COMPLETE ANALYSIS OF THE FAST FOURIER TRANSFORMED ^{1}HNMR SPECTRA OF COENZYME A, ACETYL COENZYME A AND 3′5′ADP AND THEIR SOLUTION CONFORMATION

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1. Introduction

'Coenzyme A is the most prominent acyl group transfer coenzymes in living systems. Its universal occurrence, the large number of enzymatic reactions in which it is involved, and the variety of reaction types in which its derivatives are concerned testify to its importance' [1]. Since its discovery and elucidation of its primary structure [2,3], nothing is known up to the present time, with respect to its conformation. In the present paper, we present for the first time, the complete analysis of the complex ^{1}HNMR Fourier transformed spectra of coenzyme A (see fig. 1 for structure) and acetyl coenzyme A. Also presented is the complete analysis of the 3′5′ADP spectra which is a component of these coenzymes as well as that of RNA. The NMR data in turn is used to arrive at their time averaged dynamic conformation in aqueous solutions.

2. Materials and methods

Spectra of 3′5′ADP (0.1 M), CoASH (0.05 M) and CoAS-CO-CH_3 (0.025 M), all commercial products, were obtained in a 100 MHz Fast Fourier transform NMR system at pH 8.0, 30.5°C in the ^{1}H mode. The ^{31}P spectra were recorded at 40.48 MHz

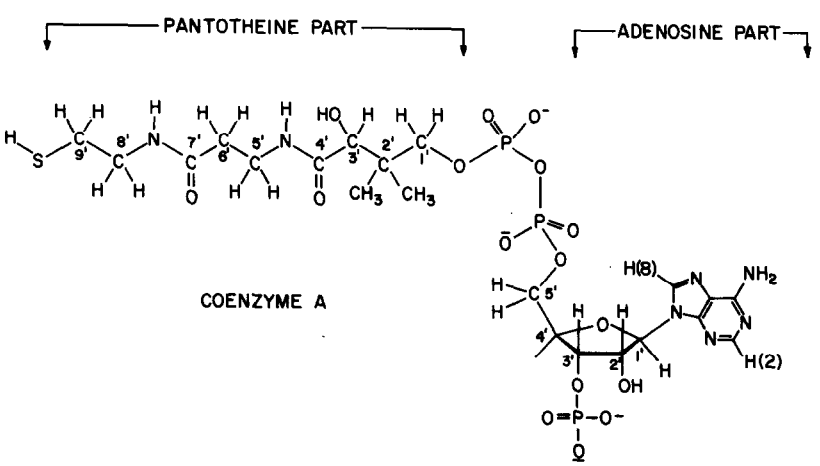


Fig. 1. Structure of coenzyme A. The numbering system employed in the discussion of the NMR data is also shown.

in Fourier mode. Details of instrumentation provided elsewhere [4,5]. The assignment of absorption peaks of the protons attached to the pantotheine part was somewhat complicated. However, the fact that protons near the free end of a long chain will experience considerable degree of freedom of motion and consequently will have sharper absorption peaks, along with consideration of electronegativity effects, enabled to identify these protons. Finally, spectra were unequivocally analysed by computer program LAOCN3 with a plotting routine designed by one of us (Che-Hung Lee). In computer simulation, the whole ribose moiety is considered as an AA'BCDMX nuclei system, while the pantotheine part is treated as three independent systems, i.e. ABX for ^{31}P(1')H(1')H(1''), A_2B_2 for H(8')H(8'')H(9')-H(9'') as well as for H(5'), H(5''), H(6') and H(6''), the numbering system for pantotheine side chain is shown in fig. 1. With appropriate bandwidths for the peaks of each proton, the entire spectrum of CoASH and CoAS-$COCH_3$ were simulated until agreement with the observed spectrum was attained. In fig. 2 we illustrate the observed and computer simulated ^{1}HNMR spectrum of CoASH. The phosphorus hydrogen couplings for the ribose region ($J_{5'-P(5')}$, $J_{5''-P(5')}$, $J_{4'-P(5')}$, $J_{3'-P(3')}$, $J_{4'-P(3')}$) and for the pantotheine region ($J_{1'-P(1')}$, $J_{1''-P(1')}$) are obtained from both ^{1}H and ^{31}P spectra. The data for the various protons of CoASH, CoAS-$COCH_3$ and 3'5'ADP are compiled in table 1.

