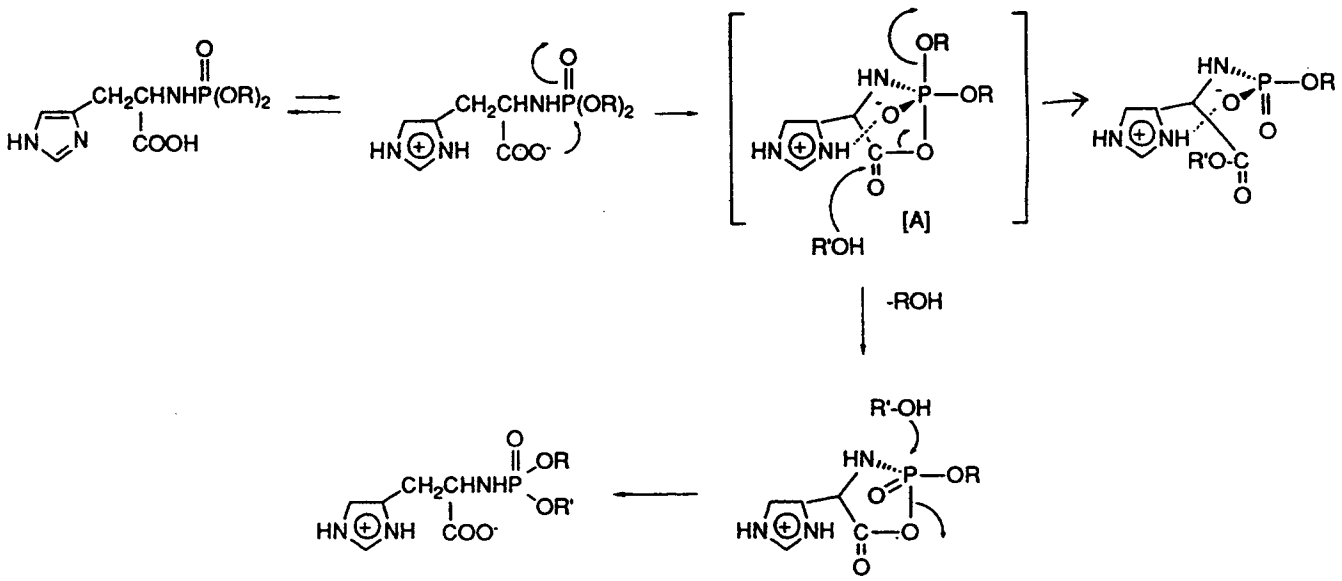
In the hexa-coordinate intermediate (Scheme 3), a five-membered phosphoryl-carboxyl mixed anhydride is fused with a six-membered ring containing two P–N bonds. One of them is the phosphoryl-imidazole bond which is very reactive. For the purpose of testing the reactivity of the phosphoryl-imidazole bond in the histidine derivative, N^{α}, N^{Im}-Bis-DIPP-His-OMe, **5**, was synthesized. When compound **5** was reacted with methanol at 40°C without any catalysis, a complete N^{Im}-phosphoryl transfer reaction occurred to give, in quantitative yield, methyl diisopropyl phosphate, **6**, and DIPP-His-OMe, **4** as confirmed by the ^{31}P NMR spectrum (Scheme 4).

CONCLUSION

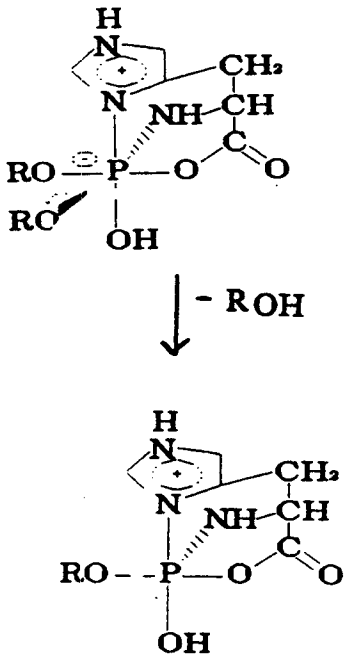
As mentioned previously, the phosphoryl group in N^{α}-DIPP-His-OMe, **4**, was inert; neither the phosphoryl transfer nor the ester exchange occurred. However, with DIPP-His, **1**, having the presence of the free carboxylic acid to activate the phosphoryl group, together with the intramolecular imidazole catalytic effect, the phosphoryl ester exchange was possible, but phosphoryl exchange was minimal.



