A Multinuclear NMR Study of the Coordination of Uranyl Oxo-cations by Adenine Nucleotides*

M. MARGARIDA C. A. CASTRO
and CARLOS F. G. C. GERALDES†

Chemistry Department, University of Coimbra, 3000 Coimbra, Portugal

The biological importance of the interaction of metal ions with nucleic acids justifies a systematic study of complex formation between nucleotides and oxo-cations which show a tendency to polymerize in aqueous solution. Multinuclear NMR spectroscopy has been shown to be useful in characterizing the interaction of Mo(VI) oxo-cations with adenine nucleotides [1]. Similar studies using the uranyl oxo-cation [2–4] show evidence of various complexes between uranyl and the nucleotides 5′-AMP, ADP and ATP in D_2O solution in the pH range of 7–11. In this work we report a more thorough structural characterization of these systems, studying complexation as a function of pH, total concentration and metal-to-ligand (M/L) ratio, using proton, ^{31}P and ^{13}C NMR spectroscopy.

*Paper presented at the Second International Conference on the Basic and Applied Chemistry of f-Transition (Lanthanide and Actinide) and Related Elements (2nd ICLA), Lisbon, Portugal, April 6–10, 1987.

†Author to whom correspondence should be addressed.

Experimental

AMP, ADP, ATP, deuterated solvents and uranyl nitrate were obtained from Sigma Chemical Co., Ltd. and from Merck. Proton ^{31}P and ^{13}C NMR spectra were obtained in deuterium oxide solutions at 22 ± 1 °C using a Varian XL-200 NMR spectrometer. Most of the ^{31}P and ^{13}C spectra were broad-band proton decoupled. DSS, trimethylphosphate and dioxane were used as internal references for the proton, ^{31}P and ^{13}C spectra, respectively.

Results and Discussion

U(VI)–AMP System

Figure 1 illustrates the spectra of U(VI)/AMP solutions at various pH values (7.9 to 11.4) and M/L ratios. At pH > 9.5 we observe signals from free AMP and up to three complexes (M1, M2, M3) in different populations, depending on the M/L ratio. While only free AMP and M1 are present for M/L ≤ 1, complexes M2 and M3 are also present when M/L > 1. The complex M1 is a sandwich-type (ST) complex [2] of 2:2 stoichiometry, where U(VI) is bound to the C-2′ and C-3′ ribose hydroxyl and the phosphate groups. This structure is justified by the observed complexation shifts, especially those of the C-13 ribose nuclei (Table I). The complexes M2 and M3 are also ST complexes of stoichiometry 4:2 formed by dis-

TABLE I. Complexation Chemical Shifts Induced by U(VI) Binding to AMP, Δδ (ppm)

Nucleus	pH > 9.5			pH < 9.5
	M_1	M_2	M_3	Non-ST complexes (e.g. M_4, M_5)
H_8	0.32	0.44	0.35	0.32; 0.38; 0.47
H_2	0.16	0.22	0.22	0.18; 0.21; 0.24
H_1'	0.02	0.41	0.17	0.18; 0.36
H_2'	3.00	3.39	[a]	[a]
H_3'	0.72	[a]	[a]	0.6
H_4'	2.26	2.35	2.23	2.16; 2.28; 2.31
H_5', H_5''	1.01; 0.24	[a]	[a]	1.08; 0.15
^{31}P		5.2[b]		5.75; 5.02; 4.84; 3.85; 2.95
C_8	0	[c]	[c]	[a]
C_6	−0.08			
C_5	0			
C_4	0			
C_2	0			
C_1'	4.50			
C_2'	10.64			
C_3'	14.08			
C_4'	4.66			
C_5', C_5''	−0.60			−0.64

[a]Not observed. [b]It was not possible to distinguish the complexes M_1, M_2 and M_3 through ^{31}P data. [c]It was not possible to distinguish the M_1, M_2, M_3 complexes through ^{13}C spectra.

