^{13}C-NUCLEAR MAGNETIC RESONANCE AND X-RAY PHOTOELECTRON SPECTROSCOPY OF Cu-AMP

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1. Introduction

Due to the dominant role of metal ions in the biochemistry of nucleic acids the reaction of metal ions with polynucleotides and their monomeric constituents was intensively studied [1–5]. It was demonstrated that metal binding can occur with the bases, the phosphate residue and even with the ribose moiety. Besides X-ray diffraction studies a wide number of spectroscopic techniques were devised and successfully employed to elucidate the metal binding sites within a metal–nucleotide complex. Cu^{2+} especially belongs to the most actively studied metal ions and considerable information regarding the binding situation was obtained [1, 4, 5]. For example it was shown by ^{1}H-NMR that Cu^{2+} binds preferentially with the N-7 of the purine base and from ^{31}P-NMR studies the second binding site was found to be the phosphate residue within the same or even more likely with the neighbouring AMP molecule [4].

The recent application of ^{13}C-NMR spectroscopy [6, 7] proved most convenient to obtain substantial information regarding the structure of purine nucleosides and nucleotides [8–12]. In this context we were interested to examine the Cu-complex of AMP in aqueous solutions employing this new technique. Of further interest was the binding situation of the core electrons of the cupric ion within the Cu-AMP complex. This question was studied using X-ray photoelectron spectroscopy.

From the present ^{13}C-NMR measurements it was clearly shown that Cu^{2+} is bound with the N-7 of the purine ring. This result supports earlier physico-chemical data of such a Cu–AMP complex. Surprisingly the binding energy of the Cu $2p_{3/2}$ core electrons was remarkably low compared to other low molecular weight copper compounds. In fact, the binding energy of the $2p_{3/2}$ level was the same as reported for the copper in erythrocuprein [13], namely 932 eV.

2. Materials and methods

AMP was from Boehringer, Mannheim; D_2O from Merck, Sharp and Dohme, Canada. The proton broad band decoupled ^{13}C-spectra were recorded at room temperature with a Bruker HFX-90-NMR spectrometer (22.628 MHz for ^{13}C, 90 MHz for ^{1}H, 13 MHz for ^{2}H). The accumulation of the pulse interferograms was carried out using a 4 K 1074 Fabritek averager and the Fourier transformation was performed with a 4 K-PDP-8-I computer (Digital). The chemical shifts were measured versus external dioxane. From these data ppm values related to tetramethylsilane (TMS) were calculated.

X-ray photoelectron spectroscopy was performed with a fully automated Varian V-IEE-15 spectrometer equipped with a dedicated computer. The energy of the exciting X-rays was 1253.6 eV (Mg-$K_{\alpha 1,2}$). For further experimental details see ref. [13].

