Studies on the Interaction of DNA and a Novel of Water-soluble Polymer-bound Schiff Base Complex

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The modes and activities of the interaction of DNA and water-soluble polymer bound Schiff base nickel complex, which has been prepared by polymer analogous reaction, have been discussed according to fluorescent probe method. It indicates that polymer matrix effect and increasing solubility in water can increase the interaction of this polymer complex with DNA.

Significant developments have been occurred in recent years in the field of biopolymers and biomaterials. Especially, interesting are investigations of pharmacologically active polymers (polymer drug) which by themselves may be active as drugs or alternatively may be used as carries for normal pharmaceutical agents.[1-4]

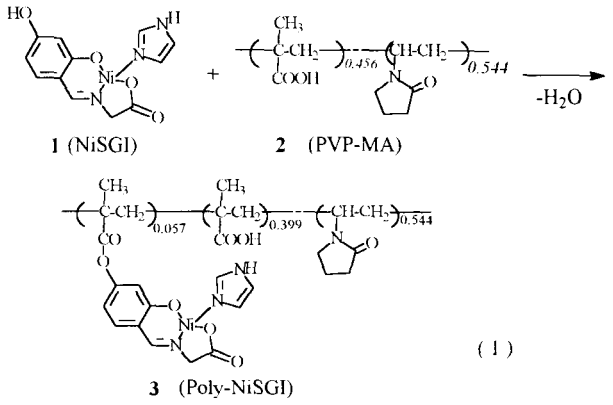
At the same time, studying metal complexes interaction with DNA is also increasing field.[5-7] DNA is the primary target molecule for most of anticancer and antiviral therapies according to cell biology and biochemistry. Moreover, metal complexes, such as Schiff base metal complexes, possess good anti-tumor and anti-viral activities as they can interact with DNA.[8,9] However, It is difficult to measure the anti-tumor activities of these low molecular weight compounds because of their low solubility in both aqueous and organic media. Furthermore, when such kinds of compounds in suspension are administered as drugs, the particle size may affect their activities.

So far, to our knowledge, very few studies have been carried out using polymer metal complexes as a polymer anticancer drug.[10] In this paper, a new kind of ternary water-soluble polymer Schiff base nickel complex has been synthesized, and the interaction of this polymer complex with calf thymus DNA has been studied.

The ternary Schiff base nickel complex **1** (NiSGI) was synthesized from 2,4-dihydroxy benzaldehyde, glycine and imidazole[11], and **1** was only slightly soluable in water. In the presence of dehydrolyzing agent and phase transfer catalyst, the novel of water-soluble polymer Schiff base metal complex **3** (Poly-NiSGI) has been synthesized successfully by **1** and a water soluble copolymer(N-vinylpyrrolidone-co-methacrylic acid) support **2** (PVP-MA) (Eq.1). The yield of **3** is 77%.

The polymer complex compositions were estimated by analysis of metal content. In the UV/Vis spectra, the water soluble copolymer **2** (PVP-MA) has no absorption from 200 nm to 400 nm. Compared with the Schiff base nickel complex **1** (NiSGI), the four absorption peaks of polymer Schiff base nickel complex **3** (Poly-NiSGI) showed red shift about 40~50 nm. In the IR spectra, the characteristic absorption bands of ν_{OH} (3067 cm^{-1}) and δ_{OH} (770 cm^{-1}) of the **1** (NiSGI) disappeared in **3** (Poly-NiSGI), and the characteristic absorption peak of **3** (Poly-NiSGI) was presented at 1030 cm^{-1} by $\nu_{s(C-O-C)}$. The polymer complex has also been characterized by thermal analysis, ^{1}HNMR and Low Angle Laser Light Scattering (LALLS).

A fluorimetric method based on ethidium bromide



(1)

(EB)/DNA system was used for investigating the interaction modes of Poly-NiSGI with calf thymus DNA, and for evaluating the anticancer activities of Poly-NiSGI. EB , a fluorescent dye, can intercalate into the double helix chains of DNA and greatly enhance the intensity of fluorescence,[12] so do some metal complexes. Furthermore when these complexes are added to the EB/DNA fluorescent system, the fluorescence intensity will be decreased. The measurement was performed in buffer [Tris-HCl (5 mmol/l) with 20 mmol/l NaCl, pH=7.4].

