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MOLECULAR ORBITAL STUDIES ON THE CONFORMATION OF THE PHOSPHOPANTETHEINE MOIETY OF COENZYME A

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Summary

Torsion angles and notations for phosphopantetheine.

Conformational study on phosphopantetheine shows that this compound has an intrinsic tendency to adopt a multitude of conformations which contain hydrogen bonds involving the sulphydryl, hydroxyl, carbonyl and amide groups. The sulphydryl group may form a hydrogen bond with the C(7′) = O carbonyl group, the latter being also involved in hydrogen bonding with the N(4′)-H group. All these hydrogen bondings occur for different conformations around the backbone. The N(7′)-H and C(4′) = O groups are not involved in hydrogen bonding. It is also found that a strong interaction occurs between N(4′)-H and O-3′ which is responsible for a rigid conformation around the C(3′)-C(4′) and C(3′)-O(3′) bonds. As far as the phosphate group is concerned the results show that this group may interact with the O(3′)-H hydroxyl group to form hydrogen-bonded rings of different sizes. A six-membered ring formed by hydrogen bonding between O(3′)-H and O-1′ appears more favorable than an eight-membered ring involving an anionic oxygen instead of an ester oxygen related to the phosphate group.

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Introduction

The solution conformation of coenzyme A (CoA) and its derivatives has been the subject of extensive investigations using nuclear magnetic resonance spectroscopy (NMR) [1—5]. These studies have indicated the possible conformations of the adenosine and the phosphopantetheine moieties of CoA and shown the existence of linear and folded conformations for the entire molecule. Nevertheless the conformations around some of the bonds (e.g. C(1′)-C(2′), C(3′)-C(4′), C(5′)-N, C(6′)-C(7′), C(8′)-N, C(9′)-S) relative to the pantetheine part of the CoA have not yet been determined by experiment. No X-ray crystal structure of CoA has yet been determined either.

The aim of this paper is the determination of the possible conformations of the phosphoantetheine part of CoA by quantum mechanical calculations, in the absence of any consideration of the solvent effect. The torsions around nearly all the bonds have been explored. The results obtained are compared with those given by the NMR expereriments.

Procedure

The method employed in this investigation is the PCILO (Perturbative Configuration Interaction using Localized Orbitals) method, the details of which can be found in the original papers [6—9]. The detailed program may now be obtained from Q.C.P.E. (Quantum Chemistry Program Exchange) at the Chemistry Department of Indiana University, Bloomington, Ind. U.S.A.

The structure of phosphopantetheine and the torsion angles which have been considered are presented in Fig. 1. A trans configuration has been assumed around the peptide bonds, this configuration being the most stable one for the peptide group [10]. A gauche conformation has been considered around the P-O(1′) bond (Φ(P-O(1′) = 300°), such a conformation being preferred in nucleic acids [11]. The terminal hydroxyl of the phosphate group has been oriented in a trans configuration with respect to P-O(1′) in order to prevent any hydrogen bonding of this hydroxyl group with the remaining part of the molecule, in view of the fact that such a possibility does not exist in the pantetheine part of the CoA.

The conventions adopted for the torsion angles are those given by Sundaralingam [11]. The zero values of the torsion angles correspond to the cis planar arrangement of the bonds adjacent to the rotating bond and the positive direction is a right-handed rotation when looking along the rotating bond, the far bond rotates clockwise relative to the near bond. The computations relative to

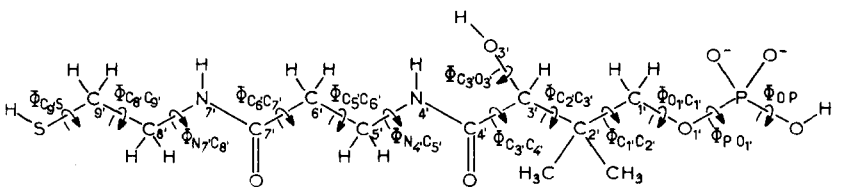

Fig. 1. Torsion angles and notations for phosphopantetheine.

