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Mechanism of cleaving DNA through hydrolysis of a novel complex of Mg containing dien ligand

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Abstract: A series of metal complexes were designed and synthesized and a novel binuclear magnesium complex has been selected, namely $[Mg_2(dien)Cl(OH_2)_2]Cl_2 \cdot H_2O$ (dien=diethylene-triamine), which can cleave the plasmid pBR322 DNA effectively in close to physiological condition without adding any external materials. Through biological and chemical methods, especially the comparative experiments, we find the interaction between the complex and DNA belongs to hydrolytic mechanism.

Keywords: pBR322 DNA, metal complexes, dien ligand, BDNPP.

A series of elements necessary for life bodies, such as Mg, Cu, Mn, Fe, Co, Zn *etc*, are chosen as center ions of complexes, because most of them act as active centers of enzymes and auxiliary factors. We select dien as ligand because nitrogen, especially muti-nitrogen coordination is general in natural enzymes and simulated systems, furthermore dien has structural flexibility.

The complexes of Mg containing dien and the activity of cleaving DNA

A series of metals, such as Mg, Mn, Fe, Co, Ni, Cu and Zn, their chlorides reacted with dien in ethanol and synthetic products were obtained. Electrophoresis was done after the synthetic products reacted with pBR322 DNA and the results show, that only synthetic products $[Mg_2(dien)Cl(OH_2)_2]Cl_2 \cdot H_2O$ (Mg_2L) can cleave DNA effectively and make it linear.

The reaction conditions and the basic mechanism of Mg_2L′s cleaving DNA.

The activity of the reaction was measured and the appropriate conditions were got as follows: the lower salt concentration of buffer solution (Tris-HCl: 5mM, NaCl: 3.1mM), 37° C, pH=6.78-9.76, $[Mg_2L] \geq 2.5 \times 10^{-3}$M, avoiding light for 2-4 hours. In order to make sure whether Mg_2L's cleaving DNA belongs to the oxidation or hydrolysis mechanism, we did different comparative experiments: 1) under aerobic or anaerobic condition, the results show oxygen is not necessary for cleaving DNA, 2) scavenger of free radical, reductant and H_2O_2 do not affect the reactions, 3)connection of the cleaved products shows the connecting enzymes named T4DNA can make linear DNA to close ring again.

Via the above experiments we can infer the mechanism of the interaction of Mg_2L′s cleaving DNA belongs to hydrolysis.

The study on the interaction pattern of metal complexes with DNA

The interaction patterns of different complexes, mainly that of Mg_2L with DNA have been analyzed by means of UV, CD, fluorescence spectrum and thermal denature experiment.

When the concentration of Mg_2L is lower, the UV spectrum shows hypochromic effect, the positive band of CD weakens and fluorescence spectrum has no apparent changes. It suggests that Mg_2L binds with DNA by oxygen on phosphate at first and stabilizes double helix structure of DNA. When the concentration of Mg_2L is higher, the different results were obtained: hyperchromic effect of UV, the strengthened positive band of CD and fluorescence quenched. It suggests that Mg_2L cleaves DNA and destroys the structure of DNA. The phenomena of the interactions of CuL, CoL, NiL with DNA are different from those of Mg_2L. The results show that they are apt to bind with the base of DNA and denature DNA by changing its conformation.

The further demonstration of Mg_2L's cleaving phosphate diester linkage of DNA Effectively

We selected the model complex BDNPP(BDNPP=2,4-dinitrobenzalphosphate) which has the similar structure of phosphate diester linkage to DNA to replace DNA, made it react with Mg_2L and studied the hydrolytic mechanism.

The kinetic process of the reaction of Mg_2L with BDNPP was studied by UV. The peak of 2,4-dinitrophenol was strengthened gradually, it means the bond between phosphorus and oxygen was broken. HPLC analysis shows that the quantity of 2,4-dinitrophenol increased gradually with time increasing. These results further prove that Mg_2L cleaved DNA according to the mechanism of breaking phosphate diester linkage. In this course, Mg^{2+} ion as Lewis acid activated the combined water molecule and OH^- ion was produced. At the same time Mg^{2+} ion bound with negative ion of oxygen on phosphate by static electricity and oxygen atom was anchored. Another Mg^{2+} ion stabilized the product through combining with the oxygen on leaving group. Based on it, activated water molecule took nucleophilic attack and made the phosphorus atom unstable transition state coordinated with five atoms. As a result the bond between phosphorus and oxygen was broken. Because of the synergistic effect of the two Mg^{2+}, the great catalytic ability was produced that made the reaction very easy to process. This is the key reason that the binuclear Mg_2L can cut the DNA effectively.

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