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Circular Dichroism Studies of Cu^{2+}–ATP Complexes. Existence of Ribose–Metal Ion Interactions

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The interactions of Cu^{2+} with the ribose moiety of ATP are studied by Circular Dichroism to understand the formation of hydroxocomplexes in the alkaline pH range. When the pH is basic, we observed two different kinds of CD spectra only for the ribonucleotides and not for dATP. These characteristic CD spectra correspond to two distinct complexes for which the 2' and 3' hydroxyls are respectively ionised.

Introduction

It is well known that metal ions of the 3d series enhance the rate of hydrolysis of adenosine-5'-triphosphate (ATP) considerably [1]. Recent studies of Sigel and coll. [2, 3] suggest that the formation of a macrochelate with the metal ions, the β and γ phophates and the N (7) of adenine ring, is crucial for hydrolysis; the reaction proceeds *via* a dimer complex species, *i.e.* $(Cu(ATP)]_2^{4-}$ [4]. Complex formation probably lowers the energy barrier of the reaction. There are two conceivable ways in which a metal ion may influence the rate of hydrolysis: conformational change and polarisation of σ or π electrons.

Many studies have been devoted to the interactions between metal ions and either phosphates or the base of the nucleotide but is seems that the ribose moiety has not been extensively investigated [5–8]. We have studied by measurements of CD spectra the role of the ribose moiety in the formation of Cu^{2+}–ATP complexes.

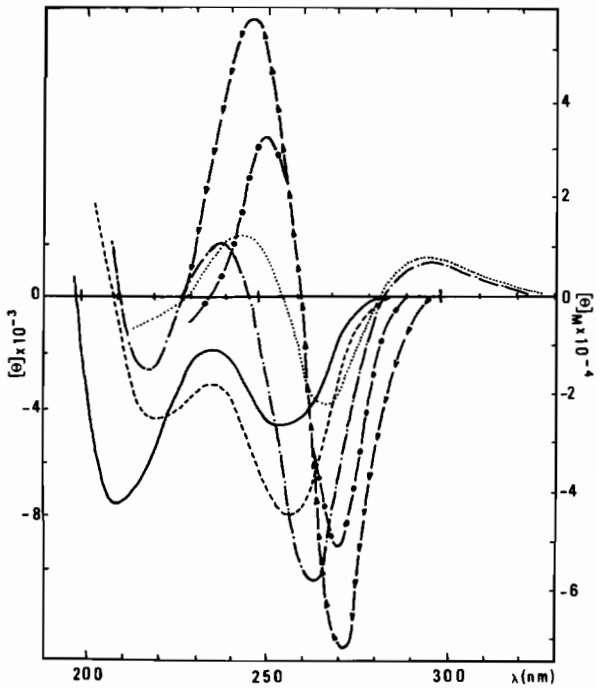

Figure 1. Circular dichroism spectra of ATP in the presence of Cu^{2+}. Molar ellipticity ($|\theta|$ in d° cm^2 $dmol^{-1}$) referred to copper concentrations. Equimolar solutions (5.10^{-4} *M*) were measured at 25 °C in 1 mm quartz cuvette at pH 3.6 (——), 7.0 (------) and 11.5 (·····). CD difference spectrum between ATP–Cu^{2+} and ATP at pH 11.5 (–·–·–). Magnetic circular dichroism spectra ($|\theta|_M$) of ATP in the presence of Cu^{2+}. Equimolar solutions (5.10^{-5} *M*) were measured at 25 °C in 1 cm quartz cuvette at pH 2.0 (–△–△–) and 4 to 10.5 (–○–○–) with a magnetic field of 7T.

Experimental

The spectrometer for the recording of the CD spectra has been calibrated with a solution of isoandrosterone in freshly distilled dioxane ($\Delta\epsilon$ = 3.310 cm^{-1} l mol^{-1} at 304 nm). The wavelength scale has been calibrated on the xenon emission at 467 nm. Magnetic circular dichroism spectra (MCD) are recorded on dichrograph equipped with a superconducting magnet of 7 Tesla [9].