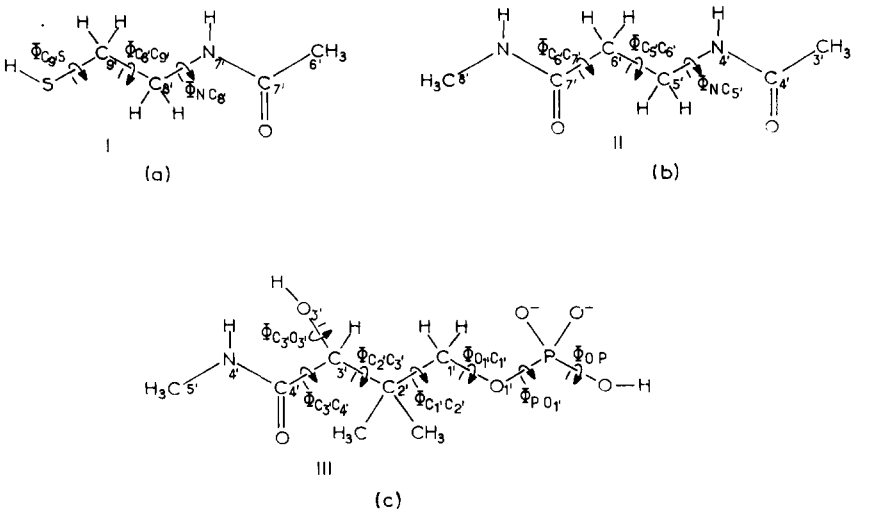

Fig. 2. The different portions of phosphopantetheine considered in this article.

the conformational energy maps and curves have been carried out in 30° increments of the angles.

Because of the high computational time required to perform a thorough conformational study on phosphopantetheine which has a large size and a large number of rotational bonds, we carried out this research in four steps as follows: in the first three steps we study successively the conformations of three portions of the phosphopantetheine (I, II and III) indicated respectively in Fig. 2a, b and c; in the fourth step we analyze the conformations of phosphopante-

TABLE I

STANDARD BOND LENGTHS AND ANGLES ADOPTED FOR THE PHOSPHOPANTETHEINE

The notation is indicated in Fig. 1.

Bond lengths (in Å)		Valence angles (in degrees)	
C—S	1.77	S—C—C	117.000
C—C	1.52	$C(H_2)$—$C(H_2)$—N	111.00
$C(H_2)$—N	1.46	$C(H_2)$—C(=O)—N	116.00
C(=O)—N	1.32	C—N—C	122.00
$C(H_2)$—O	1.44	C(7′)—C(6′)—C(5′)	114.00
C(H)—O	1.48	C(4′)—C(3′)—C(2′)	114.00
C=O	1.24	C(3′)—C(2′)—C(1′)	109.47
P—O	1.60	$C(H_3$—C—$C(H_3)$	109.47
P—O^-	1.49	C—C—$C(H_3)$	109.47
S—H	1.34	C(2′)—C(1′)—O(1′)	109.47
C—H	1.09	C—O—P	121.10
N—H	1.09	O—P—O	101.30
O—H	1.09	O—P—O^-	109.06
		N—C—O	123.50
		C(4′)—C(3′)—O(3′)	108.00
		C—S—H	96.50
		C—O—H	109.47
		P—O—H	105.00

theine taking into consideration the local conformations obtained in the previous steps.

Standard values have been used for the geometry of phosphopantetheine. These values are given in Table I.

Results and Discussion

(A) Conformation of portion I of phosphopantetheine

Conformational energy maps as a function of Φ(C(9′)-S) and Φ(N(7′)-C(8′)) have been built for three preselected values of Φ(C(8′)-C(9′)) corresponding to staggered orientations of the two methylene groups: Φ(C(8′)-C(9′)) = 60°, 180° and 300°. The global and local minima obtained in these maps are indicated in Table II. The first two stable minima correspond to gauche conformations around C(8′)-C(9′) with the thiol group forming a hydrogen bond with the carbonyl group. The remaining minima, which correspond to more extended conformations, are less than 2 or 3 kcal/mol above the global minimum. The differences in energies between the various minima are thus sufficiently low to permit the corresponding conformations to occur frequently. This conclusion is in agreement with the experimental evidence [1,2] that the cysteamine portion of CoA has a relatively high mobility.

