A Spectrophotometric Study of the Binding of Cu^{2+} Ions to Transfer Ribonucleic Acid

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In this work UV, VIS and NIR difference spectroscopy have been used in order to study Cu^{2+} ions binding to unfractionated E. coli *tRNA. The binding of Cu^{2+} ions to tRNA molecules leads to an increase in intensity of the characteristic absorption band of the $[Cu(H_2O)_6]^{2+}$ ion in the range 500–900 nm and to a small blue shift in the wavelength of its absorption maximum. Besides this, Cu^{2+} binding induces specific changes in the tRNA electronic spectrum in the 230–450 nm range. The spectral changes thus observed indicate a direct binding of the metal ion to the nitrogen atoms in the heterocyclic bases of the tRNA molecules. An analysis of these effects was used for studying Cu^{2+} binding to tRNA as a function of Cu^{2+} concentration as well as the competitive effects of other metal ions.*

Introduction

It is well known that the biological functionality of tRNA depends on the existence of a well-established tertiary structure of the molecule in solution and that the ionic environment plays a major role in determining and stabilizing that structure [1]. Transfer ribonucleic acids are polyelectrolytes with a high negative charge, so that their binding to the cations causes, above all, a reduction of the electrostatic repulsion between neighbouring residues. In this way cation binding favours a well-established conformation of the macromolecule. It is generally presumed that the biologically active conformation is achieved only in the presence of appropriate salt concentrations. Some authors consider that a wide number of mono and divalent cations, transition metals included, are equivalent in producing this effect [2]. Most of the studies on divalent cation binding to tRNA have been carried out at low ionic strength (<10 m*M* Na^+ ion). In these conditions tRNA exhibits an 'extended form' conformation, different from the 'native' one [3]. The analysis of cation binding is then complicated by two factors: a) the addition of divalent ions is coupled to a reduction of the net charge of the polyelectrolyte and b) the macromolecule conformation changes [4–6]. Both these factors are less important at high ionic strength. In fact, at ionic strength greater than 0.1 *M*, tRNA occurs in a 'native' conformation even in the absence of divalent ions and addition of these ions in this case gives rise to a stabilization of the structure without other conformational changes [3, 7]. Notwithstanding this, only a few studies have been carried out under high ionic strength conditions even though these are closer to the physiological ones [8, 9]. The studies on transition metal complexes are particularly interesting since the toxicity of some of these metals may be a result of their binding to nucleic acids.

This work reports an analysis of Cu^{2+} ion binding to unfractionated *E. coli* tRNA under high ionic strength conditions. Use has been made of a difference spectroscopic technique in UV, VIS and NIR in order to study the formation of the tRNA–Cu^{2+} complex both in the aqueous ion absorption band (at about 800 nm) and in the absorption band of the tRNA heterocyclic bases (230–350 nm).

Experimental

In order to remove divalent cations, *E. coli* MRE 600 unfractionated tRNA (Boehringer, Mannheim GMBH) was dialysed at 4 °C overnight againt 4 l of 0.1 *M* NaCl, 4×10^{-3} *M* EDTA (disodium salt) pH 7 and then against two charges of 0.1 *M* NaCl pH 7. The samples were stored at −20 °C until used. As a rule, tRNA absorption at 260 nm was 15 for the measurements carried out in the 300–990 nm range and 1.5 for the 240–300 nm measurements. The tRNA nucleotide concentration was determined by assuming a molar extinction coefficient equal to 7500 [10]. Spectral changes following the addition of divalent ions to the tRNA solution were measured with a Mod. 360 Shimadzu spectrophotometer. 3 ml

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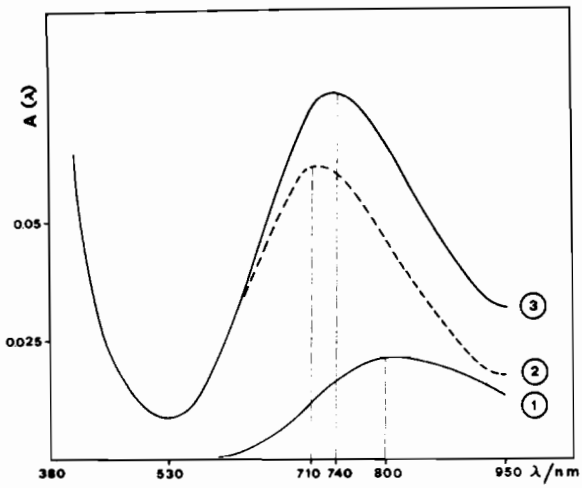