The concentration of the solution and the optical path length are choosen to obtain an optical density value near one in the aromatic range. The highest optical density achieved in the visible range is about 0.1. In all experiments, self-association of the solutes has been avoided. The highest ATP concentration

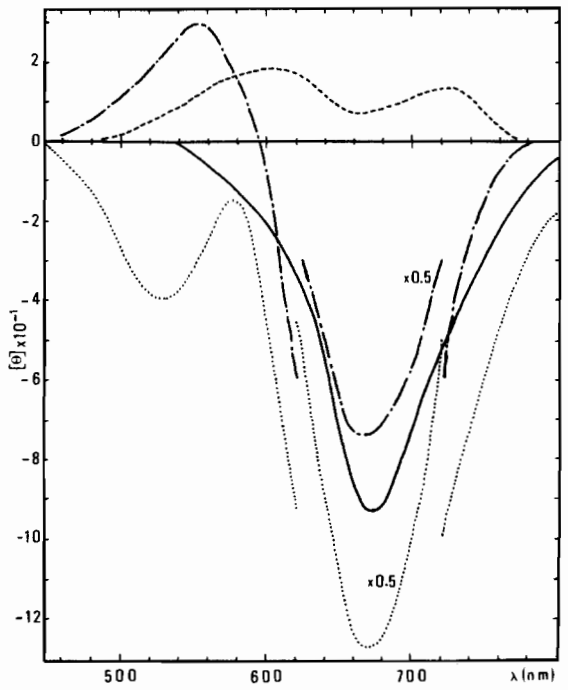

Figure 2. Circular dichroism spectra of ATP in the presence of Cu^{2+}. Equimolar solutions (2.5 10^{-3} *M*) were measured at 25 °C in 2 cm quartz cuvette at pH 5.6 (——), 9.0 (------), 10.7 (—·—·—) and 11.5 (·····).

used is 5 10^{-4} *M* in the aromatic region and 2.5 10^{-3} *M* in the visible range. Solutions are prepared in a potentiometer cell at constant temperature (25 °C) and under nitrogen.

The pH is measured with a calomel electrode and adjusted by decarbonated NaOH 0.1 N stored under nitrogen. In a typical experiment a solution of the sample to be analyzed is prepared by mixing of the suitable freshly prepared stock solutions of ligand and metal in $NaClO_4$ 0.1 N. Each spectrum is recorded in 10 minutes to avoid any nucleotide hydrolysis [2].

The nucleotides were purchased from Calbiochem, San Diego, Calif. and the metal salts were obtained from Fluka A.G., Buchs, Switzerland.

Results

The aromatic part of the ATP–Cu^{2+} CD spectra are shown in Fig. 1; they are similar to those for the nucleotide alone as far as pH 8 [10]. At basic pH values, the 260 nm band becomes stronger and a new positive band appears at 300 nm.

The band in the range near 230 nm is strongly affected. It has sometimes been attributed to an $n \rightarrow \pi^*$ transition [11, 12] although other authors situate it at higher wavelengths [13, 14]. The CD difference spectrum from Fig. 1 shows the effects arising from the Cu^{2+} binding. With lower concentration, modifications of the CD spectrum are only observed at higher pH. On the contrary, MCD spectra from ATP–Cu^{2+} complexes are only affected under very acid conditions (pH < 4).

For the d–d transitions ($\epsilon \cong 50$), the molar ellipticity is 100 times lower than for the transitions involved in the UV absorption range and is also weakly related to the pH value. We observe (Fig. 2) a negative band at 670 nm with a maximum amplitude for pH 5.6. At pH 8–9.5 a very low positive effect appears and decreases for the more basic pH values. At pH 10.5 we observe a characteristic pattern (+, –) which becomes (–, –) at pH 11.5, for the same wavelengths.

Many studies have shown that the basic medium promotes the metal–ribose interactions [7, 8, 15, 16], therefore we have been led to experiment the complexation of other adenoside derivatives with copper. These spectra are concentration independent and, at high pH values, the same characteristic patterns are observed under the same conditions with adenosine, AMP and ADP [17].

Nevertheless, with dATP the complexation of Cu^{2+} remains ineffective on the aromatic range of the CD spectra. The 300 nm band observed with ATP in basic medium (pH = 11.5) does not appear with dATP [17]. In the metallic absorption range the same kind of results are obtained. Cu^{2+} complexes of ATP and dATP have similar spectra in acid medium and as far as pH 6. When the pH value increases, the effect decreases slowly for dATP and the maximum absorption position shifts to the higher wavelengths. The characteristic spectra of ATP in basic medium (pH 10.5 and 11.5) do not appear with dATP.

Discussion

Many potentiometric studies of the Cu^{2+}–ATP complexes have been published; they have allowed for the determination of the different species in solution and the calculation of the stability constants [2, 18, 19]. Buisson and Sigel [2] have demonstrated that the dinuclear and dihydroxylated species do not exist. We chose the experimental conditions described by Buisson and Sigel to calculate the theoretical distribution of the different complexes as a function of the starting concentrations. Indeed the stability constant values are dependent upon both the solvent and the ionic strength. Therefore, the CD spectra modifications observed might be related with some peculiar species of the complexes assumed to be present in the solution.