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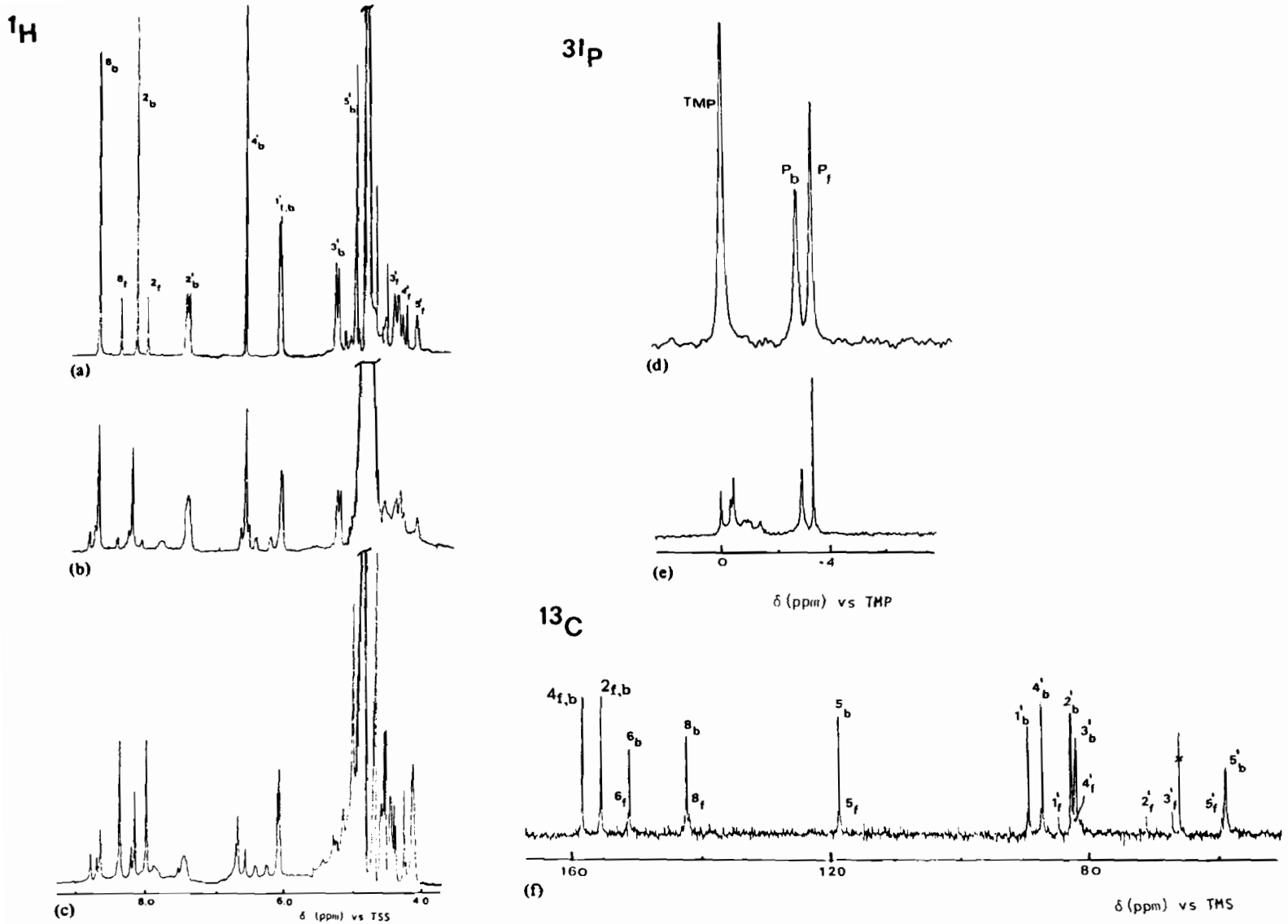

Fig. 1. Proton, ^{13}C and ^{31}P spectra of aqueous solutions of U(VI)/AMP. Proton: (a) 0.1:0.1 M, pH 10.7; (b) 0.2:0.1 M, pH 9.7; (c) 0.1:0.1 M, pH 8.3. ^{31}P: (d) 0.2:0.1 M, pH 10.3; (e) 0.05:0.05 M, pH 8.0. ^{13}C: (f) 0.2:0.2 M, pH 10.1.

proportionation of the M1 complex [2], which also yields free nucleotide. It was not possible to distinguish the three complexes by ^{31}P and ^{13}C NMR spectroscopy.

At pH < 9.5, gradual protonation of the hydroxyl groups at C-2′ and C-3′ of the ribose ring leads to formation of non-ST complexes involving interaction of U(VI) exclusively with the phosphate group (M5) and mixed complexes involving partial coordination to the hydroxyl groups (M4). Multiple proton and ^{31}P signals are thus observed (Fig. 1).

U(VI)–ADP System

The proton and ^{31}P spectra of U(VI)/ADP solutions obtained at various pH values (pH 7 to 11) and M/L ratios (1:2 to 2:1) show that at pH > 9.5 there is free ADP and up to four complexes (D1 to D4) in different populations depending on the M/L ratios. Their populations are, however, almost negligible for M/L < 1, indicating that these complexes have stoichiometries of 1:1 or higher in the metal. The values of the complexation shifts (Table II) show that these are ST complexes where U(VI) binds to the phosphate groups (P_α and P_β) and the ribose C-2′–OH and C-3′–OH groups, very similarly to U(VI)/AMP complexes, M1, M2 and M3. These complexes probably have stoichiometries of 2:2 and 4:2 and various structures are possible [3, 4].