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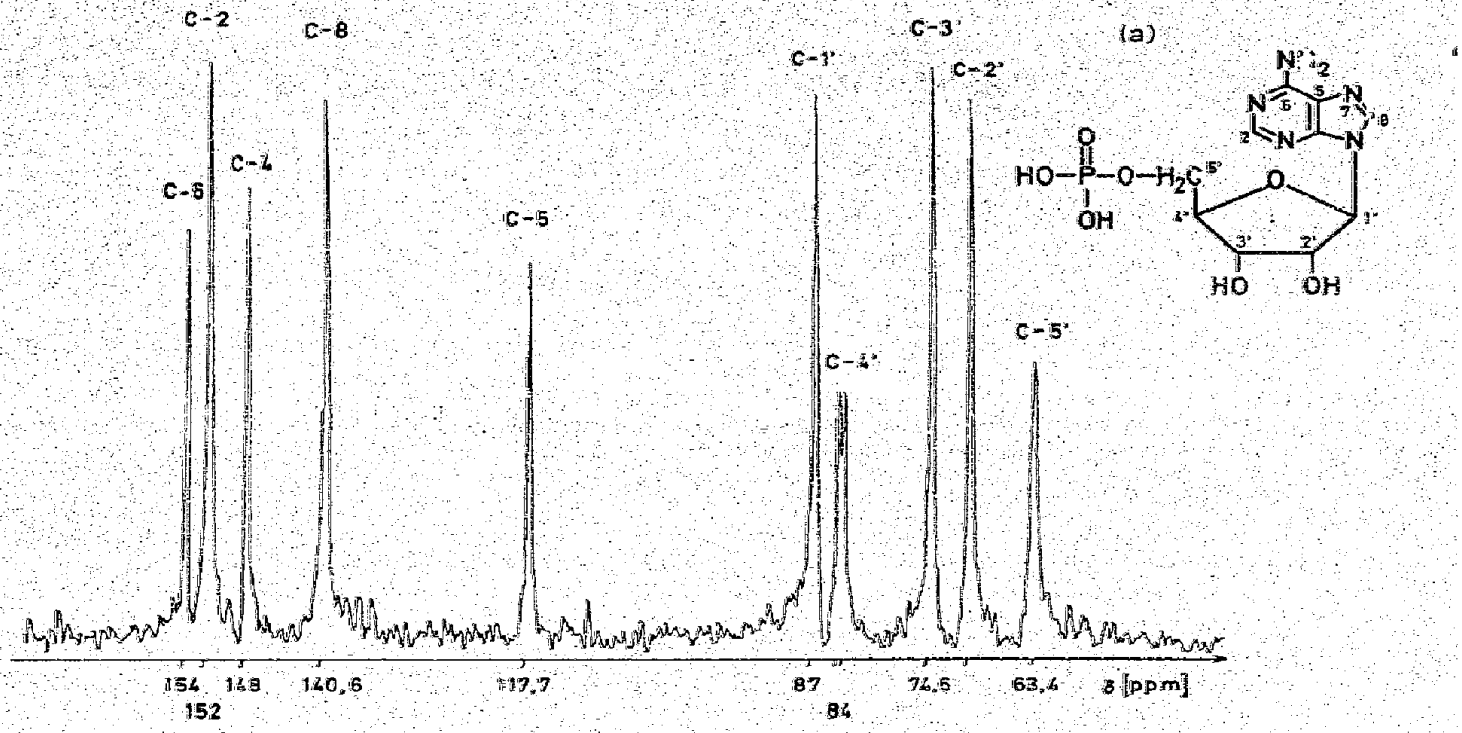
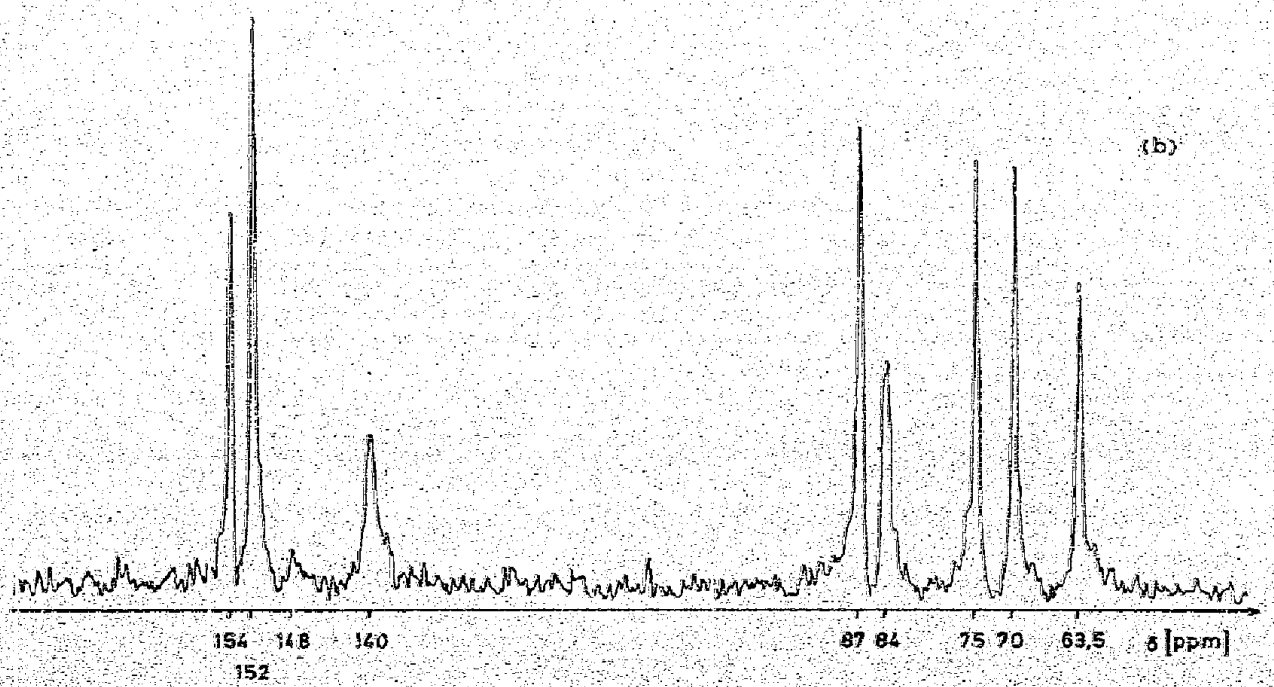
3. Results and discussion

