

Cu(II)-mediated stabilization of DNA duplexes having different numbers of 1,*N*⁶-ethenoadenine lesions as metal binding sites

(¹Graduate School of Science, The University of Tokyo) ○Silpa Chandran Rajasree,¹ Yusuke Takezawa,¹ Mitsuhiko Shionoya¹

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The interactions of ligand-type nucleobases with metal ions can result in the formation of metal-mediated unnatural base pairs, often accompanied by characteristic thermal stabilization of DNA duplexes.^[1] Metal-mediated base pairing is recently being utilized for controlling DNA structure and functions. The synthesis and optimization of aforementioned metal-mediated base pairs is an important cornerstone in the development of advanced metal-responsive DNA architectures such as allosteric DNazymes.^[2]

In this study, 1,*N*⁶-ethenoadenine (ϵ A), a well-known damaged nucleobase, was used as a ligand-type nucleobase for metal-mediated base pairing (Figure (a)).^[3] It was previously reported that ϵ A forms a Cu^{II}-mediated base pair (i.e., ϵ A–Cu^{II}– ϵ A) in DNA duplexes,^[4] but no significant duplex stabilization was observed ($\Delta T_m = +3$ °C). Since ϵ A–Cu^{II}– ϵ A base pairs are stabilized by stacking interactions, we hypothesized that the thermal stabilization of the duplex would be more pronounced with the incorporation of consecutive ϵ A–Cu^{II}– ϵ A base pairs (Figure (b)). Melting experiments showed that duplexes with one and two pairs of ϵ A– ϵ A (duplex I and II) were hardly stabilized by the addition of stoichiometric equivalents of Cu^{II} ions ($\Delta T_m = -0.2$ °C and $+1.0$ °C, respectively). In stark contrast, the thermal stability of DNA duplexes with three and four consecutive ϵ A– ϵ A pairs (duplex III and IV) was dramatically improved in the presence of stoichiometric equivalents of Cu^{II} ($\Delta T_m = +13.3$ °C and $+16.5$ °C, respectively; [duplex] = 20 μ M). The formation of multinuclear Cu^{II} complexes such as III·Cu₃ and IV·Cu₄ was confirmed by ESI mass spectrometry in the presence of Cu^{II} ions. Upon addition of Cu^{II} ions to the duplexes, the fluorescence signal of the fluorescent ϵ A nucleobases was quenched, confirming that each ϵ A base was coordinated to the Cu^{II} ion. These results indicate that the stabilization of the duplex is achieved by the formation of interstrand ϵ A–Cu^{II}– ϵ A base pairs. This study established that the incorporation of at least three consecutive ϵ A–Cu^{II}– ϵ A pairs is essential for the metal-dependent stabilization of the duplex structure. This Cu^{II}-mediated duplex stabilization assures the usefulness of the ϵ A–Cu^{II}– ϵ A pair in the construction of metal-responsive DNA architectures.

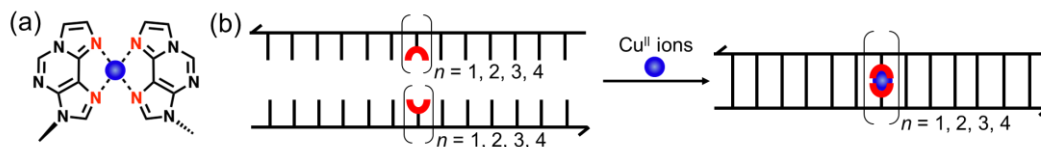


Figure (a) Chemical structure of Cu^{II}-mediated ethenoadenosine base pairs (ϵ A–Cu^{II}– ϵ A). (b) Formation of DNA duplexes containing different numbers of ϵ A–Cu^{II}– ϵ A base pairs.

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