The ^{31}P complexation shifts of bound ADP change quite dramatically from pH > 9.5 to pH < 9.5 (see Table II). Taking into account the known dependence of ^{31}P chemical shifts on the torsion angles of the phosphate groups [5], this observation is compatible with a conformational change of the phosphate moiety between ST and non-ST (e.g., D4, D5) complexes, due to a changed involvement of the ribose in U(VI) binding.

U(VI)–ATP System

Proton spectra (Fig. 2) show that at pH ~ 10 and M/L ≤ 1 there is free ATP together with two complexes, T1 and T2; for M/L > 1 another four complexes (T3 to T6), with small populations are seen. Although difficult to characterize, they are probably polymerized forms with higher M/L stoichiometries. The most abundant complexes, T1 and T2, are of the ST type, with binding sites at C-2′–OH, C-3′–OH, P_β and P_γ (see Table III) and stoichiometries 2:2 and 4:2 respectively [3, 4]. It was previously proposed [4] that at pH 10.5, 1:1 solutions, apart from U(VI)/-ATP complexes, also contain the U(VI)/AMP 2:2 complex M1, due to U(VI) induced hydrolysis of ATP to AMP and phosphate. However, our ^{31}P spectra (Fig. 2d) show that these species are not present, and only the two complexed forms of ATP, T1 and T2, are observed under these conditions. At pH ~ 8 non-ST complexes of various stoichiometries predominate, with U(VI) binding directly to the ATP phosphate groups (see complexation shifts at Table III).

TABLE II. Complexation Chemical Shifts, Δδ (ppm), Induced by U(VI) Binding to ADP

Nucleus	pH > 9.5				pH < 9.5
	D_1	D_2	D_3	D_4	Non-ST complexes (e.g. D_5, D_6)
H_8	0.65	0.60	0.48	0.39	[a]
H_2	0.20	0.20	0.17	0.16	
H_1'	0.07	0.15	0.25	0.34	
H_2'	3.21	3.21	3.04	2.80	
H_3'	[a]	[a]	[a]	[a]	
H_4'	2.52	2.44	2.21	2.34	
H_5',$''$	[a]	[a]	[a]	[a]	
P_α		1.8[b]			4.74
P_β		5.4[b]			3.71
C_8	0.6	[c]	[c]	[c]	[a]
C_6	0.4				
C_5	0				
C_4	0				
C_2	0				
C_1'	4.70				
C_2'	11.20				
C_3'	14.31				
C_4'	2.53				
C_5',$''$	0.5				

[a]Not measured. [b]Average value; it was not possible to distinguish perfectly the 4 complexes in the ^{31}P spectra. [c]It was not possible to distinguish D_2, D_3 and D_4 complexes through ^{13}C spectra.