Fig. 1. Absorption and difference spectra for the tRNA–Cu^{2+} complex: 1) the $[Cu(H_2O)_6]^{2+}$ absorption spectrum $A_1(\lambda)$; 2) the difference spectrum $\Delta A(\lambda) = A_3(\lambda) - A_1(\lambda)$; 3) the tRNA–$Cu^{2+}$ absorption spectrum $A_3(\lambda)$ for $[Cu^{2+}]/[PO_4^-] = 0.3$. Spectrum 1 was obtained adding 15 μl of 100 mM $CuCl_2$ to 2.5 ml of a 0.1 M NaCl solution and spectrum 3 by adding the same quantity of 100 mM $CuCl_2$ to 2.5 ml of a tRNA solution in 0.1 M NaCl, $A_{260} = 15$, pH 7.

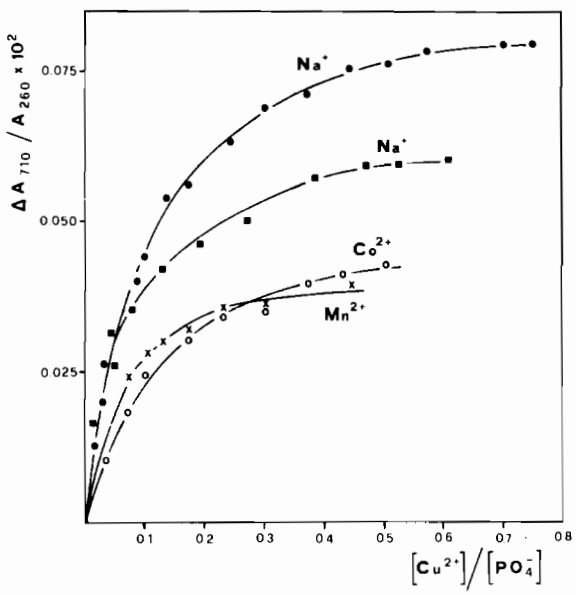

Fig. 2. Experimental curves for the ΔA_{710} dependence on the ratio $[Cu^{2+}]/[PO_4^-]$, in the presence of other metal ions: (•) $[Na^+] = 0.1$ M; (▪) $[Na^+] = 0.7$ M; (×) $[Mn^{2+}] = 0.01$ M; (○) $[Co^{2+}] = 0.01$ M. The ΔA_{710} parameter was normalized with respect to A_{260}.

quartz cells 1 cm long were used. Absorption changes were detected by a difference method as described elsewhere [11] (see also the legends to the figures). The tRNA solutions were unbuffered (with pH about 7) in order to prevent the interaction of Cu^{2+} with buffers. All the measurements were performed at room temperature.

Results and Discussion

Cu^{2+} ions in the presence of non complexing anions exhibit an absorption spectrum in the visible and NIR region (Fig. 1). The absorption band appears because of the splitting of the five-fold degenerate ^2D state of the 3d electrons induced by the ligand field, and the consequent transitions of these electrons between the ground level and the higher ones. For the Cu^{2+} aqueous ion the field is due to six water dipoles and the absorption band maximum in this case arises at about 800 nm. The addition of ligands to such aqueous solutions leads to the formation of complexes by the successive displacement of water molecules [12].

The spectrum of the copper aqueous ion $[Cu(H_2O)_6]^{2+}$ changes in the presence of the tRNA molecule: a marked increase in absorption and a shift in its maximum from 800 to 740 nm are observed (Fig. 1). This effect is similar to the one observed on coordination of Cu^{2+} with ammonia or ethylenediamine in aqueous solutions when the complex $[Cu(NH_3)(H_2O)_5]^{2+}$ forms, and is due to the stronger ligand field of the nitrogen derivatives [12]. It may therefore indicate that the copper(II) aqueous ion exchanges a water molecule with a heterocyclic base of tRNA at one of its nitrogen donor sites, creating a coordination complex.