SCHEME 1



SCHEME 2



SCHEME 3

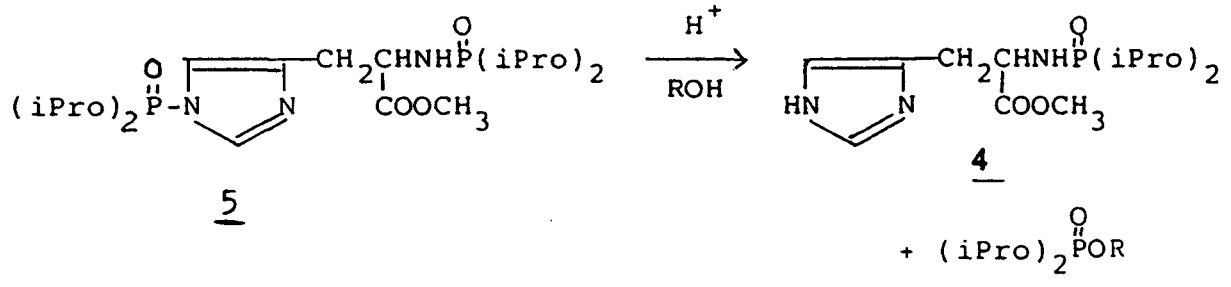
However, the phosphoryl–imidazole bond is very labile in compound **5**, affording, under neutral conditions, a phosphoryl transfer reaction as the dominant reaction. These results may provide a clue to the histidine's function in the enzymes.

EXPERIMENTAL SECTION

Methods: The ^{13}C NMR, ^{31}P NMR, and ^{1}H NMR spectra were taken on a JEOL FX-100 spectrometer. The ^{31}P NMR shifts involved 85% phosphoric acid as the external reference. The ^{31}P NMR spectra were recorded by the broadband decoupling program. The ^{13}C NMR spectra used chloroform-d as the internal reference at δ 76.9. TMS was used as the internal standard for the ^{1}H NMR spectra. The positive-ion FAB-MS data were obtained on a kyky Zhp-5 double-focusing mass spectrometer from the Scientific Instrument Factory (Beijing, China) equipped with a standard kyky fast atom gun. Infrared spectra were determined with a carlzeiss Jena specord 751IR instrument. The melting points were uncorrected.

Preparation of N^{α}-(Diisopropyloxyphosphinyl) histidine **1**

A mixture of diisopropyl phosphite (2 g, 0.012 mol) and 10 mL CCl_4 was added dropwise to a suspension of L-histidine (1.5 g, 0.01 mol) in 4.2 mL Et_3N, 10 mL H_2O, and 10 mL MeOH cooled to 0°C. The mixture was stirred at 3°C for 10 hours and then distilled under reduced pressure at 40°C. The residue was cooled to 0°C, and the solution was adjusted to pH 11 by adding 10N NaOH. The basic solution was washed with Et_2O and EtOAc, respectively, and then acidified to pH 6 by addition of 6N HCl and subsequently to pH 3 with 1.2N HCl. The water layer was extracted with a mixed solvent of *t*-BuOH and EtOAc (1:1.5) and washed with saturated aqueous NaCl, dried with $MgSO_4$, and then concentrated in vacuo; **1** (2.8 g, 88%) as a colorless viscous oily product was obtained. ^{31}P NMR: δ 5.57; ^{1}H NMR: δ 1.14 (m, 12H, $(CH_3)_2C$), 3.04 (m, 2H, CH_2), 3.90 (m, 1H, N–CH), 4.29 (m, 2H, O–CH) 5.24 (t, 1H, NH, disappeared in D_2O), 7.38 (*s*, 1H,



SCHEME 4

Im-5'-H), 9.0 (d, 1H, Im-2'-H), 11.00 (br, 2H, CO_2H and Im-NH, disappeared in D_2O); ^{13}C NMR: δ 21.94 (d, J = 5.86 Hz, $(CH_3)_2C$), 27.56 (d, J = 5.86 Hz, CH_2), 54.18 (s, N–C), 72.12 (d, J = 5.86 Hz, O–CH), 116.42 (d, J = 9 Hz, Im-5'-C), 128 (s, Im-4'-C), 132.28 (s, Im-2'-C), 174 (d, J = 5.86 Hz, CO_2); FAB-MS: m/z 320 (M + H^+, 84%); HRFAB-MS: m/z 320.3052 ($C_{12}H_{23}N_3PO_5$ requires 320.3046); IR(KBr): 3500, 2500, 1675, 1200, 980.