(B) Conformation of portion II of phosphopantetheine

The conformational energy maps of portion II of phosphopantetheine (Fig. 2b) as a function of Φ(C(6′)-C(7′)) and Φ(N(4′)-C(5′)) have been constructed for the three staggered conformations around the C(5′)-C(6′) bond. The global and local minima obtained in these conformational maps are summed up in Table III. It can be observed that the gauche conformations around the C(5′)-C(6′) bond are stabilized by the formation of a hydrogen bond between C(7′) = O and H-N(4′). The trans conformation around the C(5′)-C(6′) bond which does not permit the formation of hydrogen bonds between the carbonyl and amino groups corresponds to energies higher by about 11 kcal/mol. Such a preference for gauche conformations over the trans one around the methylene

TABLE II

CONFORMATIONAL ENERGY MINIMA OBTAINED FOR THE PORTION I OF PHOSPHOPANTETHEINE

(See Fig. 2a.) The relative energies are indicated with respect to the global minimum taken as energy zero.

Torsion angles			*E* (kcal/mol)	Interactions
Φ (C(8′)—C(9′))	Φ (C(9′)—S)	Φ (C(8′)—N)		
60	0	270	0.	S—H····O=C
300	0	90	0.6	S—H····O=C
300	180	210	1.7	—
60	180	180	1.8	—
180	60	180	2.7	—
180	300	180	2.7	—
180	180	180	2.8	

TABLE III

CONFORMATIONAL ENERGY MINIMA OBTAINED FOR THE PORTION II OF PHOSPHOPANTETHEINE

(See Fig. 2b.) The relative energies are indicated with respect to the global minimum taken as energy zero.

Torsion angles			E (kcal/mol)	Interactions
Φ (C(5′)—C(6′))	Φ (C(6′)—C(7′)	Φ (N—C(5′))		
60	120	180	0.0	C(7′)=O····H—N(4′)
300	210	210	0.0	C(7′)=O····H—N(4′)
300	270	180	0.6	C(7′)=O····H—N(4′)
180	60	180	11.1	—
180	300	180	11.1	—
180	180	180	11.2	—

group is in agreement with the solution conformations established for the β-alanyl moiety of CoA by Wilson et al. [5] by NMR experiments. These authors report a highly favoured gauche conformation around the C(5′)-C(6′) bond for the aforementioned moiety consistent with a hydrogen bonding hypothesis. On the other hand, the results reported by Lee and Sarma [1,2] indicate that all the three staggered rotamers around the C(5′)-C(6′) bond occur in equal proportions.

(C) Conformation of portion III of phosphopantetheine

It is expected that the preferred conformations of this portion of phosphopantetheine (Fig. 2c) should depend to a large extent on the possible interactions between the peptide, hydroxyl and phosphate groups. We first analyzed the interactions between the peptide and hydroxyl groups by constructing a conformational energy map as a function of Φ(C(3′)-C(4′) and Φ(C(3′)-O(3′) for an extended conformation of the remaining part of the molecule. The torsion angles Φ(C(2′)-C(3′), Φ(C(1′)-C(2′), Φ(O(1′)-C(1′), Φ(O-P) are thus fixed at 180°, with however Φ(P-O(1′) = 300°. The conformational energy map obtainned is presented in Fig. 3. It can be seen that it contains only one minimum at Φ(C(3′)-O(3′)) = 180° and Φ(C3′)-C(4′)) = 240°. This minimum corresponds to a hydrogen bond between the amino and the hydroxyl groups forming a five-membered ring. The relative energy increases rapidly with a departure from this global minimum. The conformation corresponding to this minimum can thus be considered as more or less rigid within 30° variation of the torsion angles. We adopted this conformation as the preferred one in the remaining part of our study.