When NiSGI and Poly-NiSGI were added into EB/DNA system respectively, the fluorescence intensity of EB/DNA system more quickly decreased with increasing amounts of Poly-NiSGI than that of NiSGI(Figure 1). Where, F and F_0 are respectively the fluorescence intensity of EB/DNA system in the presence and the absence of complexes. $C_{Ni}/C_{DNA(P)}$

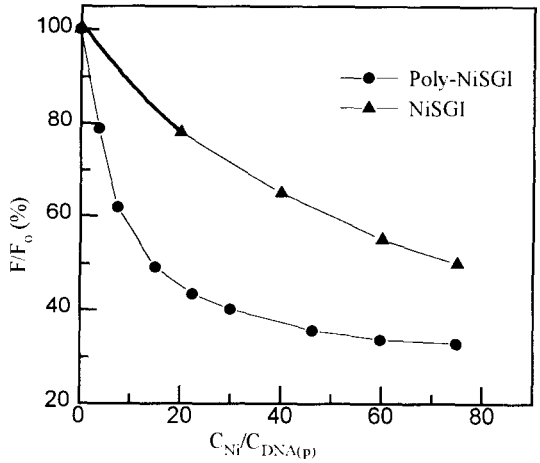


Figure 1. Relative fluorescent intensity of EB/DNA system in the presence of increasing metal concentration. Samples excitated at 534 nm.

represents ratio of concernation of nickel to that of DNA(P) in system. It suggested that there is a strong interaction of Poly-NiSGI with calf thymus DNA, it can be explained that a portion of the complex(Poly-NiSGI) intercalate to adjacent base pairs of the DNA, which inhibit EB binding to DNA competitively. After decreasing the binding ability between EB and DNA, the fluorescence intensity of system quickly decrease. From Figure 2, Plot of Fo/F versus C_{Ni} was not straight line and irrelevant to

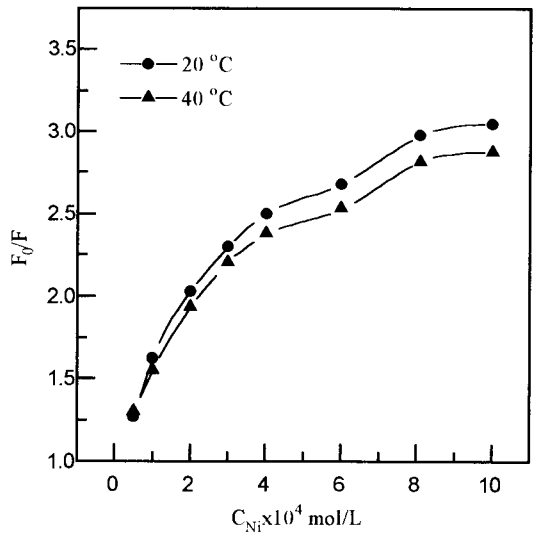


Figure 2. Fluorescence quenching of Poly-NiSGI to EB/DNA system at different temperatures.

reaction temperature. It indicated that the fluorescence of EB/DNA system was not being quenched by classical mechanism, so it can be concluded that the pattern of fluorescence quenching of Poly-NiSGI to EB/DNA system was neither simply dynamic nor simply static quenching[13]. We think, perhaps, there are two interaction modes in Poly-NiSGI/DNA system. It was found, with supposed the fluorescence intensity of pure EB/DNA system was 100% ($C_{NaCl}=2\times10^{-2}$mol/l), the fluorescence relative intensity of EB/DNA system increased with increasing of the ionic strength(C_{NaCl}) in reactive system (Table 1). It indicated that the intercalary degree of the polymer complex in DNA weakened with increasing of the ionic strength, because Na^+ atmosphere inhibit positively charged polymer

Table 1. Effects of ionic strength on the fluorescence intensity of the Poly-NiSGI /DNA-EB systems

$C_{NaCl} \times 10^3$mol/l	20	30	40	50	60
Relative fluorescence intensity(%)	63.13	64.04	71.47	74.20	71.62

$C_{DNA(P)}=1.378\times10^{-4}$ mol/l, $C_{EB}=5\times10^{-6}$ mol/l, $C_{Ni}=5\times10^{-5}$ mol/l.

complex electrostatic binding to negatively charged phosphate backbone of calf thymus DNA. The above results showed that the Poly-NiSGI interacted with calf thymus DNA by two modes. At first, the cationic complex electrostatic bind to negatively charged phosphate backbone at the periphery of the double helix, then , a portion of ligands intercalates between the base pairs on the DNA duplex strand. UV/Vis spectra are also in accord with this result.

In this work, the water solubility of Poly-NiSGI was greatly improved by introducing hydrophilic groups. This water solubility and polymer matrix effect jointly made DNA interact with Poly-NiSGI stronger than NiSGI. The fluorescence intensity of EB/DNA system decreased about 55% in low ratio of conceration of metal to DNA. So it is possible[14] to design this kind of water-soluble polymer metal complexes as polymer anticancer drug.

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