SELF-ASSOCIATION OF Co^{2+}- NUCLEOTIDE AND Co^{2+}- DINUCLEOTIDE COMPLEXES

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SUMMARY: Ultraviolet circular dichroism of 0.1 M Co^{2+}- nucleotide complexes exhibits drastic changes when the secondary terminal phosphate group is deprotonated, changes not observed in 10^{-4} M solutions. The visible C.D. of 0.1 M Co^{2+}- ADP complex, much more intense than the C.D. of Co^{2+}- ATP complex, follows the same evolution. These changes are related to a modification in self-association. Visible C.D. of Co^{2+}- dinucleotide complexes is also concentration and pH dependant: modification of intermolecular interactions occurs when the adenine N - 1 is deprotonated.

The interaction of metal ions with nucleotides and dinucleotides has been well studied by various potentiometric and spectroscopic methods. Insight into the metal ion environment can be gained by broadening studies of the proton signals of the adenine ring in the presence of paramagnetic transition metal ion (Co^{2+}, Ni^{2+}, Mn^{2+}) substituting for magnesium, for instance. This method, though restricted to $\frac{[\text{metal ion}]}{[\text{nucleotide}]}$ ratio circa $1/100$ has been widely used. Circular dichroism (C.D.) studies of the d - d transitions of the metal ion have been neglected up to now though they offer an opportunity to obtain information at $\frac{[\text{metal ion}]}{[\text{nucleotide}]}$ ratios, near unity, and concentrations comparable with those observed in living systems, as far as nucleotides and Mg^{2+} are concerned. A recent C.D. study (1) of the d - d transitions of Cu^{2+} in presence of nucleotides at low concentration, revealed an interaction Cu^{2+} - ribose at pH 11.

We report here some results of C.D. studies of the visible transitions of Co^{2+} in presence of nucleotides (ADP, ATP) or dinucleotides (NAD, NADP) at concentrations where self-association occurs. The formation of 1:1 Co^{2+}- nucleotide or 1:1 Co^{2+}- dinucleotide

complexes has been well studied and their stability constants are known (2); the metal ion is bound to the phosphate moiety and, through a water molecule, to N(7) of the adenine ring (3,4,5).

EXPERIMENTAL PROCEDURE AND RESULTS: The C.D. spectra were measured on a Roussel-Jouan dichrograph model CD 185 or a Jobin-Yvon CNRS dichrograph model Mark III. The nucleotides and dinucleotides purchased from Sigma were used without further purification.

1) C.D. spectra in the visible region.

- 1:1 mixtures of 0.05 M ATP and Co^{2+} exhibit C.D. spectra between 450 and 600 nm showing pH dependance, but with a constant order of magnitude ($\Delta\epsilon_{Max} \sim 2 \times 10^{-3}$).
- 1:1 mixtures of 0.05 M ADP and Co^{2+} give very different results: when pH varies from 2.5 to 7, an intense C.D. signal (figure 1) appears progressively, the maximum C.D. ($\Delta\epsilon_{520} \sim 48 \times 10^{-3}$) being obtained for pH 7.
- 1:1 mixtures of 0.1 M NAD (or NADP) and Co^{2+} show the same evolution as a function of pH as ADP - Co^{2+} mixtures, the inflexion point being situated near pH 3.7 and the C.D. signal intensity reaching a plateau near pH 5 ($\Delta\epsilon_{520} = 10 \times 10^{-3}$).

2) Evolution of C.D. spectra as a function of $\left[Co^{2+}\right] / \left[\text{ADP}\right]$ or $\left[Co^{2+}\right] / \left[\text{dinucleotide}\right]$.

Due to the high stability constant of the $1/_1$ studied complexes, the added Co^{2+} is totally complexed and the observed C.D. ought to increase linearly until a ratio $\frac{[Co^{2+}]}{[ADP]} = 1$ is reached and then to remain constant.

Actually this is not observed; the observed signal reaches a maximum for $\left[Co^{2+}\right] / \left[\text{ADP}\right] = 0.25$ and tends asymptotically towards a limit for $\left[Co^{2+}\right] / \left[\text{dinucleotide}\right] > 2$.