3. Results and discussion

3.1. *The sugar-base torsion angle*

The ^{1}HNMR spectra of 0.01 M CoASH and CoAS-$COCH_3$, pH 8.0, were taken in the presence of Mn(II) ions 5×10^{-6}, 10×10^{-6}, 15×10^{-6}, 25×10^{-6} and 35×10^{-6}, molar concentrations. It was noticed that the C(8)H of adenine was selectively broadened by the Mn(II) ions whereas no significant effect on the line width of C(2)H is noticed. This indicates in CoASH and CoAS-$COCH_3$, the adenine is preferentially oriented in anti-conformation as has been shown to be the case by identical studies for 5'AMP [6,7], pyridine coenzymes [8] and adenosinediphosphoglucose [9].

3.2. *Conformation of the ribofuranose moiety*

It has been pointed out that, a $^2E \rightleftarrows {}^3E$ equilibrium best describes the solution conformation of the ribofuranose moiety of nucleosides and nucleotides in aqueous solutions [4,10,11]. Recently Altona and Sundaralingam [12] suggested that ribose conformation in aqueous solution can be quantitatively described in terms of pseudorotation parameters and provided equations to compute the percentage populations of N and S [12] conformers. Evans and Sarma [13], made a detailed error analysis of the Altona-Sunderalingam [12] approach and suggested that the pseudorotational methods do not have the claimed reliability. Whether one uses the traditional Karplus method [4,10,11] or the Altona–Sunderalingam method [12], one has to invoke at least an error of 10% in computed populations. To deter confusion, one may use the terms ^{2}E and S as well as ^{3}E and N synonymously. The computed value of S conformer for 3'5'ADP, CoASH and CoAS-$COCH_3$ from $J_{3'4'}$ in table 1 is $\cong$ 80%. The traditional Karplus equation, as used by Schleich et al. [14], gives a value of $\cong$ 70% for the population of ^{2}E con-

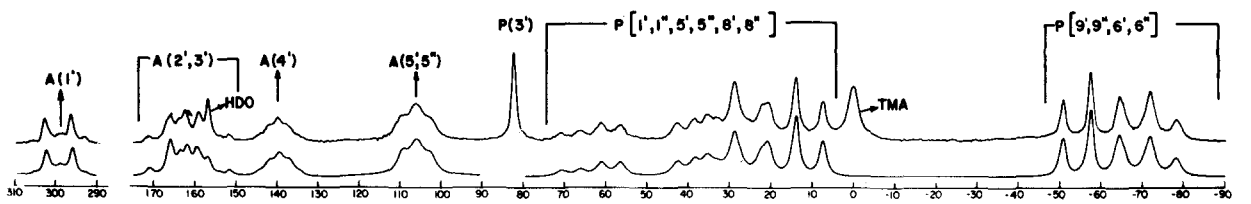


Fig. 2. Top. Observed ^{1}H NMR spectrum, recorded in Fourier mode of 0.05 M, pH 8.0, Coenzyme A. The peaks corresponding to adenine C(8)H, C(2)H, as well as pantotheine C(2')Me_2 are not shown. Internal standard was tetramethylammonium chloride (TMA). In the assignments indicated the letter A refers to adenosine part protons and P refers to protons of the pantotheine part, the numbering system employed being that shown in fig. 1. Bottom: Computer simulated spectrum of CoASH. A comparison of spectra on top and bottom show excellent agreement between the observed and simulated spectra. Because proton P(3') is a singlet, it was not included in the simulation.

Table 1
NMR parameters for 3′5′ADP, coenzyme A and acetyl coenzyme A[a]