We next analyzed the conformational possibilities around the O(1′)-C(1′) bond for all the combinations of staggered arrangements around the C(1′)-C(2′) and C(2′)-C(3′) bonds. The conformational energy curves as a function of Φ(O(1′)-C(1′)) for all these combinations are presented in Fig. 4. It can be seen that the allowed conformations occur for an angle of Φ(O(1′)-C(1′) between 90° and 270°. The energies are very high outside these limits indicating steric hindrance between the phosphate group and the two methyl or the hydroxyl groups. All the minima obtained are situated between Φ(O(1′)-C(1′)) = 120°

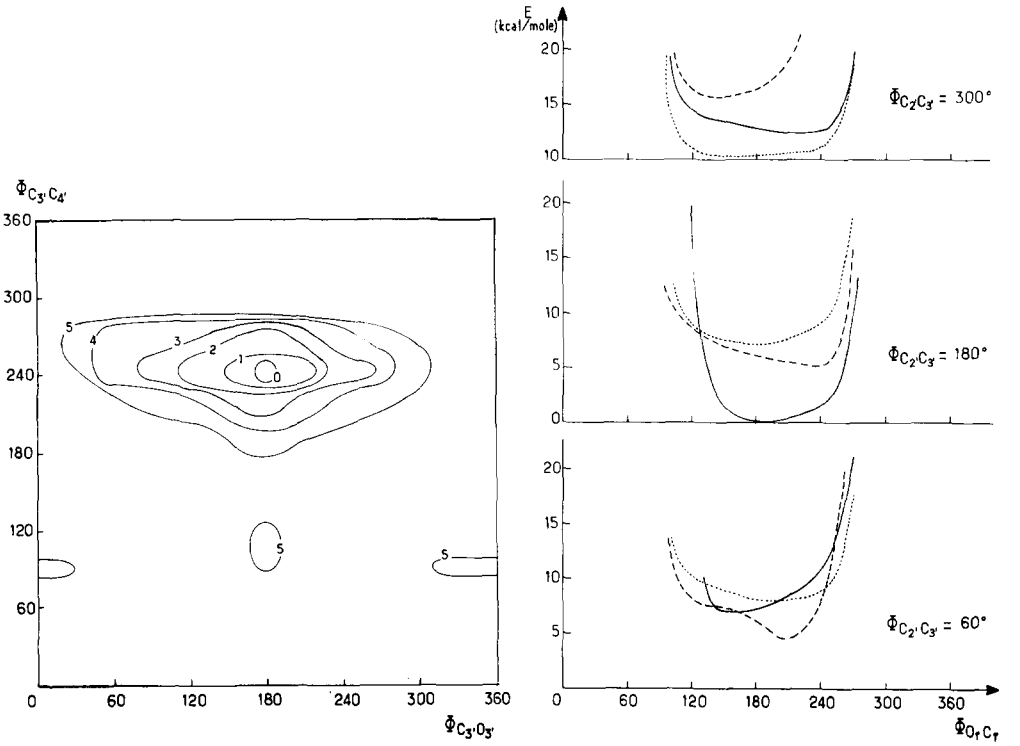

Fig. 3. Conformational energy map as a function of Φ(C(3′)-O(3′)) and Φ(C(3′)-C(4′)) for the portion III of phosphopantetheine (Fig. 2c), with Φ(C(2′)-C(3′)) = Φ(C(1′)-C(2′)) = Φ(O(1′)-C(1′)) = Φ(O-P) = 180° and Φ(P-O(1′) = 300°. The global minimum has been taken as energy zero.

Fig. 4. Conformational energy curves as a function of Φ(O(1′)-C(1′)) for all the combinations of staggered conformations (60°, 180° and 300°) around C(1′)-C(2′) and C(2′)-C(3′). The curves are: ------ for Φ(C(1′)-C(2′)) = 60°, · · · · · · for Φ(C(1′)-C(2′)) = 180° and ——— for Φ(C(1′)-C(2′)) = 300°, the remaining angles are fixed at Φ(C(3′)-C(4′)) = 240°, Φ(C(3′)-O(3′)) = 180°, Φ(P-O(1′)) = 300° and Φ(O-P) = 180°. The global minimum has been taken as energy zero.

and 240°. The global minimum corresponds to Φ(O(1′)-C(1′)) = 180° with Φ(C(1′)-C(2′)) = 300° and Φ(C(2′)-C(3′) = 180°. All the other minima have a relative energy of more than 4 kcal/mol above the energy minimum.

