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STUDIES ON PHOSPHOSERINE AND PHOSPHOTHREONINE DERIVATIVES: N-DIISOPROPYLOXYPHOSPHORYL-SERINE AND -THREONINE IN ALCOHOLIC MEDIA

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SUMMARY: N-diisopropyloxyphosphoryl-serine and -threonine in alcoholic media were found to proceed phosphoric ester exchanges, with the concomitant transfer reaction of the phosphoryl group from the amino to the hydroxyl. The participation of the amino, the hydroxyl and the carboxyl groups in the molecules is essential for these reactions to occur.

It has been established that the activities of many enzymes are regulated through phosphorylation-dephosphorylation at a serine residue which, in most cases, is flanked by both basic and acidic amino acid residues.[1-3] To understand the chemistry of the phosphorylation-dephosphorylation processes, it is of great interest to carry out the studies on the properties of phosphoserines.

In our syntheses of N-diisopropyloxyphosphoryl(DIPP)-amino acids, it is interesting to find that N-DIPP-serine (1a) exhibits some unusual properties different from most of other N-DIPP-amino acids.[4] When compound 1a was kept warmed in 1-butanol at 40°C for 2 hours, it was found by fast atom bombardment mass spectroscopy (FAB-MS) that a small amount of a product was formed which gave a peak at m/z 284 (MH^+ of 1a + 14, 13%). The ^{31}P-NMR analysis for this solution gave two signals, one at 5.7 ppm which is the resonance of the material 1a and the other at -3.0 ppm (Table 1). It seems that the latter NMR signal could come from the resonance of the formed product which has the molecular structure of O-butyloxyisopropyloxyphosphoryl-serine (2a). When the warming time of this solution was prolonged to 15 hours, it was found by FAB-MS that in addition to the above peak of 2a which increased to 39%, a second product with a peak at m/z 298 (MH^+ of 1a + 28, 12%) was formed. ^{31}P-NMR showed a new resonance at -1.9 ppm corresponding to this new product which should be proposed as O-dibutyloxyphosphoryl-serine (3a). The structural assignments of the above two products are based on both FAB-MS and ^{31}P-NMR analyses, and it seems reasonable to exclude the formation of other species such as N-butyloxyisopropyloxyphosphoryl-serine and N-dibutyloxyphosphoryl-serine since the ^{31}P-NMR chemical shifts at -3.0 ppm and -1.9 ppm of these two products are typical of phosphoryl triesters, but not phosphoramidates.[5] The change of the media from 1-butanol to 1-hexanol gave analogous results for compound 1a as given in Table 1.

In order to understand whether the above results are of generality for other hydroxy amino acids, we extended the above experiments to a homologue,

Table 1 Relative Peak Intensity of FAB-MS and Chemical Shifts of ^{31}P-NMR for N-DIPP-Amino Acids Which were Warmed in Alcohol at 40°C

Entry	Compound [a]	Media (pH=3)	Time (hr)	FAB-MS(%) [b] M+1	(M+1)+14 or+42	(M+1)+28 or+84	^{31}P-NMR(ppm) [c,d] 1a-h	2a-h	3a-h
1a	N-DIPP-Ser M=269	1-butanol	0	100	0	0	5.7	—	—
			2	87	13	0	5.7	-3.0	—
			15	49	39	12	5.6	-2.9	-1.9
		1-hexanol	0	100	0	0	5.7	—	—
			15	53	36	11	5.7	-2.9	-1.9
1b	N-DIPP-Thr M=283	1-butanol	0	100	0	0	6.1	—	—
			3	83	17	0	6.1	-2.4	—
			15	50	41	9	6.2	-2.8	-1.9
		1-hexanol	0	100	0	0	6.1	—	—
			15	42	50	8	6.2	-2.8	-1.9
1c	N-DIPP-Pro M=279	1-butanol	0	100	0	0	4.4	—	—
			15	100	0	0	4.4	—	—
1d	N-DIPP-Gly M=239	1-butanol	0	100	0	0	7.0	—	—
			15	100	0	0	7.0	—	—
1e	N-DIPPSerOMe M=283	1-butanol +HOAc	0	100	0	0	5.6	—	—
			15	100	0	0	5.6	—	—
1f	3-DIPP-HPA M=254	1-hexanol	0	100	0	0	-3.3	—	—
			15	100	0	0	-3.5	—	—
1g	$(i\text{-PrO})_2\overset{O}{\overset{\|}{P}}OEt$ M=210	1-hexanol +HOAc	0	100	0	0	-2.8	—	—
			15	100	0	0	-2.8	—	—
1h	N-DIPP-Hyp M=295	1-butanol	0	100	0	0	4.5	—	—
			15	100	0	0	4.5	—	—

a). Amino acids are of the L configuration unless otherwise indicated. Abbreviations used are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature. HPA=3-hydroxypropionic acid; Hyp=trans-4-hydroxy-L-proline.

b). The (M+1)+14 and (M+1)+28 peaks refer to those obtained with 1-butanol as the media, and the (M+1)+42 and (M+1)+84 peaks refer to those obtained with 1-hexanol as the media.