Although the abundance of ^{13}C in natural carbon atoms is only 1.1% the development of the pulse Fourier transform technique made it possible to measure the ^{13}C resonances of unlabeled organic compounds in reasonable amounts of time. For this reason the pulse Fourier ^{13}C-NMR spectroscopy proved one





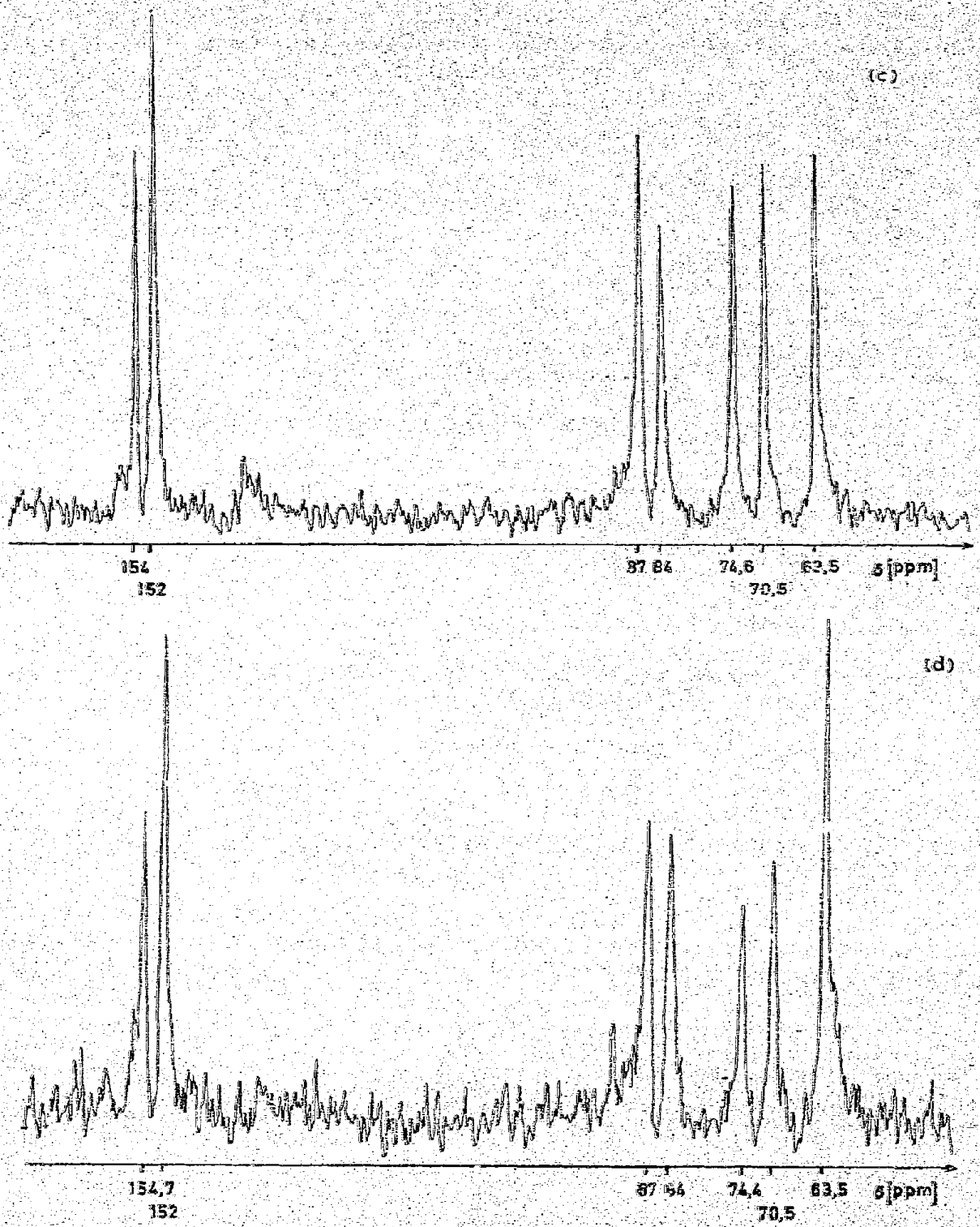


Fig. 1. 22.63 MHz pulse Fourier transform ^{13}C-NMR spectra of AMP. *1a:* 700 µMol 5'-AMP in one ml D_2O, no $CuSO_4$ added. *1b:* + 0.3 µMol $CuSO_4$. *1c:* + 1.0 µMol $CuSO_4$. *1d:* + 3.0 µMol $CuSO_4$. 8192 pulse interferograms; pulse width 8 µsec; temperature 28°C. ^{13}C-chemical shift related to TMS (tetramethylsilane) taken as 0 ppm. External standard dioxane δ = −66.4 ppm versus TMS.

of the most powerful and promising tools for the structure elucidation of many biochemically active compounds [6, 7].