The choice of staggered conformations corresponding exactly to 60°, 180° and 300° for Φ(C(2′)-C(3′)) and Φ(C(1′)-C(2′)) could hinder the search of specific interactions between the phosphate group and the peptide or hydroxyl groups. We have therefore extended the conformational analysis of portion III (Fig. 2c) by constructing conformational energy maps as a function of Φ(C(2′)-C(3′)) and Φ(C(1′)-C(2′)) for different values of Φ(P-O(1′)) varying from 90° to 270° with a variational increment of 30°. The remaining torsion angles are fixed at the same values as in the preceding study. The conformations corresponding to the different minima are indicated with their relative energies in Table IV.

The most stable conformations obtained correspond on the one hand to a six-membered ring formed by a hydrogen bond between O(3′)-H and O(1′), and on the other to an eight-membered ring formed by a hydrogen bond between

TABLE IV

CONFORMATIONAL ENERGY MINIMA OBTAINED FOR THE PORTION III OF PHOSPHOPANTETHEINE

(See Fig. 2c.) Minima obtained with Φ(C(3')-C(4')) = 240°, Φ(C(3')-O(3')) = 180°, Φ(P-O(1')) = 300° and Φ(O-P) = 180°. The relative energies are indicated with respect to the global minimum taken as energy zero.

Torsion angles			E (kcal/mol)	Interactions
Φ (C(2')—C(3'))	Φ (C(1')—C(2'))	Φ (O(1')—C(1'))		
180	200	180	0	O(3')—H···O(1')—P
180	30	240	0	O(3')—H···$^-$O—P
180	300	150	1.6	—
150	60	210	2.4	—

O(3')-H and $^-$O-P. These two conformations correspond to a value of 180° for Φ(C(2')-C(3'), and to values of 300° and 30° respectively for Φ(C(1')-C(2')); the Φ(O(1')-C(1')) torsion angle corresponds to a value of 180° in the six-membered ring structure, but adopts a larger value, of about 240°, in the eight-membered ring structure.

The results presented in this section are in agreement with those reported by Lee and Sarma [1,2] for the solution conformations of the free phosphopantetheine. These authors suggest, on the basis of their NMR results, an intramolecular interaction between the double ionized phosphate and the O(3')-H group and advance the hypothesis that the O(3')-H is involved in hydrogen bonding with one of the phosphate oxygens to form an eight-membered ring. Our results confirm this hypothesis, showing however that the occurence of a structure consisting of a five-membered ring formed by hydrogen bonding between O(3')-H and O-1' is also highly probable.

(D) Conformations of phosphoantetheine

Finally, we have explored all the conformations of the phosphopantetheine which can be generated by combining the different stable conformations obtained for portions I, II and III of this molecule (Fig. 2) in the preceding sections. The relative energies of the most stable conformations of phospopantetheine obtained in this way are indicated up to 1.4 kcal/mol in Table V. The first two conformations, which are the preferred ones, correspond to a structure which is depicted in Fig. 5. It presents a six-membered ring formed by

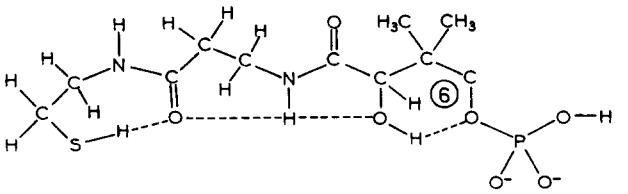

Fig. 5. Molecular structure of phosphopantetheine corresponding to the conformations 1 and 2 of Table V.

TABLE V

RELATIVE PCILO ENERGIES OF THE PREFERRED CONFORMATIONS OF PHOSPHOPANTETHEINE

The values of some of the torsion angels are $\Phi(C(9')\text{-}S) = 0^\circ$, $\Phi(C(3')\text{-}C(4')) = 240^\circ$, $\Phi(C(3')\text{-}O(3')) = 180^\circ$. The relative energies are indicated with respect to the global minimum taken as energy zero.

