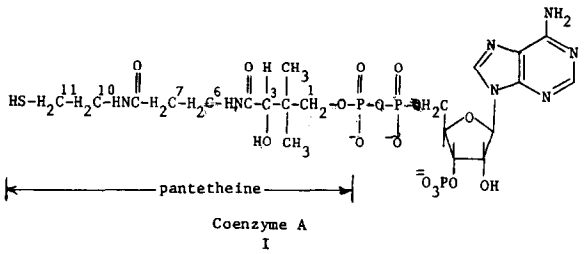
MAGNETIC RESONANCE STUDIES OF THE CONFORMATION OF MANGANESE (II) COENZYME A

G. Edwin WILSON, jr
Department of Chemistry, Washington University St Louis, MO 63130, USA

Received 11 June 1979
Revised version received 3 July 1979

1. Introduction

Several magnetic resonance studies have been directed toward determining the dominant conformation of coenzyme A (I) in solution, the interest stemming in part from the high specificity of enzymes for the unaltered coenzyme. From coupling constant studies it has been suggested that the β-alanine segment of the molecule adopts a predominantly *gauche* conformation, and that all three rotamers of the cysteamine fragment seem of about equal energy [1]. An ^2E pucker of the ribose ring appears to be highly preferred [2], and this information, together with complete coupling constant data was used to formulate a folded model in which the pantetheine chain coils around the adenine base. A coiled arrangement of the pantetheine of benzoyl coenzyme A was also suggested as the structural feature allowing positioning of the aromatic ring over the adenine thus explaining a set of upfield shifts of H-1′, H-2 and H-8 of the adenosine portion of the molecule [3]. Lanthanide shifts were used to calculate distances between the bound metal ion and several protons of dephosphocoenzyme A [4] but an interpretation of such data is made difficult by the necessity to consider the angular dependence of the shifts with respect to an unknown magnetic anisotropy axis and the contribution from other than 1:1 complexes. From this shift data, a structure possessing a ribose in the ^2E conformation with a predominantly *anti* adenine base-sugar torsional angle and an extended pantetheine chain emanating from the metal binding site was deduced. Finally, the distance dependence of the paramagnetic effects of Co(II) has been used to calculate a structure for propionyl coenzyme A bound to transcarboxylase [5].



Coenzyme A
I

A common deficiency in the structural proposals to data is the failure to recognize and take into account structural features which would lead to stabilizing a folded conformation relative to extended ones, and a failure to relate structure to biological specificity. In this paper we describe the use of manganese (II) as a relaxation probe of the structure of coenzyme A and we propose a conformation of the manganese (II) complex with structural features which tend to produce conformational integrity of a coiled structure and to explain observed biochemical specificities.

2. Materials and methods

The sodium salt of coenzyme A was purchased from Sigma Chemical Co. For the NMR measurements, coenzyme A was dissolved in D_2O, adjusted to pH 8.2 with sodium deuteroxide, then lyophilized three times from D_2O, and finally taken up in D_2O with TSP added as a chemical shift standard. The concentration of coenzyme A was calculated from A_{260}

Table 1
Molar relaxivities at various Mn(II) concentrations and average Mn–H distances

	$T_{1M}^{-1} \times 10^{-3} s^{-1}$			
	13.4 μM Mn^{2+}	50.6 μM Mn^{2+}	113 μM Mn^{2+}	r_{avg} (A°)
H-8	27.3 ± 4.8	10.8 ± 1.8	–	4.1
H-2	–	5.06 ± 0.79	5.63 ± 0.86	4.6 ± 0.2
H-1′	5.00 ± 0.94	4.91 ± 0.79	4.62 ± 0.72	4.7 ± 0.2
H-4′		1.98 ± 0.45	1.14 ± 0.24	5.7 ± 0.5
H-5′	13.2 ± 3.7	9.45 ± 1.88	16.9 ± 2.7	4.0 ± 0.4
P–H-3	10.5 ± 1.9	10.2 ± 1.6	–	4.1 ± 0.2
$P-CH_3A$	3.86 ± 1.43	4.37 ± 0.88	4.18 ± 0.73	4.8 ± 0.5
$P-CH_3B$	5.49 ± 1.58	3.57 ± 0.74	3.61 ± 0.63	4.8 ± 0.4
P–H-7	4.03 ± 1.71	2.25 ± 0.63	1.45 ± 0.33	5.2 ± 0.8
P–H-11	1.01 ± 1.08	1.28 ± 0.44	2.69 ± 0.53	5.6 ± 0.6