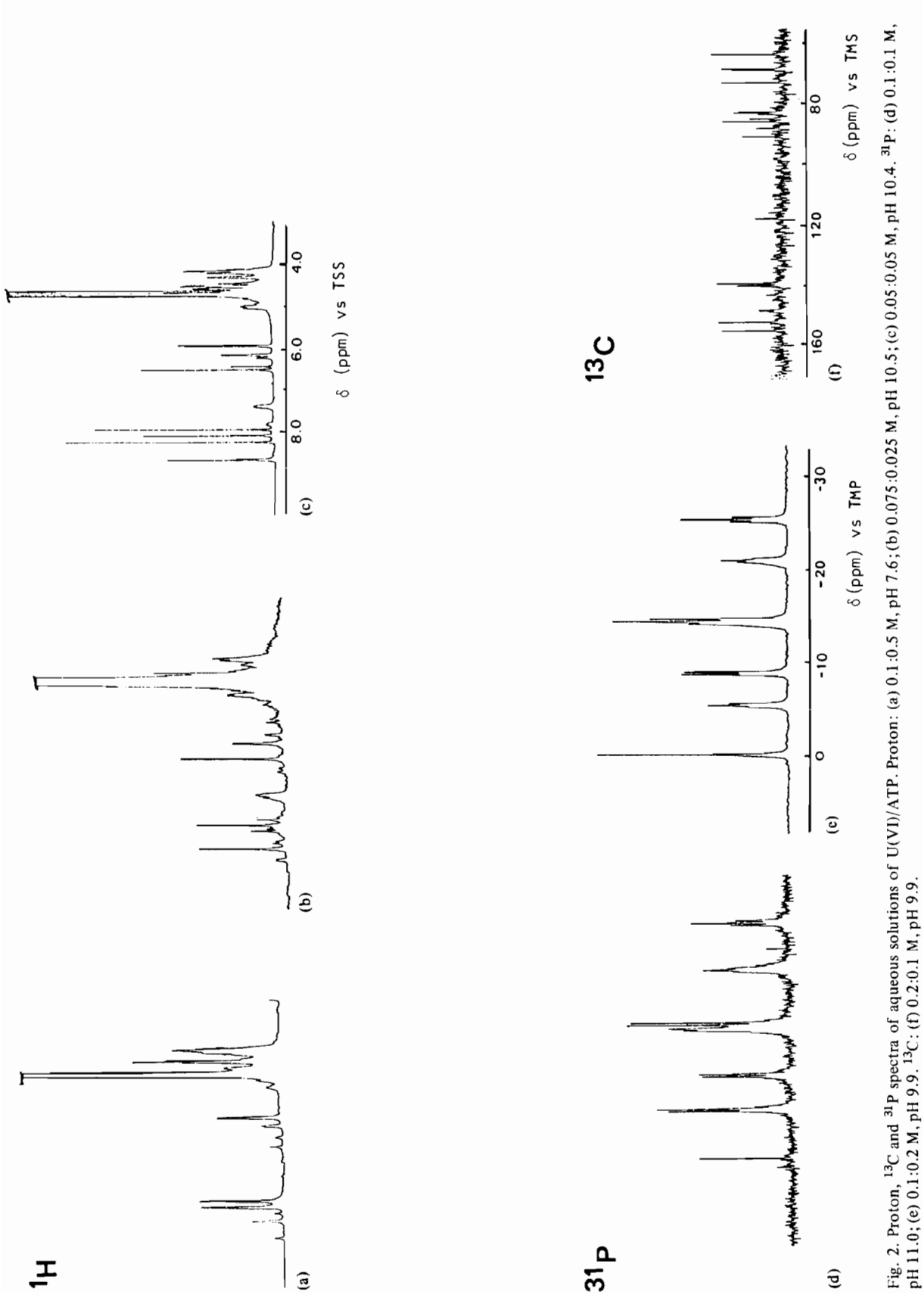

Fig. 2. Proton, ^{13}C and ^{31}P spectra of aqueous solutions of U(VI)/ATP. Proton: (a) 0.1:0.5 M, pH 7.6; (b) 0.075:0.025 M, pH 10.5; (c) 0.05:0.05 M, pH 10.4. ^{31}P: (d) 0.1:0.1 M, pH 11.0; (e) 0.1:0.2 M, pH 9.9. ^{13}C: (f) 0.2:0.1 M, pH 9.9.

TABLE III. Complexation Chemical Shifts, $\Delta\delta$ (ppm), Induced by U(VI) Binding to ATP

Nucleus	pH ≃10 (pH > 9.5)						pH ≃8 (pH < 9.5)
	T_1	T_2	T_3	T_4	T_5	T_6	Non-ST complexes (e.g. T_7, T_8)
H_8	0.45	0.41	0.73	0.70	0.30	0.24	0.15; 0.36; 0.78
H_2	0.16	0.15	0.22	0.25	[a]	[a]	0.03
H_1'	0.22	0.30	[a]	[a]	[a]	[a]	0.15
H_2'	2.83	3.28	3.40	[a]	[a]	[a]	1.65; 1.92
H_3'	0.50	0.48	[a]	[a]	[a]	[a]	0.3
H_4'	2.28	2.16	2.52	2.61	[a]	[a]	1.26; 1.41
H_5',$''$	[a]	[a]	[a]	[a]	[a]	[a]	0.48
P_α	0.23	0.46	[b]	[b]	[b]	[b]	0.24; 0.48
P_β	4.06	4.47	[b]	[b]	[b]	[b]	3.85; 4.09
P_δ	3.10	3.31	[b]	[b]	[b]	[b]	1.92; 3.19
C_4	0	[b]	[b]	[b]	[b]	[b]	[a]
C_2	0						
C_6	0.56						
C_8	0.70						
C_5	0						
C_1'	4.86						
C_2'	11.76						
C_3'	14.32						
C_4'	0.56						
C_5',$''$	0						

[a]Not observed. [b]It was not possible to distinguish the various complexes through ^{13}C or ^{31}P spectra.

References

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