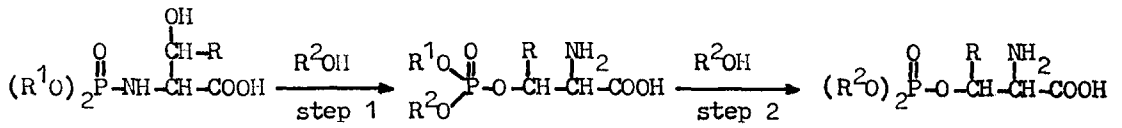
c). Compounds 2a-h and 3a-h correspond to those with (M+1)+14 or +42 and (M+1)+28 or +84 peaks in FAB-MS spectra respectively.

d). ^{31}P-NMR spectra were measured using 85% H_3PO_4 as external reference.

N-DIPP-threonine (1b). After compound 1b was heated in 1-butanol or 1-hexanol at 40°C for different duration, similar results were observed as indicated in Table 1.

From the above results, it is possible to propose that the products 2a-b and 2a'-b' be derived from the phosphoric ester exchanges of the phosphoramidates 1a-b with the media together with the transfer reaction of the phosphoryl group from the amino to the hydroxyl (step 1 in scheme 1), and the products 3a-b and 3a'-b' result from further phosphoric ester exchanges of the phosphoryl triesters 2a-b and 2a'-b' with the media (step 2 in scheme 1).

SCHEME 1



1a-b 2a-b, 2a'-b' 3a-b, 3a'-b'

R^1=isopropyl
1a: R=H, N-DIPP-Ser
1b: R=CH_3, N-DIPP-Thr

2a: R=H, R^2=n-butyl
2b: R=CH_3, R^2=n-butyl
2a': R=H, R^2=n-hexyl
2b': R=CH_3, R^2=n-hexyl

3a: R=H, R^2=n-butyl
3b: R=CH_3, R^2=n-butyl
3a': R=H, R^2=n-hexyl
3b': R=CH_3, R^2=n-hexyl

However, the N-DIPP derivatives of nonhydroxylated amino acids such as N-DIPP-glycine (1c) and N-DIPP-proline (1d), and an amino acid ester devoid of a free carboxyl group such as N-DIPP-serine methyl ester (1e) did not undergo ester exchanges under the same conditions (Table 1). This indicates that the hydroxyl and carboxyl groups in the molecules 1a-b are necessary for the reaction in step 1 to take place, and conclusion can be drawn that the N→O migration of the phosphoryl group in step 1 should occur intramolecularly since no phosphoryl triesters were found by ^{31}P-NMR spectroscopy in the heated alcoholic solutions of 1c-d. The studies on two model compounds lacking the amino group or both the amino and carboxyl groups, namely, 3-(diisopropyloxyphosphoryloxy)-propionic acid (1f)[6] and ethyl diisopropyl phosphate (1g) revealed that both the amino and carboxyl groups are indispensable for the exchange reaction in step 2 to occur, because 1f-g were unchanged under the same treatment (Table 1). From these results, it seems that the reaction intermediates in step 1 and step 2 might involve the participation of the three groups of the amino, the hydroxyl and the carboxyl in a molecule, and the attack of an alcohol at these intermediates will result in the formation of compounds 2a-b, 2a'-b', 3a-b and 3a'-b'.

Although N-DIPP-trans-4-hydroxy-L-proline (1h) which has a hydroxyl group

in the molecule can meet the above requirement, no rearrangement took place after it was warmed at 40°C in 1-butanol for 15 hours. This fact can be accounted for from the stereochemical point of view, since the hydroxyl group and the carboxyl group in this molecule are transoid on its rigid ring skeleton, and hence it is impossible for 1h to form any intermediate which involves the participation of all three groups. It is therefore concluded that a suitable configuration in addition to the presence of all three functional groups in a system is also required for the phosphoric ester exchange and phosphoryl transfer reactions of a N-phosphoryl-amino acid in alcoholic media. The result for compound 1h further confirms that the phosphoryl transfer reaction should be an intramolecular rather than intermolecular one.

It is worthwhile to mention that the N→O migration of the phosphoryl seems irreversible because no signals in the region of 0-20 ppm which are typical of phosphoramidates were observed in the ^{31}P-NMR spectra after 1a-b were heated at 40°C in alcohol for 48 hours, and this means that the starting materials 1a-b were completely transformed into the phosphoryl triesters within this duration.

Our preliminary results illustrate the distinguished reactivities of N-phosphoryl-serine and -threonine, which are not possessed by other simple amino acids. The studies on the mechanisms of these reactions in more details are under progress in our laboratory.

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REFERENCES AND NOTES:

1. P. Cohen Ed., Recently Discovered Systems of Enzyme Regulation by Reversible Phosphorylation, Elsevier/North Holland, New York, 1980.
2. O.M. Rosen and E.G. Krebs, Eds., Protein Phosphorylation, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1981.
3. G. Taborsky, Adv. Protein Chem., 28, 1(1974).
4. All N-DIPP-amino acids mentioned in this paper were prepared by an improved phosphorylation method directly from diisopropyl phosphite and amino acids in aqueous solution, and they were characterized by ^{31}P-, ^{1}H- and ^{13}C-NMR, FAB-MS and elemental analysis (Yu-Fen Zhao et al., to be published).
5. D.G. Gorenstein, Progr. NMR Spectrosc., 16, 1(1983).
6. Compound 1f was prepared from the monophosphorylation of propane-1,3-diol with diisopropyl phosphite, followed by oxidation with potassium permanganate solution.

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