using $\epsilon_{260} = 16.8\ mM^{-1}\ cm^{-1}$ [5]. Longitudinal relaxation times (T_1) were measured at 99.6 MHz using a 90–τ(HSP)–90 sequence and an internal deuterium lock. Manganese (II) chloride was added from calibrated stock solutions in D_2O. Chemical shifts for coenzyme A have been determined [2,3].

3. Results

The molar paramagnetic relaxation rates (T_{1M}^{-1}) for experiments at three manganese (II) concentrations are shown in table 1. Distances between the metal ion and the relaxed protons were calculated from the modified Solomon Bloembergen equation [6];

$$T_{1M}^{-1} = \frac{A}{r^6}\left[\frac{3\tau_c}{1+\omega_I^2\tau_c^2} + \frac{7\tau_c}{1+\omega_S^2\tau_c^2}\right]$$

where A is a constant characteristic of the nucleus with a value of 2.878×10^{-31} for protons, ω_I and ω_S are the proton and electron Larmor frequencies and τ_c is the rotational correlation time. The quantity T_{1M}^{-1} is derived from the observed paramagnetic relaxation rates, T_{1p}^{-1} by:

$$T_{1M}^{-1} = T_{1p}^{-1}/p$$

where p is the bound metal–substrate ratio. Using the value $K_d = 1.1$ mM for the Mn–coenzyme A complex [7] one calculates that 95% of the manganese is bound to the coenzyme. A value of $\tau_c = 6 \times 10^{-11}$ s was adopted for the calculations. This represents the average value obtained experimentally from the difference in T_{1M}^{-1} values at 100 and 220 MHz [8]. It is also the value calculated from Stokes law assuming rotation about the long axis in our proposed structure ($r \simeq 4$ A°). Calculated distances averaged from the three measurements are given in table 1. To verify that the diphosphate is the metal binding site, we repeated the experiments with dephosphocoenzyme A. The relative distances to all protons except H-4′ agreed well with those for coenzyme A. The longer Mn–H distance to H-4′ in coenzyme A is not compatible with metal binding to the 3′-phosphate group of coenzyme A.

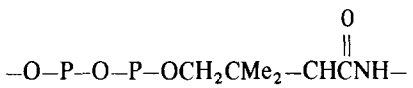
4. Discussion

There are several essential criteria which must be satisfied in any proposed structure derived from the relaxation data. The Mn–H distance to H-5′ must be shorter than that to H-4′ and about the same as that to H-1′. Since the primary metal binding site is the diphosphate, this requires that the chain fold back from C-4′ over the ribose ring. The average Mn to P–H-3* distance is about the same as that to the two

* The nomenclature P–H-x refers to the hydrogen on atom x of the pantetheine portion of coenzyme A as shown in I

methyl groups. Thus, considering the intervention of only one methylene group between the diphosphate metal binding site and the CH–CMe_2 sequence, P–H-3 and the two methyl groups must lie away from the manganese and on the same side of the intervening P–C-2 to P–C-3 bond. This places P–OH-3 close to the Mn; and we propose that it occupies one of the manganese ligand positions. To allow positioning of the Mn equidistant from adenine H-2 and H-8, the manganese must lie above the ring. The Mn–H distances to P–H-7 and P–H-11 are relatively short, and not consistent with extended conformations of the β-alanine and cysteine units stretching away from the pantoyl group. Thus this unit must be coiled.

Using these restrictions a model (fig.1) was constructed with measured distances reasonably in accord with the calculated values. An ^2E ribose conformation best fits the data. The base–C-1′ bond appears to have great torsional freedom assuming no spatially preferred Mn–base arrangement. The unit:



appears to be free to rock with respect to the rest of the chain. The main chain torsional angles through P–C-2 in the proposed structure are given in table 2 and compared with those proposed by Lee and Sarma [2]. Beyond P–C-2 there is only one possible anchor point for conformational control, the possible hydrogen bond to adenine. This segment has already been discussed at length [1,2]; the current data is not compatible with a predominantly stretched out chain, but agrees with our previous finding of a preferred *gauche* rotamer in the β-alanine segment.