In the present study the Cu binding with AMP was investigated. The ^{13}C-NMR spectra of AMP and the titration with $CuSO_4$ is depicted in fig. 1a–d.

The resolution of the signals in all spectra was of remarkable quality. In some earlier published ^{13}C-NMR measurements the assignments [8–12] for the resonances of the ribose residue carbon atoms were made by application of general ^{13}C-NMR shift rules, by spectral comparison of different analogous sugars and by observing a doublet for the resonance of C-4 attributable to phosphorous–carbon coupling (fig. 1a).

For the signal assignment of the resonances of the purine moiety especially the proton 'off-resonance' partially decoupled spectrum gave valuable information. Out of all carbon atoms of the adenine residue only C-8 and C-2 bear one hydrogen atom, respectively. In the off-resonance spectrum the signals of these carbons appear as doublets. Spectral comparison with other purine residues [8–12] led to the assignment marked in fig. 1.

Upon the addition of increasing amounts of $CuSO_4$ the resonances of C-4, C-5 and C-8 are broadened and finally levelled off. The line broadening of H-atoms being present in close proximity to bound paramagnetic ions was demonstrated in 1962 [14] using the Cu-chelates of amino acids and peptides. Similar to this phenomenon observed during the above ^{1}H NMR spectroscopy the signals of ^{13}C-atoms in the neighbourhood of the binding sites of Cu^{2+} in copper (II) chelates are broadened due to the rapid relaxation of these ^{13}C-atoms. The present ^{13}C-NMR measurements clearly demonstrate that Cu^{2+} added to aqueous solutions of AMP is preferentially bound to the N-7 of the purine ring. This conclusion supports independently earlier ^{1}H-NMR measurements [15].

It is interesting to note that for comparable broadening of the resonances of the carbon atoms of the sugar moiety much higher concentrations of Cu^{2+} are required. This would indicate some binding of the Cu^{2+} to the 2′, 3′-OH groups of the ribose moiety.

The question remains open regarding the binding situation of the cupric ion during complexation with AMP. The X-ray photoelectron spectrum of Cu–AMP revealed an unusual low binding energy of the Cu $2p_{3/2}$ core electrons of 932.0 eV which was almost identical to the corresponding binding energy of the copper in erythrocuprein (931.9 eV [13]). In contrast, the Cu $2p_{3/2}$ – values for $CuSO_4 \cdot 5H_2O$ and $Cu(NH_3)_4SO_4 \cdot H_2O$ were 934.5 and 933.9 eV [16], respectively.

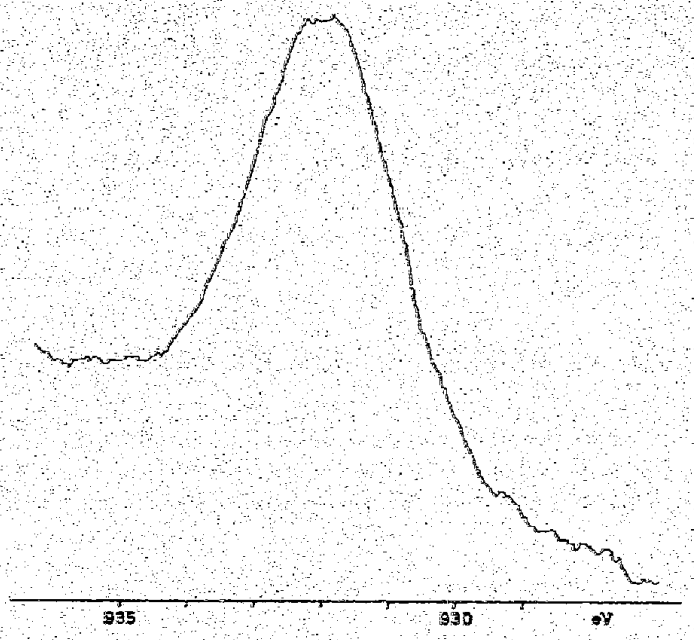


Fig. 2. X-ray photoelectron spectrum of the Cu $2p_{3/2}$ level of Cu-AMP (1:1 complex). X-ray source: Mg-$K_{\alpha_{1,2}}$ (1253.60 eV). Smoothing over 13 points; work function 6.6 eV; analyser energy 100 eV; sweep width 10 eV; sweep time 20 sec; number of scans 200; number of channels 200. The C-1s line of cellotape at 284.0 eV served as reference.

Acknowledgements

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