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ABSTRACT

The binding site of Cu(II) with Adenosine-triphosphate (ATP) has been studied at pH 3 and pH 12 by analysis of both 1s electronic transitions (XANES spectra) and d-d transitions (optical spectra) of the Cu ions. We have determined the tetragonal distortion of the exacoordinated Cu(II) ion by XANES calculation with multiple scattering approach. A comparison with $CuCl_2$, Cu-ribose and Cu-tripolyphosphate indicates that Cu(II) binds to the oxygen atoms of the phosphates with a distorted octahedral symmetry in Cu-ATP at pH 3, while at pH 12 the metal is bound to the ribose moiety with an almost planar symmetry.

1. INTRODUCTION

The adenosine 5'- triphosphate (ATP) plays a fundamental role in many cellular processes. ATP acts as an activator in several enzymatic reactions, requiring for this function the presence of divalent metal ions which can give specific conformations to the molecule. The knowledge of the metal-ATP complex structure in aqueous solution is therefore of great importance for understanding the action of the molecule in living cells.

Studies of metal-ATP complexes are complicated by the metal ability to bind to a number of different sites (e.g. phosphate, base and ribose hydroxyl groups) (Fig. 1) and there is considerable disagreement about the sites where a given metal ion is coordinated[1].

Only very few studies have been carried out on the formation of these complexes as a function of pH, though this may be an important factor in determining the nature of the interaction sites. In fact a competition may exist between metal ions and protons for the ionizable groups which act as ligands.The metal-ATP interaction may be expected to depend on the ionization of the adenine ring (pK≈4), of the terminal phosphate group (pK≈7) and of the ribose hydroxyl group (pK≈12.5). Most of these studies, however, were aimed to investigate the effect of the metal binding on the

Fig. 1 - Schematic of ATP molecule

physico-chemical properties of the nucleotide, while it may be useful to look at the effect that the ligand field has on the electronic properties of the metal itself. We used the X-ray spectroscopy with synchrotron radiation at the Cu K-edge to probe the 1s electronic transitions[2-3] and the optical absorption spectroscopy to probe the d-d transitions[4] of the cupric ions in the Cu-ATP complexes at two extreme pH values (pH 3 and pH 12). The results are compared with the spectra of $CuCl_2$ in aqueous acidic and basic solution.
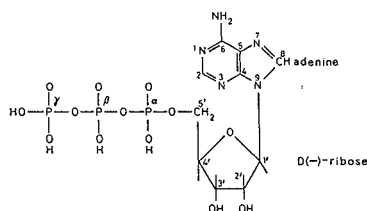
2. MATERIALS AND METHODS

Copper solutions were made from $CuCl_2 \cdot 6H_2O$ reagent grade. ATP was purchased from Boehringer-Mannheim GmbH. No buffers were used, in order to avoid interaction of Cu(II) with other ligands. Solutions were prepared by slowly adding concentrated (2N) NaOH to aqueous solution initially at pH 3. The volume change was negligible. The concentrations used were as a rule 15 mM for the Cu(II) salt and 30 mM for the ATP, tripolyphosphate, and D-ribose. Optical absorption spectra were measured with a Mod 360 Shimadzu spectrophotometer using 3-ml, 1-cm long quartz cells.

X-ray absorption spectra in the Cu XANES region were collected at the Wiggler beam line of the Adone storage ring with the transmission technique. High resolution was achieved by using a Si (111) monochromator with 1-mm entrance slit. Special teflon cells with kapton windows, 2-mm thick were employed. The spectra were recorded immediately after the preparation of solutions. All the measurements were performed at room temperature. XANES spectra were analyzed with multiple scattering theory, using model calculations for a CuO_6 cluster to obtain the most likely bond lengths according to the method already described[2].

3. RESULTS AND DISCUSSION

3.1. $CuCl_2$ in aqueous solution

3.1.1. d-d absorption spectra

The cupric ions are in a $3d^9$ electronic configuration, their optical properties being higly sensitive to the symmetry and strength of the ligand field. In the free state the ground electronic level of the Cu^{2+} has fivefold degeneracy, and the degree of its removal depends on the symmetry of the ligands in the Cu^{2+} complex formed in solution.