Letting $A_1(\lambda)$ be the absorbance of the aqueous cupric ion and $A_3(\lambda)$ be the absorbance of the complex tRNA–Cu^{2+}, we can prove that the difference $\Delta A(\lambda) = A_3(\lambda) - A_1(\lambda)$ is proportional to the bound copper concentration. In fact:

$$A_3(\lambda) = [Cu^{2+}]_b \epsilon_b l + [Cu^{2+}]_f \epsilon_f l$$

$$A_1(\lambda) = ([Cu^{2+}]_b + [Cu^{2+}]_f)\epsilon_f l$$

hence

$$\Delta A(\lambda) = A_3(\lambda) - A_1(\lambda) = [Cu^{2+}]_b(\epsilon_b - {}_f\epsilon) \cdot l \propto [Cu^{2+}]_t$$

where ϵ_b and ϵ_f mean the respective molar extinction coefficient for Cu^{2+} bound in the tRNA complex and free in solution; l indicates the optical pathlength.

The difference $\Delta A(\lambda)$ has a maximum at 710 nm, indicated by ΔA_{710}. Figure 2 shows that ΔA_{710} increases immediately as Cu^{2+} ions are added and that a saturation process occurs only for a $[Cu^{2+}]/[PO_4^-]$ ratio higher than 0.5. An increase in the NaCl concentration from 0.1 to 0.7 M has little influence on the formation of the tRNA–Cu^{2+} complex, the effect

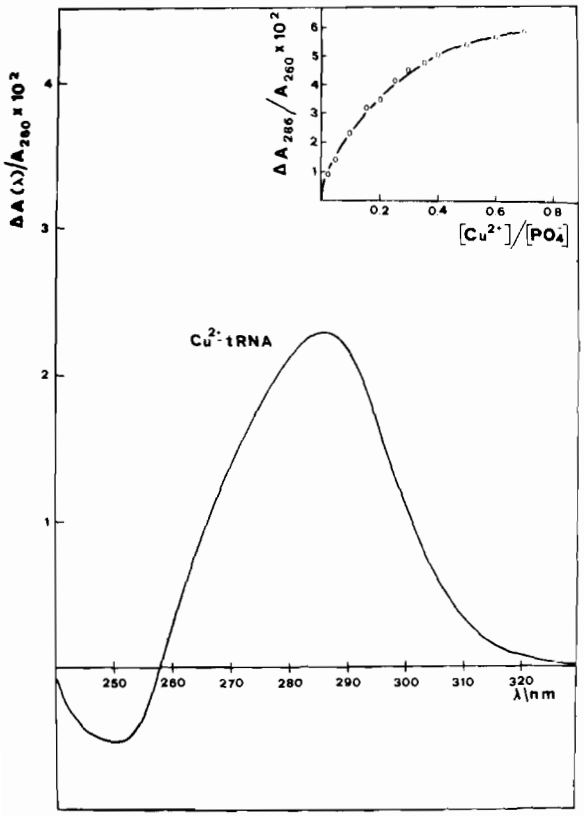

Fig. 3. UV difference spectrum for the tRNA–Cu^{2+} complex. $[Cu^{2+}]/[PO_4^-]$ = 0.3, pH 7. The sample and reference cells were filled with 2.5 ml of a tRNA solution in 0.1 *M* NaCl (A_{260} = 1.5, pH 7); 15 μl of 10 m*M* $CuCl_2$ were then added to the sample cell and the same amount of solvent was added to the reference cell to avoid any correction for dilution. This difference spectrum has a maximum at 286 nm whose value, indicated by ΔA_{286}, increases with the added copper concentration (see inset).

