NMR Studies on UO_2^{2+} Complexes with Pyridoxal

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Abstract

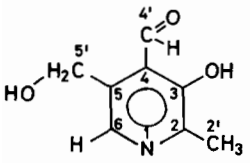
The interaction of pyridoxal with dioxouranium(VI) acetate was studied by ^1H and ^{13}C NMR measurements in D_2O and CD_3OD.

The results indicate that the preferred bonding site is the C-3–O^- donor, and the major species obtained under the experimental conditions used is the equimolar complex.

Introduction

The function of pyridoxal catalysis and metal ions in several enzymic reactions of aminoacid metabolism is well known [1, 2].

In our laboratory we have undertaken studies on the interaction of dioxouranium(VI) with pyridoxal, which yields solid complexes which were purified and characterized [3]. We report here some results obtained by ^1H and ^{13}C NMR studies on the dioxouranium(VI)/pyridoxal system in aqueous and methanol solutions.



Pyridoxal

Experimental

Materials

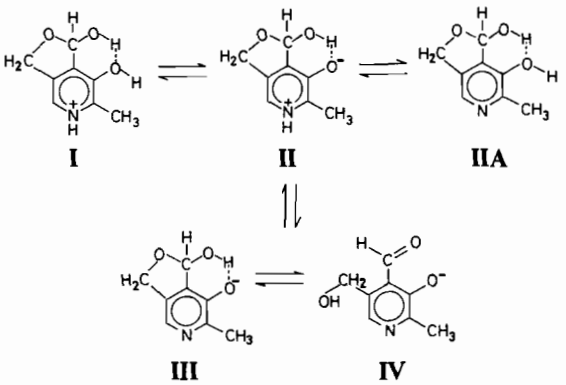
Pyridoxal hydrochloride was purchased from Merck and $UO_2(CH_3COO)_2 \cdot 2H_2O$ was obtained from Carlo Erba. Deuterium oxide (99.8%) and tetradeuteromethanol (99.5%) were Ega–Chemie products.

Methods

^1H and ^{13}C NMR spectra of 0.1 M pyridoxal hydrochloride in D_2O or CD_3OD were recorded at 28 °C at varying dioxouranium(VI)/pyridoxal molar ratios by using a FT-80 Varian spectrometer. The spectra in D_2O of pyridoxal upon UO_2^{2+} addition were measured at pH = 3.25, since precipitation occurred at higher pH. The pH values, adjusted with NaOD, were measured with a Radiometer TTT2 pH meter. No correction for D_2O solvent was applied. Chemical shifts expressed in δ/ppm were related to dioxane converted to TMS scale for D_2O solutions, and to TMS as internal standard for CD_3OD solutions.

Results and Discussion

At low and medium pH pyridoxal exists in hemiacetal form, both in aqueous and methanol solutions [4–7]. This form of ligand undergoes a two step deprotonation process according to Scheme 1:



Scheme 1.

In methanol the presence of the non-polar form **IIA** was observed by a spectrophotometric method by Martell *et al.* [6]. The pK values found by ^1H NMR

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TABLE I. ^{1}H NMR[a] Chemical Shifts (δ/ppm) of free Pyridoxal Hydrochloride and UO_2 Acetate Containing Solutions in D_2O at pH 3.25.

Compound	C-6–H	C-4′–H	5′-CH_2	2′-CH_3	-$CH_{3acet.}$
Pyridoxal	8.10, 1H	6.76, 1H	5.26, 2H	2.61, 3H	
Pyridoxal + UO_2acetate 1:0.5	8.04, 1H	6.78, 1H	5.25, 2H	2.68, 3H	2.20
Pyridoxal + UO_2acetate 1:1	8.02, 1H	6.81, 1H	5.27, 2H	2.77, 3H	2.23
Pyridoxal + UO_2acetate 1:1.5	8.00, 1H	6.81, 1H	5.27, 2H	2.79, 3H	2.27
Pyridoxal + UO_2acetate 1:2	7.99, 1H	6.83, 1H	5.27, 2H	2.82, 3H	2.34
Δ ppm =	−0.11	+0.07	+0.01	+0.21	

[a] ^{1}H NMR chemical shifts are measured downfield from TMS, using dioxane as an internal standard.

TABLE II. ^{13}C NMR[a] Chemical Shifts (δ/ppm) of Free Pyridoxal Hydrochloride and UO_2 Acetate Containing Solutions in D_2O at pH 3.25.

Compound	C-3	C-2	C-4	C-5	C-6	C-4′	C-5′	C-2′
Pyridoxal	150.4	144.4	140.0	138.3	125.1	99.0	70.3	14.8
Pyridoxal + UO_2acetate 1:1	147.2	142.1	138.1	138.1	122.9	99.4	70.9	14.8
Pyridoxal + UO_2acetate 1:2	146.5	141.3	138.1	138.1	123.3	99.3	70.9	14.8
Δ ppm =	−3.9	−3.1	−1.9	−0.2	−1.8	+0.3	+0.6	–

[a] ^{13}C NMR chemical shifts are measured downfield from TMS, using dioxane as an internal standard.

for aqueous solutions are 4.4 and 8.7 respectively, and they fit well with the values obtained by potentiometric measurements [5].

In aqueous solution the proton decoupled ^{13}C NMR spectra consist of eight resonances with assignments recently given by Jenkins *et al.* [7] (Table II). The position of C-4′ resonance at around 100 ppm corresponds to the hemiacetal form of pyridoxal.

NMR Spectra in Aqueous Solutions

Tables I and II show respectively the ^{1}H and ^{13}C NMR chemical shifts of free pyridoxal and uranyl acetate/pyridoxal solutions at varying molar ratios.

