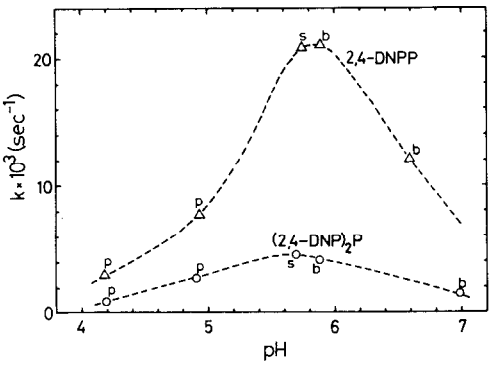

Fig. 1. trpnCo^{III}(aq) promoted hydrolysis of 2,4-DNPP and $(2,4\text{-DNP})_2P$. Ester, 5.0×10^{-5} *M*; Co^{III}, 1.25×10^{-3} *M*; buffer, 0.025 *M*; T = 25.0 °C; ionic strength = 0.50 *M*. Self-buffered, s; bis-tris, b; PIPPS, p.

It may be emphasized that in these experiments the rate is not close to saturation for the trpnCo^{III}(aq) concentrations used. The results therefore imply specific rate constants for the more active complexes which are much larger than the rate constants reported here.

The extraordinary effectiveness of trpnCo(OH)$(OH_2)^{2+}$ in promoting these phosphoryl transfer reactions relates to: (i) the *cis* requirement for the coordinated waters; (ii) the rapidity with which complexation takes place through water substitution; (iii) the involvement of complexes containing more than one cobalt center; (iv) the availability and reactivity of coordinated hydroxide on a suitably positioned cobalt.

Acknowledgement. Research supported by the U.S. National Science Foundation and the Petroleum Research Fund.

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T11

Binding of Dioxouranium(VI) to Thiamine and Its Pyrophosphate Ester (Cocarboxylase) in Dimethylsulfoxide

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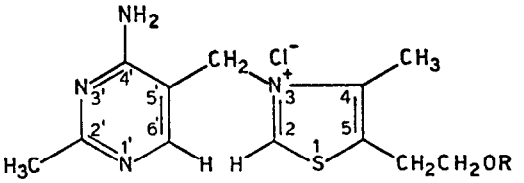
Thiamine hydrochloride (Vitamin B_1) is a necessary dietary constituent and its pyrophosphate ester (TPP) is the coenzyme cocarboxylase which catalyzes the decarboxylation of α-ketoacids in the presence of divalent metal ions (Mg(II), Mn(II), Co(II) *etc.*).



R=H Thiamine; $P_2O_7^{3-}$ Thiamine pyrophosphate (TPP)

A general mechanism of action of thiamine pyrophosphate has been proposed but there are still uncertainties on the role of metal ions and the nature of the bonding sites. In fact, Hadjiliadis *et al.* [1], on the basis of ^1H and ^{13}C NMR studies in DMSO-d_6 on the interaction of thiamine and its pyrophosphate ester with Pt(II) and Pd(II) concluded that the role of metal ions is to coordinate pyrimidine at N-1′ position in agreement with the results obtained by the X-ray structure of the Cd(thiamine)Cl_3 complex [2], whereas Gary and Adeyemo [3] through the same NMR and IR spectra showed that Zn(II), Cd(III) and Hg(II) are bound through the N-3′ position of the pyrimidine ring.

The binding of dioxouranium(VI) with thiamine and cocarboxylase, already studied by us in methanol solution [4–8], has been now analyzed by ^1H and ^{13}C NMR measurements in deuterated dimethylsulfoxide.

^1H NMR Spectra. Coordination of thiamine to dioxouranium(VI) caused a remarkable variation of the proton chemical shifts towards higher field, in particular of C-2′-CH_3 and C-6′-H protons. Such an upfield shift depends on the UO^{2+}/thiamine molar ratio and is very relevant for the protons adjacent to the N-1′ nitrogen of the ligand whereas no important effect is observed for the $-CH_2-CH_2-OH$ side-chain protons.