No.	Torsion angles								
	Φ (O(1′)—C(1′))	Φ (C(1′)—C(2′))	Φ (C(2′)—C(3′))	Φ(N(4′)—C(5′))	Φ (C(5′)—C(6′))	Φ (C(6′)—C(7′))	Φ (N(7′)—C(8′))	Φ (C(8′)—C(9′))	*E* (kcal/mol)
1	180	300	160	210	300	210	270	60	0
2	180	300	180	210	300	210	90	300	0
3	240	30	180	180	60	120	270	60	0.8
4	240	30	180	180	60	120	90	300	0.9
5	180	300	180	180	60	120	270	60	0.9
6	180	300	180	180	60	120	90	300	1.0
7	240	30	180	210	300	210	90	300	1.1
8	240	30	180	210	300	210	270	60	1.2
9	240	300	180	210	300	210	270	60	1.3
10	240	300	180	210	300	210	90	300	1.4

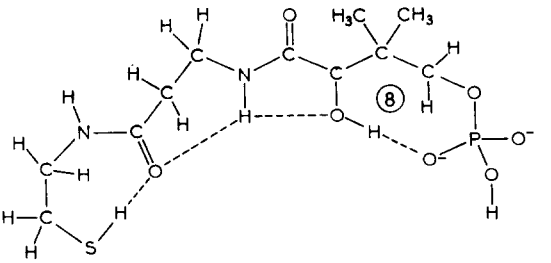

Fig. 6. Molecular structure of phosphopantetheine corresponding to conformation 3 of Table V.

hydrogen bonding between O(3′)-H and O-1′, and three other rings which involve the participation of the O(3′)-H, N(4′)-H, C(7′) = O and S-H groups in hydrogen bonding. The next most stable conformation which is 0.8 kcal/mol above the preceding ones (No 3 in Table V) is depicted in Fig. 6. This structure differs from that indicated in Fig. 5 by an eight-membered ring instead of six formed by hydrogen bonding between O(3′)-H and one anionic oxygen of the phosphate group. It is worthwile to indicate that the conformational study performed on protion III of phosphopantetheine (Fig. 3c) in the preceding section has shown that these two types of rings have equal probability of occurence, and that the consideration of the whole molecule of phosphopantetheine in the present section gives a different result; the six-membered ring being preferred to the eight-membered one. The remaining conformations indicated in Table V which have altogether similar structures as those indicated in Figs. 5 and 6 should have an appreciable probability of occurrence.

The theoretical calculations indicate (see Table V) that there are two kinds of preferred conformations around O(1′)-C(1′) corresponding respectively to Φ(O(1′)-C(1′) = 180°. (trans conformation) and Φ(O(1′)-C(1.)) = 240°. The conformations corresponding to Φ(O(1′)-C(1′) = 240° present an interaction between O(3′)-H and the anionic oxygens of the phosphate group. It seems conceivable that the protonation of the phosphate group should exclude such an interaction described above and consequently the trans conformation should be predominant. Our results seem in agreement with the NMR results on ionized phsophopantetheine given by Lee and Sarma [1,2] which indicate that these compounds adopt in solution both trans and gauche conformations and that protonation of the phosphate group increases the population of the trans conformation.

An interesting feature of the results is the indication that the proton of the N(4′)-H amino group is simultaneously involved in two hydrogen bonding. Such a possibility seems not to be excluded by the quantum mechanical calculations.

Conclusions

The conformational study on phosphopantetheine shows that this compund has an intrinsic tendancy to adopt a multitude of conformations which present hydrogen bonds involving the sulphydryl, hydroxyl, carbonyl and amide groups. The sulphydryl group may form a hydrogen bond with the C(7′)

= O carbonyl group, the latter to be also involved in hydrogen bonding with the N(4′)-H group. All these hydrogen bondings occur for different conformations around the backbone. The N(7′)-H and C(4′) = O groups are not involved in hydrogen bonding. It is also found that a strong interaction occurs between N(4′)-H and O(3′) which is responsible for a rigid conformation around the C(3′)-C(4′) and O(3′)-H bonds. As far as the phosphate group is concerned the results show that this group may interact with the O(3′)-H hydroxyl group to form hydrogen bonded rings of different size. A six-membered ring formed by hydrogen bonding between O(3′)-H and O(1′) appears more favorable than an eight membered ring involving an anionic oxygen instead of an ester oxygen related to the phosphate group.

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