Interaction of Ribonuclease A with Cu^{++} in the Presence of Nucleotides. An ^1H NMR and ESR Study.

L.Sportelli and V.Viti[+]

Dipartimento di Fisica, Università della Calabria, 87100 Cosenza, Italy and [+]Laboratorio di Biologia Cellulare ed Immunologia, Istituto Superiore di Sanità, Viale Regina Elena, 299; Rome-Italy.

The reassignment of the ^1H NMR C-2 histidine resonances of RNase A has imposed a revision of the role of the different histidines in their interaction with Cu^{++} (1). From the examination of the ESR and ^1H NMR data it appears that, at pH 5.5, the Cu^{++}-enzyme complex exhibits nearly a square planar symmetry and that a nitrogen atom of the His-119 is present in the coordination sphere of the metal ion. On the other hand, at pH 7.0 the complex shows a rhombic symmetry and two nitrogen ligand atoms are provided by His-105 and -119.
The strongest interaction site of the RNase A with the nucleotides 2'-CMP and 3'-CMP is the His-12. When at pH 5.5 Cu^{++} and 2'-CMP are both present in the solution it is possible to observe a competition for the Cu^{++} interaction site located at the His-119 depending on the preparation of the ternary complex while, on the contrary, when 2'-CMP is replaced by 3'-CMP only additive effects are observable on all the histidines of RNase A.
Similar effects are observable at pH 7.0, too.
Furthermore, the modification induced by 2'-CMP and 3'-CMP on the ESR spectra of the Cu^{++}-RNase A complex allows to obtain useful informations on the geometry of the ternary complexes.

1. Sportelli, L. and Viti, V. (1979) Biochim. Biophys.Acta 580, 100-107.