Chemical shifts							Coupling constants						
Nuclei	Adenosine part			Nuclei	Pantotheine part		Nuclei	Adenosine part			Nuclei	Pantotheine part	
	3′5′ADP	CoA-SH	CoA-Act		CoA-SH	CoA-Act		3′5′ADP	CoA-SH	CoA-Act		CoA-SH	CoA-Act
AH(1′)	298.7	299.0	298.7	PH(1′)	62.8	63.5	$J_{1'2'}$	6.8	6.7	6.7	$J_{1'1''}$	−9.7	−9.7
AH(2′)	170.3	164.5	163.1	PH(1″)	36.8	36.8	$J_{2'3'}$	5.0	5.2	5.2	$J_{1'-P(1')}$	4.6	4.6
AH(3′)	160.0	159.7	158.1	$PCH_3(2')$	−231.4	−231.4	$J_{3'4'}$	2.4	2.4	2.4	$J_{1''-P(1')}$	4.2	4.2
AH(4′)	138.7	139.6	138.6	$PCH_3(2'')$	−244.5	−244.5	$J_{3'-P(3')}$[f]	7.3	7.3	7.3	Σ''[e]	8.8	8.8
AH(5′)	88.4	108.2	107.2	PH(3′)	82.0	82.9	$J_{4'5'}$	3.1	2.9	2.9	$J_{5'5''}$	−10.0[b]	−10.0[b]
AH(5″)	84.0	104.1	103.0	PH(5′)	28.7	26.9	$J_{4'5''}$	3.1	2.7	2.7	$J_{5'6'}$	6.6	6.6
AH(2)	500.8	507.3	508.8	PH(5″)	28.7	26.9	Σ[c]	6.2	5.6	5.6	$J_{5'6''}$	6.6	6.6
AH(8)	544.8	536.4	537.4	PH(6′)	−71.2	−75.2	$J_{4'-P(3')}$	0.8	0.8	0.8	$J_{5''6'}$	6.6	6.6
				PH(6″)	−71.2	−75.2	$J_{4'-P(5')}$	1.4	2.0	2.0	$J_{5''6''}$	6.6	6.6
				PH(8′)	13.8	14.2	$J_{5'5''}$	−10.0[b]	−10.0[b]	−10.0[b]	$J_{6'6''}$	−10.0[b]	−10.0[b]
				PH(8″)	13.8	14.2	$J_{5'-P(5')}$	4.2	4.1	4.1	$J_{8'8''}$	−10.0[b]	−10.0[b]
				PH(9′)	−56.8	−21.5	$J_{5''-P(5')}$	4.2	4.1	4.1	$J_{8'9'}$	6.6	5.9
				PH(9″)	−56.8	−21.5	Σ'[d]	8.4	8.2	8.2	$J_{8'9''}$	6.6	6.7
				$-COCH_3$	–	−83.1					$J_{8''9'}$	6.6	6.7
											$J_{8''9''}$	6.6	5.9
											$J_{9'9''}$	−10.0[b]	−10.0[b]

[a] The coupling constants and chemical shifts are expressed in Hz (100 MHz NMR system) upfield from internal standard tetramethylammonium chloride, pH 8.0, temp. 30.5°C; concentration employed are: 3′5′ADP, 0.1 M; CoA-SH, 0.05 M and CoA-Act, 0.025 M. The numbering system followed is shown in the diagram of CoA-SH in Fig. 1.

[b] The J value selected for geminal coupling is arbitrary.

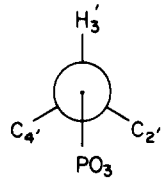
[c] $\Sigma = J_{4'5'} + J_{4'5''}$;

[d] $\Sigma' = J_{5'-P(5')} + J_{5''-P(5'')}$;

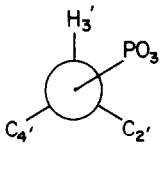
[e] $\Sigma'' = J_{1'-P(1')} + J_{1''-P(1')}$;

[f] This value is arbitrary.

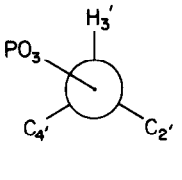
CONFORMERS ON THE ADENOSINE PART



I
trans



II
gauche



III
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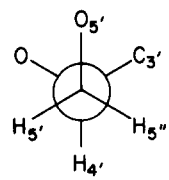
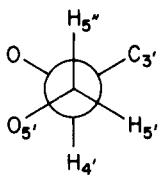
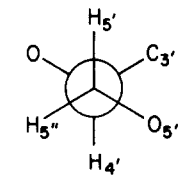


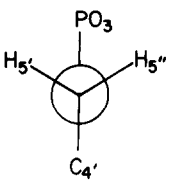
IV
gauche-gauche



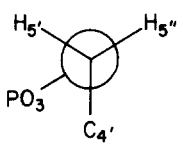
V
gauche-trans



VI
trans-gauche



VII
gauche'-gauche'