If the ligand field has an octahedral symmetry, the 3d level of the central ion is split in two sublevels, e_g and t_{2g}, and only one symmetrical absorption band is expected . It is well known that the environment of the metal ion in hexacoordinate Cu(II) complexes is frequently tetragonally distorted. This distortion splits again the e_g and t_{2g} levels so that more than one d-d transition for the Cu ion in its complexes is to be expected. This is the

case of Cu^{2+} in water at acidic pH's (Fig. 2) where the ligand field is due to six water dipoles and the observed spectrum in the visible range has been resolved [5]into the sum of two symmetrical bands with maxima at 790 nm and 1060 nm. So, this spectrum is greatly influenced by tetragonal fields. At neutral pH a light blue precipitate of $Cu(OH)_2$ is formed. Above pH 12, however, the precipitate gradually dissolves and a clear, deep blue solution is obtained. The ESR properties indicate that this soluble complex is $Cu(OH)_4^{2-}$ with an approximately square-planar structure[6]. This absorption spectrum exhibits an asymmetrical band whith a maximum at about 640 nm (Fig. 2).

Fig. 2 - Optical spectra for Cu(II) in water at pH 3 (dashed) and 12 (solid)

3.1.2 XANES spectra

The change in Cu coordination with pH, seen by optical spectroscopy, can be also revealed by the

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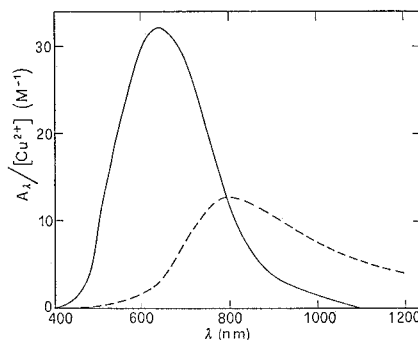XANES spectra[2], reported in Fig. 3.The distance of the axial oxygens has been found to be 2.38 ± 0.05 Å for $CuCl_2$ at acidic pH, and 2.8 ± 0.2 Å at basic pH, as compared with the 1.95 ± 0.05 Å distance of the four equatorial oxygen atoms.

3.2 Cu(II)-ATP complexes

3.2.1 d-d absorption spectra

At pH 3 the Cu(II)-ATP spectrum in the region of the optical d-d transitions (Fig. 4) shows a maximum at 800 nm, similar to the spectrum of hexaqueo cupric ion reported in Fig. 2 but the molar extinction coefficient is

Fig. 3 - Experimental (dotted) and calculated (solid) XANES spectra of Cu(II) ion in aqueous solutions and their derivatives.

somewhat greater . The same spectrum was found at pH 3 for the Cu(II) -tripolyphosphate anions, suggesting that at pH 3 the copper binds to the phosphate portion of the ATP. At pH 3 the adenine ring is protonated and this seems to hinder the interaction of copper ions with the base. The results from Raman [9], EPR [8] and UV differential spectroscopy [4] studies are consistent with these conclusions.

On increasing the pH from 3 to 12 the absorption maximum is shifted from the initial 800 nm down to 640 nm (Fig. 4). A similar dependence of the d-d optical absorption spectra on pH is observed for Cu-AMP, Cu-ADP and Cu-ATP[10-11]. Their absorption spectra at pH 12 are identical to those of the Cu(II)-adenosine and Cu(II)-D-ribose complexes. This indicates an interaction between the copper ions and the hydroxyl groups of the ribose moiety of ATP. Similar conclusions are found in the published literature[12-13]. Note that the absorption spectrum at pH 12 is similar,but not identical, to the spectrum of the soluble Cu(II) species at high pH (Fig. 2).

This result indicates similarity of the nearest environment of the Cu(II) ion in the two cases, and in particular suggests a square-planar geometry for the Cu-ATP complexes at pH 12.