At pH 6, $(Cu–ATP)^{2-}$ is the only complex formed in the solution whatever the metal or ligand concentration; consequently the 680 nm negative band reflects the structure of this complex. On the other hand, no modification of CD spectra is detected in

the aromatic absorption range (Fig. 1); therefore, as has been assumed by Schneider and Brintzinger [1], the adenylic nucleus of $(Cu–ATP)^{2-}$ is probably in the coordination plane of the metal. Such a conformation is in agreement with the phosphate and N(7) binding sites detected by NMR studies [20–24].

For higher pH values the concentration of $Cu(ATP)(OH)^{3-}$ increases slowly, this species is related with a positive low intensity dichroic absorption near 680 nm which probably reflects an unfolded structure without an N(7) binding site; indeed it has been shown that for such a complex, the Cu^{2+} catalytic effect on the ATP hydrolysis is quenched [2].

The distribution curves are only available for pH values lower than 9. Beyond this value, the analysis of the CD spectra leads us to assume the presence of two new complex species. Whatever the nucleotide (AMP, ADP or ATP) the CD curves at high pH values are similar [17]; consequently, the CD spectra would be only due to the ribose–metal interactions. Indeed Reinert and Weiss [15] have previously shown that the analysis of the titration curves of an Ado–Cu^{2+} solution suggest, at pH 10.5 and 12, two kinds of complexes, which necessarily involve sugar–metal bindings only. The possibility of ribose residue to copper binding for the Cu^{2+} complexes has furthermore been suggested by R. P. Martin and J. Pradel [8] in agreements with the results of equilibrium studies with deoxyadenosine.

In the first complex (A) prevalent at pH 10.5, only one hydroxyl is ionised and may bind the metal ion, whereas at higher pH (complex B), the second hydroxyl is also ionised and binds strongly the metal ion. In this complex (B) the phosphate groups do not bind the metal ions; indeed a similar study of the ternary complexes ATP–Cu^{2+}–2,2′bipyridyl shows only the characteristic CD spectrum of the second complex (B) [17]. The reverse of the 520 nm band which became positive at pH 10.5 may be related with the formation of a phosphate–metal–sugar hydroxyl macrochelate.

The CD difference spectrum (Fig. 1) which is only significant at pH 10, is similar to the CD spectra obtained with sugar metal complexes, when both hydroxyls are on the same side of the sugar plane [25, 26]. Moreover, the CD spectra of the complexes AMP–Cu^{2+}–spermine and Ado–Cu^{2+}–$(NH_3)_4$ show also a 300 nm band due to the interaction between sugar hydroxyls and the metal ion [16, 24].

For dATP–Cu^{2+}, in the basic range, none of the characteristic CD spectra previously described is obtained. Therefore, this indicates that sugar cannot bind Cu^{2+}. Moreover, Reinert and Weiss have suggested that the ionisation of the 3′ hydroxyl is prevented by the removal of the 2′ oxygen [15].

The Cu^{2+}–Cordycepin (3′-dAdo) complex does not possess a hydroxyl in the 3′ position. The characteristic CD difference spectrum (Fig. 1) of $(ATP–Cu)^{2-}$ in alkaline medium does not exist [17]. This means that the absence of either of the two sugar hydroxyls cancels the metal ion–ribose binding. The MCD spectra do not allow a conformational study but the bisignate MCD of Figure 1 strongly indicates a pseudo A-term, composed of two oppositely-signed B-terms due to two $\pi \rightarrow \pi^*$ transitions with a mutual perpendicular polarisation but adjacent on the frequency scale, in the adenine residue. The modifications observed in a very acid medium would be related with the ionisation of the phosphate groups or with the $n \rightarrow \pi^*$ transitions [16, 27] as suggested by some authors at shorter wavelengths [11, 12].

In conclusion, this study shows that ribose–metal interactions occur in systems containing Cu^{2+} and adenosine, AMP, ADP or ATP; these interactions should be considered in solutions with a pH higher than 10. The occurrence of such ribose–metal interactions in nucleotide complexes results in modifications of the CD spectra which are large enough to be used in more complex biological media.

Such interactions might be involved in the enzymatic mechanism and in the coenzyme binding processes of the metalloenzymes and kinases.

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