VIII
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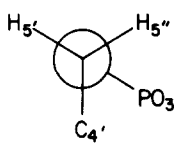

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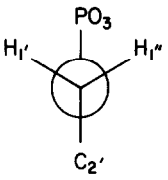
CONFORMERS ON THE PANTOTHEINE PART



X
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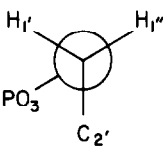

XI
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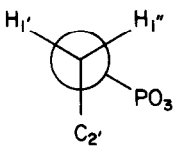
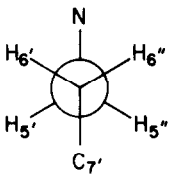


XII
trans"-gauche"



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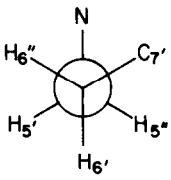
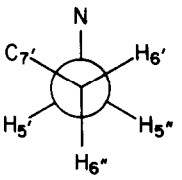
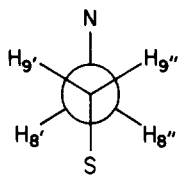


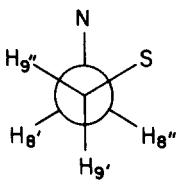
XIV
gauche'



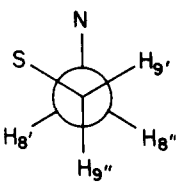
XV
gauche'



XVI
trans"



XVII
gauche"



XVIII
gauche"

former is 3'5'ADP, CoASH and CoAS-$COCH_3$. It appears that these molecules show a clear preference for the ^{2}E/S pucker.

3.3. *Conformation of the 3'-phosphate group*

The minimum energy torsional diastereomers constrained to C(3')–O(3') are shown by Newman projections in I, II and III. The expected three bond coupling constant, $J_{H(3')-P(3')}$, is 21 Hz for I and 3 Hz for II and III [15,16]. The observed value of 7.3 Hz (table 1) for this coupling suggests that, the 3' phosphate group exists as a time-averaged equilibrium mixture of I, II and III with the population of I being 29%, the combined populations of II and III being 71%. It may be noted that ^{1}H NMR data cannot distinguish between II and III. However, the values of $J_{C(4')-P(3')}$ and $J_{C(2')-P(3')}$ in 3'AMP and other 3' nucleotides [17] suggest that in general, in 3'nucleotides such as 3'5'ADP, 3'5'GDP, CoASH, CoAS-$COCH_3$ etc., the predominant conformer is most likely II. It should be pointed out in all these 3' phosphate derivatives the magnitude of $J_{H(3')-P(3')}$ is extremely similar to simple 3' phosphate derivatives such as 3'AMP.

3.4. *The conformation of the C(4')–C(5') bond*

Population distribution of the conformers constrained to the C(4')–C(5') bond (IV–VI) can be evaluated from the experimental sum $J_{4'5'} + J_{4'5''}$ (Σ) using equations developed elsewhere [18–21]. In 3'5'ADP, CoASH and CoAS-$COCH_3$, the estimated values for IV and V/VI, from Σ values in table 1 are 70 and 30% respectively. This observation of the predominance of the gg conformer (IV) in these compounds is consistent with such findings in crystalline 5' nucleotides [22,23] and in other nucleotide coenzymes [4,8,9,24].

3.5. *The conformation of the C(5′)–O(5′) bond*

The rotamers about C(5′)–O(5′) are gauche′–gauche′ (VII), gauche′–trans′ (VIII) and trans′–gauche′ (IX). It has been shown elsewhere that estimates of the conformational distribution about the C(5′)–O(5′) bond of a nucleotide fragment can be obtained from the magnitude of the sum Σ' ($J_{5'-P} + J_{5''-P}$) [18–21]. The Σ' values for 3′5′ADP, CoASH and CoAS-$COCH_3$ lie in the range 8.2–8.4 (table 1) indicating that these systems share an overwhelming bias (≅ 90%) for the g′g′ conformer (VII). This has been demonstrated to be the case in 5′-β-nucleotides in solids [22,23], as well as well as in solution [13, 20]; also nucleotide coenzymes such as pyridine nucleotides, adenosinediphosphoglucose and uridinediphosphoglucose, in solution, show an overwhelming preference for the g′g′ conformer [4,8,9,24].