3.2.2. XANES spectra

Fig. 5 shows that the Cu-ATP and Cu-tripolyphosphate complexes at pH 3 have a tetragonal distortion very close to that found for $CuCl_2$ in solution at the same pH. At pH 12 the XANES for Cu-ATP and Cu-ribose are virtually identical and the Cu binding site is almost planar. The calculated spectra reported In Fig.3 and Fig 5 have been obtained by looking for the best agreement between the experimental derivative spectra and the calculated ones leaving as free parameter the axial Cu-O bond. The comparison between the derivative spectra reduces the difficulty to predict experimental amplitudes. The quantitative results for the tetragonal distortion and the axial distances of the Cu ions are reported in Table I. The equatorial bond length distance has been fixed at 1.95 Å to give account of the main peak b. The errors have been estimated by the fact that a change of 0.05 Å in the axial distance give an appreciable disagreement between experimental data and the calculated values and that the muffin-tin approximation involves errors of the same order of magnitude. We have found the best fit using 1.00 Å and 0.95 Å for the muffin-tin radii of Cu and O respectively.
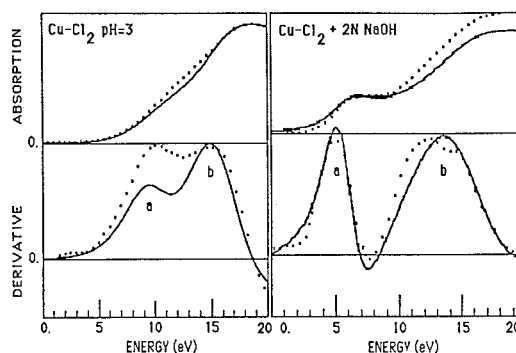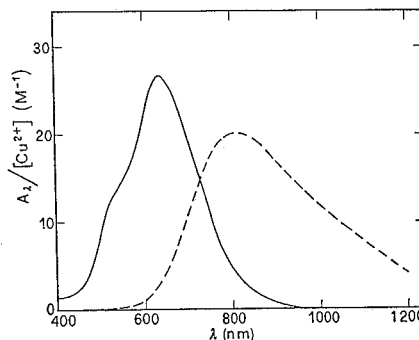
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Cu-ATP Ph=3   Cu-TRIPHOSPHATE

ENERGY (eV)   ENERGY (eV)

Cu-ATP Ph=12   Cu-D-RIBOSE

ENERGY (eV)   ENERGY (eV)

Cu-ATP Ph=3   Cu-TRIPHOSPHATE

ENERGY (eV)   ENERGY (eV)

Cu-ATP Ph=12   Cu-D-RIBOSE
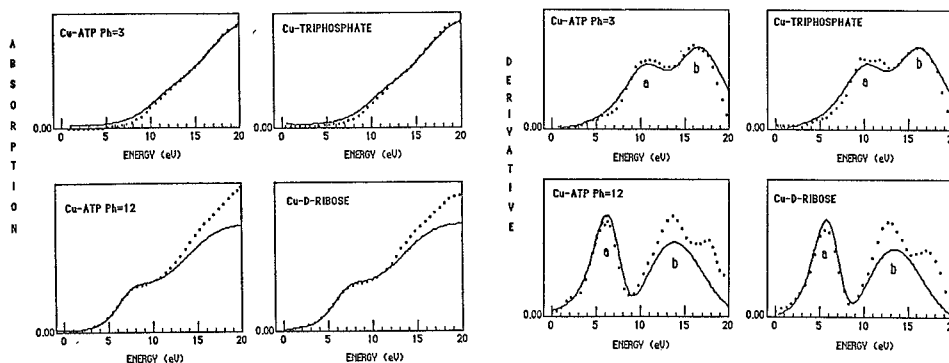
ENERGY (eV)   ENERGY (eV)

Fig.5 - Experimental (dotted) and calculated (solid) XANES spectra, and their derivatives, of Cu-ATP, Cu-triphosphate, Cu-D-ribose at various pH's.

Tab. I

Solution	-Derivative- E_a(eV)	E_b(eV)	DE (E_b-E_a)	R_{axial} (Ang)
Cu-Triph.	10.9	16.3	5.4	2.34
Cu-ATP Ph=3	11.1	16.4	5.3	2.34
Cu-ATP Ph=12	6.1	13.9	7.8	2.50
Cu-Ribose	5.8	13.2	7.4	2.50

4. CONCLUSIONS

The comparison of optical d-d spectra and of XANES shows a complete agreement in the evidentiation of a tetragonal distortion of the copper site in Cu-ATP and Cu-Trypolyphosphate, which at pH 3 is similar to the Jahn-Teller effect in $CuCl_2$ aqueous solution. At pH 12 the similarity of the spectra for Cu-ATP and for Cu-ribose confirms that in these conditions the metal ion is bound to the ribose part of the ATP molecule, and that the site symmetry is planar in this case, as for the hydrated $Cu(OH)_4^{2-}$ complex formed by $CuCl_2$ at high pH. Our quantitative analysis of the XANES allows determination of the Cu-O distances in the axial direction and in the equatorial plane for the studied complexes, and the results are consistent with the qualitative interpretation of the optical spectra.

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