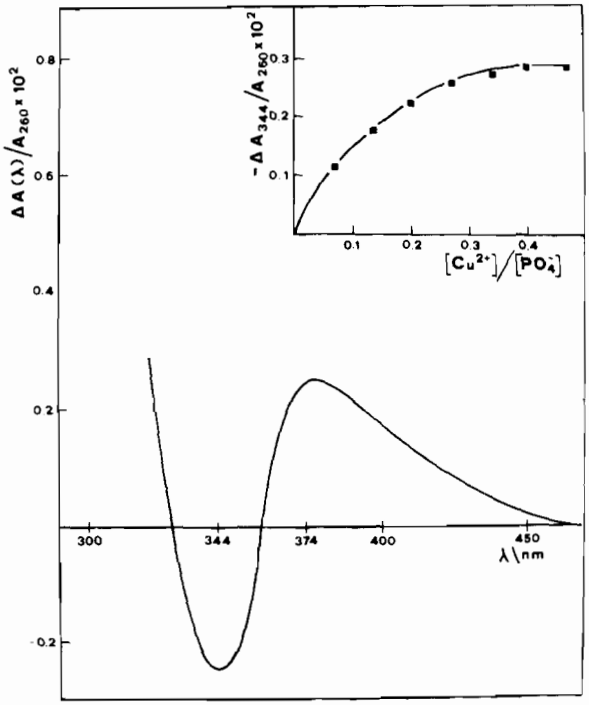

Fig. 4. UV difference spectrum for the tRNA–Cu^{2+} complex in the region where the 4-TU base absorbs. This difference spectrum was obtained with the same procedure as those in Fig. 3, except for the copper and tRNA concentrations: 15 μl of 100 m*M* $CuCl_2$ were added to a tRNA solution whose A_{260} = 15. It presents a minimum at 344 nm whose value indicated by $-\Delta A_{344}$ increases with copper concentration (see inset).

being manifest mainly at $[Cu^{2+}]/[PO_4^-]$ ratios higher than 0.05. An increase in the ionic strength should inhibit the formation of those complexes which involve an electrostatic component of the interaction energy (for example binding to the phosphate groups). Our data may therefore indicate the formation of two kinds of Cu^{2+} complex with tRNA: the formation of one complex, which appears at low Cu^{2+} concentrations ($[Cu^{2+}]/[PO_4^-] < 0.05$), is not influenced by an increase in the ionic strength; the second complex proves to be partially inhibited by an inrease in the sodium concentration. It may be noted that the 0.05 value for the $[Cu^{2+}]/[PO_4^-]$ ratio corresponds to four Cu^{2+} ions for each tRNA molecule. This number is of the same order of magnitude as the one for strong binding sites found by various authors for divalent ions in the tRNA complexes [1]. The curves in Fig. 2 show, moreover, that the formation of the tRNA–Cu^{2+} complex is hindered by the presence of small quantities of Co^{2+} or Mn^{2+} ions (at a concentration of 0.01 *M*) and this fact suggests a competition of the Cu^{2+} ions with the other transition metals for the same sites on the tRNA molecule.

A further indication for the binding of the transition metal to the tRNA heterocyclic bases may be obtained from an analysis of the changes in the optical properties of bases which accompany the binding of Cu^{2+}, Mn^{2+} and Co^{2+} ions. When Cu^{2+} is added to the tRNA solution a change in the tRNA electronic spectrum is observed, with the occurrence of an absorption band in the 240–300 nm range (Fig. 3). This effect is similar to the one observed for other transition metals such as Mn^{2+} and Co^{2+} [13]. The occurrence of the absorption band in this spectral range was attributed to a perturbation of the electronic system of the bases due to interaction with the ions.

The formation of the Cu^{2+}-tRNA complex is also accompanied by small changes in absorbance in the 300–450 nm spectral range. The absorption in this case is due to the naturally occuring modified base 4-thiouracil (4-TU) in position 8 of many *E. coli*

tRNAs [14]; the small changes, observed in the difference spectrum shown in Fig. 4 upon adding Cu^{2+} ions are to be ascribed to local variations of the stacking interactions of this base environment. The minimum in this difference spectrum occurs at 344 nm and its value ΔA_{344} exhibits a variation with the $[Cu^{2+}]/[PO_4^-]$ ratio (see inset of Fig. 1) similar to the one observed at 710 nm (Fig. 2) and 286 nm (inset of Fig. 3). This suggests that the binding of Cu^{2+} ions gives rise to subtle variations at the level of the tertiary structure of the macromolecule which affect the local stacking around the 4-TU nucleotide. These results are in agreement with those of other authors [15, 16] and indicate that the 4-TU base is a natural spectroscopic probe, sensitive to the tertiary tRNA structure.

In conclusion, we should like to underline the utility of the differential spectroscopic technique, when working in conditions of high sensitivity: with a total deflection corresponding to 0.01 units of optical density, optical density differences may be measured with an accuracy of 10^{-4}. In these conditions we were able to detect the small changes in absorbance which appear when the tRNA–Cu^{2+} complex forms, and to bring out some of the binding mechanisms. In particular our data furnish evidence for the binding of Cu^{2+} ions with the atoms of tRNA bases. The same data provide indications for the existence of two kinds of binding, one of which, not influenced by ionic strength, presumably involves the atoms of bases only while the other one, partially inhibited by an increase in the ionic strength, may involve binding with the phosphate groups too. Moreover, even in the presence of high sodium concentrations, binding with Cu^{2+} ions induces some changes in macromolecule conformation, as observed in the region of the 4-TU base absorption.

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