The spectrum of cocarboxylase upon UO_2^{2+} complexation exhibits a marked downfield shift of the protons proximal to the pyrophosphate group together with a slight change of the C-6′H chemical shift.

^{13}C NMR Spectra. The data for thiamine were in agreement with the coordination at the N-1′ nitrogen since significant downfield chemical shifts are exhibited by carbon atoms near to the pyrimidine N-1′ donor (C-6′, C-2′ and 2′-CH_3).

The present data strongly support the binding of dioxouranium(VI) to thiamine at the pyrimidine N-1′ site. The major bonding site of cocarboxylase to uranyl(VI) seems to be the pyrophosphate group even if a possible involvement of the N-1′ donor should be considered.

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T12

Chelation of the *cis*-$Pt^{II}(NH_3)_2$ Moiety by the Guanines of the Oligonucleotides d(T-G-G-C-C-A), d(A-T-G-G) and d(C-C-A-T-G-G)

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It is generally accepted that, within the cell, DNA is the primary target of the active aquated forms of the antitumor *cis*-$[Pt(NH_3)_2Cl_2]$ drug (*cis*-DDP) [1, 2]. The fact that only the *cis* isomer of the complex exhibits antineoplastic activity, suggests that the cytotoxic lesion could result from a particular bifunctional coordination of the *cis*-$Pt^{II}(NH_3)_2$ moiety [1]. Intrastrand cross-linking is one possibility [3] and platinum chelation by two adjacent guanines has received much support from studies with various DNAs [4–8] and oligonucleotide models [9–14]. We report here that the three d(T-G-G-C-C-A), d(A-T-G-G) and d(C-C-A-T-G-G) oligonucleotides give GG-platinum chelates.

We have studied the stoichiometric reactions between *cis*-$[Pt(NH_3)_2(H_2O)_2](NO_3)_2$ and the deoxyoligonucleotides d(T-G-G-C-C-A), d(A-T-G-G) and d(C-C-A-T-G-G) (1 Pt per oligonucleotide) in the 10^{-5}–10^{-4} *M* concentration range, in water at 37 °C. In the reaction conditions ^1H NMR shows that the self complementary hexanucleotides are essentially in the single strand form. For the three reactions, HPLC and ^1H NMR analyses show that the oligonucleotide is completely converted to a single complex. The same complex is obtained from the reaction with *cis*-DDP. High pressure gel permeation chromatography and atomic absorption spectroscopy coupled with the UV absorption of the complex, show that one platinum atom is bound per oligonucleotide.

^1H NMR (400 and 500 MHz) of the two hexanucleotide complexes ($1–5 \times 10^{-3}$ *M*) shows that they are single stranded in conditions where the free self-complementary oligonucleotides adopt a duplex structure [14, 15].

The metal binding sites in the d(T-G-G-C-C-A)[Pt], d(A-T-G-G)[Pt] and d(C-C-A-T-G-G)[Pt] complexes have been determined by the analysis of the pH dependence of the chemical shifts of the non exchangeable base protons. In the three cases, on going from basic to acidic pH, one observes the successive protonations of the thymine N3 (apparent pK_a *ca.* 10), of the N1 of the two guanines (app. $pK_a \approx 8.3$–8.5 instead of *ca.* 10 for the free oligonucleotides), of the N3 of the two cytosines (app. $pK_a \approx 4.1$–4.5) and of the adenine N1 (app. $pK_a \approx 3.4$–3.5). These data, together with the two different GH8 downfield shifts already encountered for the d(G-G)[Pt] and d(C-C-G-G)[Pt] chelates [10, 11], show that the *cis*-$Pt^{II}(NH_3)_2$ moiety is chelated by the N7 atoms of the adjacent guanines.

T_1 relaxation times of the base protons, nuclear Overhauser enhancements between the GH8 and deoxy-ribose H2′ and H3′, allowed the assignment of the external and internal guanines and together with two dimensional NMR (J-δ) allowed the identification of the C3′-*endo* deoxy-ribose that is characteristic of the diguanosine chelates [10, 16].

Acknowledgments. This work was supported in part by a grant from the 'Association pour le développement de la Recherche sur le Cancer'.

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