Further at pH 5.3 no C.D. signal is observed in solutions with a $\left[Co^{2+}\right] / \left[\text{ADP}\right]$ ratio = $5 \times 10^{-2}/5 \times 10^{-4}$ whereas an intense signal ($\Delta\epsilon_{520}$ per Co^{2+} atom = 0.30) is observed for solutions where $\left[Co^{2+}\right] / \left[\text{ADP}\right]$ ratio = $5 \times 10^{-4}/5 \times 10^{-2}$. The same discrepancy is observed for mixtures of Co^{2+} and dinucleotides. This would indicate that the interaction followed by C.D. is not a 1:1 one and moreover that self-association is necessary to observe it.

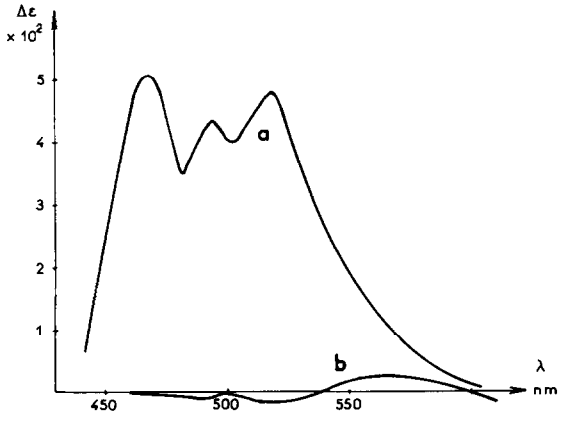

Fig. 1. C.D. spectra of 0.1 M 1/1 Co^{2+}- ADP (a) and Co^{2+}- ATP (b) solutions at pH 7

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3) C.D. spectra in the U.V. region.

Between 300 and 240 nm, the spectrum of monomeric ATP only exhibits a negative band at 255 nm. When self-association occurs, an extra band, positive, appears at 270 nm; changes in the magnitude of this band allow to follow changes in self-association (6,7). For ADP we found similar results, in particular the same evolution as a function of pH, i.e. a maximum C.D. near pH 2.8.

In presence of Co^{2+}, the evolution of U.V.D.C. of 0.1 N ADP or ATP solutions as a function of pH is quite different: the magnitude of the 255 and 270 nm bands follows the same evolution as the 520 nm band one in Co - ADP complex: it increases as pH is increased and reaches a maximum near pH 7, where it is about five times larger as for the free nucleotide.

INTERPRETATION: The evolution of the U.V.C.D. spectra as a function of pH and concentration is quite comparable to the one observed by proton magnetic resonance for Mn - ATP and Cu - ATP complexes (8,9). As pH varies around the secondary ionization of γ phosphate group, drastic changes occur in spectra but only at high nucleotide concentrations. This may be accounted for (8) by a change in the ligand exchange mechanism accompanying the structural changes in close relation to the formation of higher nucleotide polymers.

If the amplitude of the C.D. signal is interpreted in terms of the degenerate exciton model, the increase of the intensity of the

doublet near 260 nm may indicate a sound modification of the angles between the transition moments of the adenine groups of the polymers.

The comparable evolution, as a function of pH, of the U.V.C.D. of Co^{2+}- nucleotides complexes and of the visible C.D. of the Co^{2+}-ADP complex as also the smaller magnitude of the visible D.C. for mixtures $\frac{Co^{2+}}{ADP} = \frac{10^{-1}}{10^{-3}}$ versus the magnitude of mixtures $\frac{Co^{2+}}{ADP} = \frac{10^{-3}}{10^{-1}}$, indicate that the optical activity of d - d transitions in Co^{2+}- ADP complex is related to self-association of the nucleotide.

The self-association of dinucleotides cannot be studied by the U.V.C.D. because in this region, to the optical activity originating in the intermolecular interaction of the transition moments of adenines, is added the optical activity originating in the intramolecular interaction of adenine and nicotinamide transition moments. On the contrary, we think that the visible D.C. monitors the intermolecular association in Co^{2+}- NAD and Co^{2+}- NADP complexes without being influenced by the folding of adenine and nicotinamide, because the C.D. follows the same evolution as in Co^{2+}- ADP complex (except that the drastic changes occur near the pK of protonation of adenine).

Therefore one can say that in the course of protonation of adenine N - 1 no gross change in molecular shape occurs (10) but that intermolecular association is modified.

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