3.6. *Evidence for a preferred gg–g′g′ (IV–VII) conformation for the exocyclic CH_2OPO_3-group of 3′5′ADP, CoASH and CoAS-$COCH_3$ from four bond $^{31}P-^{1}H$ W coupling*

A stereochemical consequence of the C(4′)–C(5′) and C(5′)–O(5′) bonds being simultaneously oriented gg(IV) and g′g′(VII) is that the atoms H(4′), C(4′), C(5′), O(5′) and P(5′) lie in one plane and the geometric relationship between H(4′) and P(5′) is an in-plane 'W' et al. [25,26] have shown that in phos phate esters such an in-plane 'W' relationship will generate a $^4J_{(^1H-^{31}P)}$ of 2.7 Hz and this value reduces to zero when the planarity is destroyed. Sarma and coworkers [21] have shown that in 5′-β-nucleotides, at pH 8.0, an observed value of $^4J_{(^1H-^{31}P)}$ greater than 1.0 indicates that the backbone preferentially exists in a gg–g′g′ conformation. The data in table 1 show that for 3′5′ADP, CoASH and CoAS–$COCH_3$, the observed $^4J_{H(4')-P(5')}$ range from 1.4–2.2, clearly showing that in this group of compounds, the preferred backbone geometry is gg(IV)–g′g′(VII). Such a conclusion is amply supported by the discussion of the three bond coupling data in sections 3.4 and 3.5 above. With respect to the geometry of the 3′ phosphate group, there is no possibility for the occurrence of a 'W' relation between H(4′) and P(3′) in I, II or III and the observed $^4J_{H(4')-P(3')}$ in table 1 is consistent with this.

3.7 *The conformation about C(1′)–O(1′) bond of pantotheine moiety of CoASH and CoAS-$COCH_3$*

The preferred rotamers about the C(1′)–O(1′) bond of the pantotheine (see fig. 1 for the numbering scheme employed for the pantotheine residue) are shown in X, XI and XII. Manipulation of the data for $J_{H(1')-P(1')}$ and $J_{H(1'')-P(1')}$ in table 1 as discussed in section 3.5, show that the bond C(1′)–O(1′) show an outspoken preference for the g″g″(X) conformer (≅ 85%).

3.8. *The conformation of C(5′)–C(6′) bond of the pantotheine moiety of CoASH and CoAS-$COCH_3$*

Newman projections XIII, XIV and XV show the preferred rotamers about this bond. One cannot by NMR methods distinguish between the $H_{5'}$ and $H_{5''}$ as well as $H_{6'}$ and $H_{6''}$ germinal protons. However, the observation that they have identical value for the vicinal coupling (6.6 Hz, table 1) show that, all the three possible rotamers, XIII, XIV and XV are equally populated in CoASH and CoAS-$COCH_3$ [27,28].

3.9. *The conformation of C(8′)–C(9′) bond of the pantotheine moiety of CoASH and CoAS-$COCH_3$*

The Newman projections XVI, XVII and XVIII show the preferred conformers about this bond. As discussed in section 3.8, one cannot by NMR methods make a distinction between $H_{8'}$, $H_{8''}$ or between $H_{9'}$ and $H_{9''}$. However, the data for CoASH in table 1 ($J_{8'9'}$ etc.) show that the three possible rotamers XVI–XVIII are equally populated [27,28]. In the case of acetyl coenzyme A, a small perturbation in the magnitude of $J_{8'9'}$, $J_{8'9'}$, $J_{8''9''}$ and $J_{8''9''}$ is observed compared to that in CoASH. This indicates that acetylation of the -SH group of coenzyme A, slightly perturbs the distribution of the rotamers XVI–XVIII.

Acknowledgement

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Note added in proof

Comparison of the chemical shifts of acetyl coenzyme A at pH values 1.5, 5.0 and 8.0 as well as the chemical shifts of acetyl coenzyme A with that in S-acetyl pantotheine phosphate indicate that detectable amounrs of hairpin-folded conformations exist for coenzyme A and acetyl coenzyme A in aqueous solutions. It appears that these coenzymes in aqueous solutions exist as dynamic mixtures of linear and folded conformations. Details of these will be presented at the Harry Steenbock Symposium (June 16–19, 1974), University of Wisconsin, Madison, Wisc, USA. The proceedings of this symposium will be published in exenso.

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