

# A study on the effect of $\text{Cu}^{2+}$ ions on the stability of triple helix poly[(riboadenylic acid):2 poly(riboinosinic acid)]

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## SUMMARY:

The effect of  $\text{Cu}^{2+}$  on the stability of the triple-stranded helical structure of poly[(riboadenylic acid):2poly(riboinosinic acid)] poly(A:2I) was studied by means of circular dichroism spectroscopy and ultraviolet spectroscopy. The experimental results show that  $\text{Cu}^{2+}$  induces the hyperchromicity of poly(A:2I) and causes the melting temperature  $T_m$  of poly(A:2I) to decrease with increasing  $\text{Cu}^{2+}$  concentration. At the ratio of the concentration of  $\text{Cu}^{2+}$  to that of phosphate in poly(A:2I),  $r_1 = 0$ , poly(A:2I) is stable, at  $r_1 = 0.27$ , poly(A:2I) decays in poly(A) and 2 poly(I), and at  $r_1 = 1.6$ , there is a racemate.

## Introduction

When Felsenfeld et al. reported a triple helical structure for poly[(riboadenylic acid):2poly(riboinosinic acid)] poly(A:2I)<sup>1)</sup>, a great deal of physicochemical study has been done to examine the spectroscopic and thermodynamic properties of synthetic triple helical polymers of nucleic acids<sup>2–5)</sup>. Because of the special structure and biological functions of the triple helix, many attempts are being made to probe the intimate structural details<sup>3,6–9)</sup> and biological functions<sup>10–16)</sup>. Moreover, it is known that metal ions play an important role in maintaining the conformational stability of nucleic acids and in controlling their biological functions<sup>17–20)</sup>. Among metal ions, the effect on the properties of nucleic acids by copper ions is the object of the most comprehensive studies<sup>21)</sup>. As a part in our long-term interest in the effect of metal ions on the structure of synthetic polynucleotide acids, we focused our attention on the interaction of  $\text{Cu}^{2+}$  with poly(A:2I) which has been extensively studied<sup>22–24)</sup>.

## Experimental part

Poly(riboadenylic acid) (poly(A)) and poly(riboinosinic acid) (poly(I)) were purchased from Sigma Chemical Co., St. Louis, Mo, U.S.A. Poly(I) and poly(A) were exhaustively dialyzed against 0.01 mol/L ethylenediaminetetraacetic acid (EDTA), doubly distilled water and 0.1 mol/L NaCl, followed by several changes of 0.1 mol/L NaCl, pH = 6.6, for 48 h to remove any contaminants at 4 °C. Concentrations of these substances were determined spectrophotometrically using the absorption coefficients described previously<sup>25)</sup>. The poly(A:2I) solution was prepared by mixing poly(A) with poly(I), in which the mole ratio of poly(A) to poly(I) is 1/2. Poly(A:2I) was also dialyzed against 0.001 mol/L

EDTA, and doubly distilled water several times for 3 days to remove NaCl, then lyophilized. All other materials were of analytical reagent grade. The buffer solution was  $5 \times 10^{-3}$  mol/L Tris-HCl solution<sup>a</sup>, pH = 7.0.

Circular dichroism (CD) spectra were recorded on a J-500 Circular Dichroism spectrophotometer (JASCO, Japan) by using a stoppered 5 mm cell. The ultraviolet spectra and melting curves were recorded on a UV-365 spectrophotometer (Shimadzu, Japan) and the temperature was controlled by a UV-365 TB-85 Thermoregulator (Shimadzu, Japan) with 1 cm cell.

All the reactions were carried out at different  $r_1$  which is the ratio of the concentration of  $\text{Cu}^{2+}$  to that of phosphate in poly(A:2I) at 4°C in a refrigerator for at least 5 days, but the measurement was at 25°C.

## Results and discussion

### Circular dichroism studies

The CD spectra of the interaction of  $\text{Cu}^{2+}$  with the triple helix poly(A:2I) were recorded. As shown in Fig. 1, the characteristic peaks of the triple helix poly(A:2I) are those at 243 nm, 258 nm and 279 nm, respectively, which are the same as those of the triple-stranded helical structure reported by K. Fujioka et al.<sup>23</sup>. When the

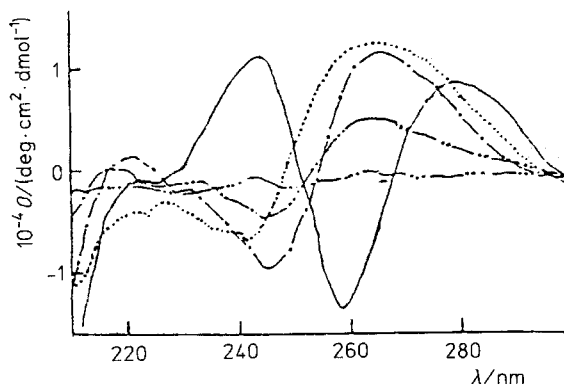


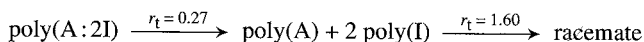
Fig. 1. Circular dichroism spectra of the interaction of  $\text{Cu}^{2+}$  with poly(A:2I) at 0.1 mol/L NaCl in  $5 \times 10^{-3}$  mol/L Tris-HCl, pH = 7.0; concentration of poly(A:2I) indicated as the concentration of phosphate in poly(A:2I)  $C_{\text{poly(A:2I)(P)}} = 1.87 \times 10^{-4}$  mol/L, ratio of the concentration of  $\text{Cu}^{2+}$  to that of phosphate in poly(A:2I)  $r_1$ : 0 (—); 0.27 (---); 0.80 (-·-·-); 1.60 (- - - -); after adding slightly excessive EDTA at  $r_1 = 1.60$  (·····)