The presence of dioxouranium(VI) ions in aqueous solutions containing pyridoxal at pH 3.25 causes changes of proton and carbon chemical shifts which may indicate the direct involvement of uranyl ion in the binding to the ligand. The major changes of chemical shifts are observed for 2′-CH_3, C-4′–H and C-6–H protons (Table I) and C-2, C-3, C-6 and C-4 carbons (Table II). Since such carbons were found to be sensitive to the deprotonation process of the phenolic group C-3–OH [7] of pyridoxal molecule, it is conceivable that the dioxouranium(VI) ion binds pyridoxal via the C-3–O^- donor.

The ^{1}H and ^{13}C NMR spectra of the solutions at different UO_2^{2+}/pyridoxal molar ratios indicate that the major species formed at this pH is the equimolar complex.

The hemiacetal form, in which pyridoxal exists both in aqueous and methanol solutions, appears to be preserved in the complexed ligand molecule since no variation of the chemical shifts is observed for C-4′ and C-5 carbons upon metal binding.

The minor change of chemical shift of C-4′ carbon upon metal coordination to pyridoxal molecule suggests that the C-4′–OH donor is not involved in metal binding in the studied solutions. Thus pyridoxal binds the UO_2^{2+} ion as a monodentate ligand at the C-3–O^- site.

NMR Spectra in Methanol Solutions

In methanol, pyridoxal maintains its hemiacetal form up to moderately basic medium [6]. The deprotonation process, however, could be different from

TABLE III. ^1H NMR[a] Chemical Shifts (δ/ppm) of Free Pyridoxal Hydrochloride and UO_2 Acetate Containing Solutions in CD_3OD.

Compound	C-6–H	C-4′–H	5′-CH_2	2′-CH_3	-$CH_{3acet.}$
Pyridoxal	8.35, 1H	6.45, 1H	5.30, 2H	2.72, 3H	
Pyridoxal + UO_2acetate 1:1	8.00, 1H	6.52, 1H	5.21, 2H	2.92, 3H	2.33
Pyridoxal + UO_2acetate 1:2	8.00, 1H	6.55, 1H	5.23, 2H	2.96, 3H	2.45
Δ ppm =	−0.35	+0.10	−0.07	+0.24	

[a] ^1H NMR chemical shifts are measured downfield from TMS used as an internal standard.

TABLE IV. ^{13}C NMR[a] Chemical Shifts (δ/ppm) of Free Pyridoxal Hydrochloride and UO_2 Acetate Containing Solutions in CD_3OD.

Compound	C-3	C-2	C-4	C-5	C-6	C-4′	C-5′	C-2′
Pyridoxal	151.13	145.13	141.01	140.28	126.20	105.92	71.14	14.74
Pyridoxal + UO_2acetate 1:1	148.50	142.70	140.15	140.15	122.49	107.00	71.07	15.49
Pyridoxal + UO_2acetate 1:2	148.50	142.70	140.15	140.15	122.49	107.00	71.07	15.49
Δ ppm =	−2.60	−2.30	−0.83	−0.10	−3.79	+1.18	−0.01	+0.79

[a] ^{13}C NMR chemical shifts are measured downfield from TMS used as an internal standard.

that found in aqueous solutions [6, 8]. Matsushima and Martell [6], on the basis of spectrophotometric studies, suggested the presence of the non-polar species **IIA** which coexists with species **II** (Scheme 1). The dioxouranium(VI) ion may affect the protonation equilibrium when bound to the pyridoxal molecule, and the chemical shifts of pyridoxal in methanol solutions could be considerably different from that in aqueous solutions.

Tables III and IV show ^1H and ^{13}C NMR chemical shifts for free pyridoxal and its solutions with uranyl acetate.

The significant upfield chemical shifts of C-2 and C-3 carbon atoms upon metal ions binding to pyridoxal in methanol solutions are quite similar to those found in aqueous solutions. This could indicate the same C-3–O^- binding of pyridoxal to UO_2^{2+} in methanol as well. The considerable upfield shift (3.79 ppm) of C-6 carbon in the presence of uranyl acetate suggests that the metal ion bound to the pyridoxal molecule via C-3–O^- may influence the equilibrium process between forms **II** and **IIA** of pyridoxal (Scheme 1).

The stronger change of chemical shift of the C-4′ carbon (1.18 ppm) observed upon UO_2^{2+} addition in methanol solution, compared to that observed in aqueous solution (0.3 ppm), could also suggest the involvement of the aldehydic function C-4′–OH in the binding to uranyl ions.

The equimolar complex seems to be the major species present under the experimental conditions used in this study.

In conclusion, the present results support the binding of UO_2^{2+} to pyridoxal both in aqueous and methanol solutions; C-3–O^- seems to be the major binding site of dioxouranium(VI) ion, and the equimolar complex seems to be the major species formed under the experimental conditions used.

References

1 D. E. Metzler, M. Ikawa and E. E. Snell, *J. Am. Chem. Soc., 76,* 648 (1954).
2 M. Ikawa and E. E. Snell, *J. Am. Chem. Soc., 76,* 653 (1954).
3 A. Marzotto, *Inorg. Chim. Acta, 62,* 183 (1982).
4 D. Heyl, E. Luz, S. A. Harris and K. Folkers, *J. Am. Chem. Soc., 73,* 3430 (1951).
5 V. R. Williams and J. B. Neilands, *Arch. Biochem. Biophys., 53,* 56 (1967).
6 Y. Matsushima and A. E. Martell, *J. Am. Chem. Soc., 89,* 1322 (1967) and refs. therein.
7 R. C. Harruff and W. T. Jenkins, *Org. Magn. Reson., 8,* 548 (1976).
8 O. A. Gansow and R. H. Holm, *Tetrahedron, 24,* 4477 (1968).