Preparation of N^{α}-(Diisopropyloxyphosphinyl)histidine methyl ester **4**

To an ice-salt cooled solution of histidine methyl ester hydrochloride (1.2 g, 5 mmol) in Et_3N (2.8 ml) and MeOH (10 ml) was added dropwise a mixture of diisopropyl phosphite (1 mL, 6 mol) and CCl_4 (10 mL), and then the mixture was stirred at 0°C for 10 hours. The mixture was evaporated in vacuo, the residue mixed with EtOAc, and then the organic layer extracted with 1.2N HCl solution (3 × 20 mL). Then the water layer was adjusted to pH 8–9 by adding saturated $NaHCO_3$ and extracted with EtOAc (3 × 20 mL). The combined extracts were washed with saturated aqueous NaCl and dried ($MgSO_4$) and concentrated in vacuo. A colorless viscous oily product was obtained (1.5 g, 90%). ^{31}P MNR: δ 5.09; ^1H NMR: δ 1.3 (d, 1H, J = 5 Hz, $(CH_3)_2C$), 3.1 (d, 2H, J = 5 Hz, CH_2), 3.73 (s, 3H, OCH_3), 3.88 (t, 1H, J = 12 Hz, NH, disappeared in D_2O), 4.1 (m, 1H, N–CH), 4.57 (m, 2H, O–CH), 6.82 (s, 1H, Im-5'-H), 7.55 (s, 1H Im-2'-H) 8.29 (bs, 1H, Im-NH, disappeared in D_2O); ^{13}C NMR: δ 23.63 (d, J = 4.4 Hz, $(CH_3)_2C$), 31.32 (d, J = 5.9 Hz, CH_2), 52.09 (s, OCH_3), 54.54 (s, N–C), 71.24 (d, J = 4.4 Hz, CH–O), 118.37 (s, 5'-C), 131.83 (s, 4'-C), 135.04 (s, 2'-C), 172.5 (d, J = 5.86 Hz, CO_2); FAB-MS: m/z 334 (M + H^+, 68%). Anal. calcd for $C_{13}H_{24}N_3PO_5$: C, 46.85; H, 7.20; N, 12.61. Found: C, 45.08; H, 7.20; N, 12.61. IR: 3300, 2950, 1705, 1400, 1200, 1000, 780 cm^{-1}.

Preparation of N^{α}, N^{Im}-Bis-(Diisopropyloxyphosphinyl) Histidine Methyl Ester **5**

To a stirred solution of L-histidine methyl ester hydrochloride (1.2 g, 5 mmol) in Et_3N (3.5 mol) and MeOH (10 mL) at −10°C was added dropwise a mixture of diisopropyl phosphite (2 mL, 12 mmol) and CCl_4 (10 mL) within 1 hour. The mixture was stirred at −5–0°C for 10 hours and then evaporated under reduced pressure at the temperature below 40°C. The residue was dissolved in a saturated aqueous NaCl (10 mL), and this water layer was washed with petroleum ether (3 × 10 mL) and then extracted by Et_2O (3 × 20). The combined Et_2O extract was washed with saturated NaCl, dried ($MgSO_4$), and concentrated at 40°C in vacuo. A crude crystalline product was obtained, which was placed in the freezer and then recrystallized from Et_2O and petroleum ether (1:10) giving a colorless crystalline product MP: 64–65°C (2.15 g, 87%). ^{31}P NMR: δ 5.63, −8.42; ^1H NMR: δ 1.27 (m, 24H, $(CH_3)_2C$), 3.04 (d, 2H, J = 6 Hz, CH_2) 3.64 (m, 1H, NH, disappeared in D_2O), 3.70 (s, 3H, OCH_3), 4.2 (m, 1H, N–CH), 4.65 (m, 4H, CHO), 6.90 (s, 1H, Im-5'-H), 7.8 (d, 1H, J = 4 Hz, Im-2'-H); ^{13}C NMR: δ 22.92, 23.34 (d, J = 4.4 Hz, $(CH_3)_2C$), 32.18 (d, J = 5.86 Hz, CH_2), 51.61 (s, OCH_3), 53.72 (s, N–C) 70.54, 73.96 (d, J = 4.4 Hz, CH–O), 116.29 (d, J = 7.3 Hz, Im-5'-c), 138.9 (d, J = 5.86 Hz, Im-4'-C), 139.54 (s, Im-2'-C), 172.57 (d, J = 4.4 Hz, CO_2), FAB-MS: m/z 498 (M + H^+, 67%). Anal. calcd. for $C_{19}H_{37}N_3P_2O_8$: C, 45.88; H, 7.44; N, 8.45. Found: C, 45.55; H, 7.61; N, 8.45; IR(kBr) 3329, 2978, 1744, 1654, 1568, 1461, 1384, 1282, 1000 cm^1.

Ester Exchange Reaction of N^{α}-(Diisoproploryphosphinyl) Histidine **1**

A solution of **1** (0.319 g, 1 mmol) in BuOH (10 mL) was kept at 40°C for 24 hours. There were four products formed as checked by the ^{31}P NMR and FAB-MS in Scheme 1 and Figure 1.

Ester Exchange Reaction of N^{α}-(Diisoprophloxyphosphoryl) Histidine Methyl Ester **4**

A solution of **4** (0.333 g, 1 mmol) in BuOH (10 mL) and HOAc (0.5 mL) was kept at 40°C for 24 hours. It was found that the HOAc could not catalyze the ester exchange reaction of **4** as checked by ^{31}P NMR and FAB-MS.

Phosphoryl Transfer Reaction of N^{α}, N^{Im}-Bis-(Diisopropyloxyphosphinyl) Histidine Methyl Ester **5**

The N^{α}, N^{Im}-bis-(diisopropyloxyphosphoryl) histidine methyl ester **5** (1 g, 2 mmol) was dissolved in 10 mL 1 MeOH. It was kept at 40°C for 24 hours, the reaction products were checked by ^{31}P NMR and FAB-MS, and it was found that compound **4** was produced. Column chromatography on silica gel using $CHCl_3$ as eluant afforded **4** (0.6 g, 90%).

ACKNOWLEDGMENTS

This work was supported by the National Chinese Natural Science Foundation, the National Science and Technology Committee of China, and Tsinghua University.

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