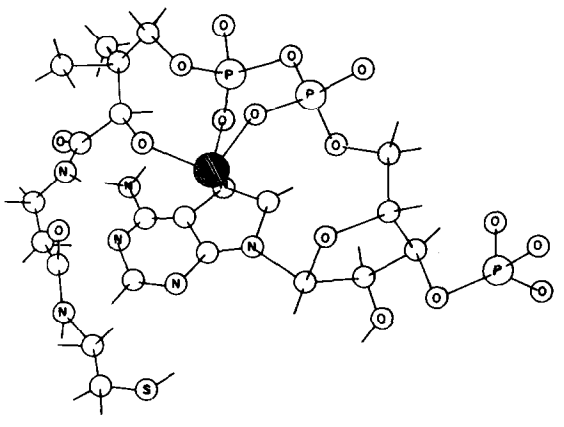


Fig.1. A possible conformation of the manganese (II) complex of coenzyme A in solution

Table 2
Torsional angles in coenzyme A

	This work	Ref. [2]
C-5′–C-4′	*g,g*	*g,g*
O–C-5′	*b*	*t*
P_α–O	*t*	
O–P_α	g^-	
P_β–O	g^+	
O–P_β	*t*	
P–C-1–O	g^+, sc^+	t[c]
P–C-2–P–C-1	g^+ or g^-	
P–C-3–P–C-2	g^+ or sc^-	

[a] Angles are measured along the main chain with g^+ defined by

[b] Highly flexible
[c] An increase in the g^+ and g^- rotamers was found at pH 8

The essential new feature in the model we propose, the coordination of a metal to the P–C-3 hydroxyl group, provides an explanation for previous biological activity studies on altered coenzyme A molecules [9]. It was observed that the diastereomer with an epimeric pantoic acid segment has only 5.5% of the activity of the natural compound while the P–C-3 keto derivative possesses 37% of the native coenzyme A phosphotransacetylase activity [9]. Epimerization at P–C-3 will prevent complexation in a molecule with an unaltered adenosine diphosphate conformation because either the carbonyl group or one of the *gem*-dimethyls would be forced into close contact with one of the diphosphate oxygens.

Coordination of the metal atom with the adenine ring provides a chiral enzyme recognition site and maintains the 3′-phosphate group exposed for easy binding to an enzyme surface. These results suggest that coenzyme A recognition by enzymes may well be facilitated by association with divalent metal ions even though subsequent unfolding of the solution conformation might take place on the enzyme surface.

Acknowledgements

The nuclear magnetic resonance spectrometer was purchased under NSF grant no. CHE77-02068.

References

[1] Wilson, G. E., jr Bazzone, T. J., Kuo, C. H. and Rinaldi, P. J. (1975) J. Am. Chem. Soc. 97, 2907–2908.
[2] Lee, C. H. and Sarma, R. H. (1975) J. Am. Chem. Soc. 97, 1225–1236.
[3] Mieyal, J. J., Webster, L. T., jr and Siddiqui, U. A. (1974) J. Biol. Chem. 249, 2633–2640.
[4] Fazakerley, G. V., Linder, P. W. and Reid, D. G. (1977) Eur. J. Biochem. 81, 507–514.
[5] Buyske, D. A., Handschumacher, R. E., Schilling, E. D. and Strong, F. M. (1954) J. Am. Chem. Soc. 76, 3575–3577.
[6] Solomon, I. (1955) Phys. Rev. 99, 559–565; Bloembergen, N. (1957) J. Chem. Phys. 27, 572–573; 595–596.
[7] Buttlaire, D. H., Cohn, M. and Bridger, W. A. (1977) J. Biol. Chem. 252, 1957–1964.
[8] Fung, C. H., Feldman, R. J. and Mildvan, A. S. (1976) Biochemistry 15, 85–92.
[9] Shimizu, M., Suzuki, T., Hosokawa, Y., Nagase, O. and Abiko, Y. (1970) Biochem. Biophys. Acta 222, 307–319.