$\text{Cu}^{2+}$  concentration is increased to a ratio of the concentration of  $\text{Cu}^{2+}$  to that of phosphate in poly(A:2I), at  $r_1 = 0.27$ , the characteristic peaks of the triple-stranded helical structure of poly(A:2I) completely disappear and are transformed into two positive peaks at 221 nm and 265 nm and a negative peak at 245 nm, these peaks are similar to those synthesized by adding the CD spectrum of only poly(I) to that of only poly(A), which suggests no formation of the triple helix poly(A:2I)<sup>22</sup>, indicating that  $\text{Cu}^{2+}$  may cause the triple helix to unwind into the single-stranded poly(I) and poly(A). When the  $\text{Cu}^{2+}$  concentration continues to be increased above  $r_1 = 0.27$ , the CD spectrum of poly(A:2I) exhibits a decrease in intensity at  $r_1 = 0.80$ ,

<sup>a</sup>) Tris = tris(hydroxymethyl)aminomethane (systematic IUPAC name: 2-amino-2,2-bis(hydroxymethyl)ethanol).

suggesting that the more relaxed structure may appear at a higher  $\text{Cu}^{2+}$  concentration<sup>26)</sup>, whereas at  $r_t = 1.60$ , the CD spectrum becomes almost a straight line, indicating that a racemate could exist at an ellipticity  $[\theta] \approx 0$ . When slightly excessive ethylenediaminetetraacetic acid (EDTA) is added to the solution at  $r_t = 1.60$ , the CD spectrum is somewhat similar to that of the single-stranded poly(A) and poly(I), i. e., after  $\text{Cu}^{2+}$  is completely complexed by EDTA, the racemized poly(A:2I) cannot regain the triple-stranded helical structure.

The above results may be represented by the following scheme:



### Thermal denaturation and ultraviolet spectra

The thermal denaturations of solutions of poly(A:2I) with various  $r_t$  are studied. As shown in Fig. 2, a significant decrease in the melting temperature ( $T_m$ ) is observed with increasing  $r_t$  at  $r_t = 0.27, 0.80$ , and  $1.60$  as compared with  $T_m$  of

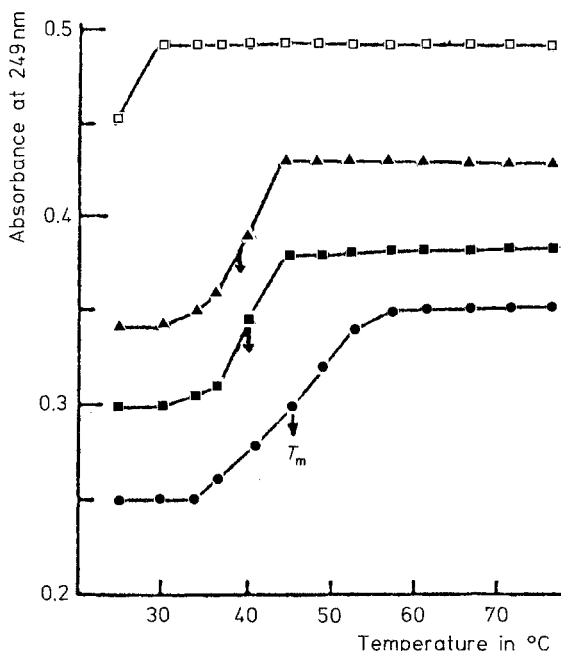


Fig. 2. Effect of  $\text{Cu}^{2+}$  on the melting temperature  $T_m$  of poly(A:2I) at 0.1 mol/L NaCl in  $5 \times 10^{-3}$  mol/L Tris-HCl, pH = 7.0; concentration of poly(A:2I) indicated as the concentration of phosphate in poly(A:2I)  $C_{\text{poly(A:2I)(P)}} = 5.70 \times 10^{-5}$  mol/L; ratio of the concentration of  $\text{Cu}^{2+}$  to that of phosphate in poly(A:2I)  $r_t$ : 0 (●—●); 0.27 (■—■); 0.80 (▲—▲); 1.60 (□—□)

poly(A:2I). This indicates that the helical structure of poly(A:2I) is destabilized by adding  $\text{Cu}^{2+}$  ions<sup>27,28)</sup>. In addition, the ultraviolet absorption spectrum of poly(A:2I) and  $\text{Cu}^{2+}$  shows that  $\text{Cu}^{2+}$  induces the hyperchromicity of poly(A:2I) with increasing  $\text{Cu}^{2+}$  concentration. The hyperchromicity could arise from a destabilization and partial opening of the hydrogen-bonded helical structure in poly(A:2I)<sup>28,29)</sup>, and from a structural transition of the triple helix into the coil form<sup>30)</sup>. The above results

show that  $\text{Cu}^{2+}$  can break the hydrogen bonds of base-paired residues and destabilize the triple-stranded helical structure of poly(A:2I). This confirms further that  $\text{Cu}^{2+}$  can cause the triple-stranded structure of poly(A:2I) to unwind by breaking the hydrogen-bonded structure in poly(A:2I). This result is consistent with that obtained from CD studies.

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