Studies on the Interaction of DNA and Water-Soluble Polymeric Schiff Base–Nickel Complexes

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ABSTRACT: The modes and activities of the interaction of DNA and water-soluble polymeric Schiff base–nickel complexes, which have been prepared by polymeric analogous reaction, have been discussed according to absorption spectra, circular dichroism spectra, and fluorescent probe method. It is indicated that polymeric matrix effect and increasing solubility in water can increase the interaction of these water-soluble polymeric metal complexes with DNA. © 2002 Wiley Periodicals, Inc. J Appl Polym Sci 84: 887–893, 2002; DOI 10.1002/app.10000

Key words: DNA; interaction; water-soluble polymeric metal complex; modes and activities

INTRODUCTION

The effective clinical use of cis-diammine dichloro platinum(II) complex (DDP) and other metal complexes[1] in the treatment of human cancer have stimulated the studies of interactions of nucleic acid (DNA) with different metal complexes, in which DNA was regarded as the primary target molecule for most of anticancer and antiviral therapies according to cell biology and biochemistry.[2–4] For example, some Schiff base metal complexes are considered to be a new kind of potential anticancer and antivirus reagent.[5,6] However, the antitumor activities of these low molecular weight compounds are measured with difficulty because of their low solubility in both aqueous and organic media; furthermore, they are administered as suspensions that the particle size may affect their activities.

At the same time, significant developments have 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.[7–10] So far, to our knowledge, very few studies have been carried out using polymeric metal complex as a polymer anticancer drug.[11] For the first time, our laboratories have successfully synthesized a novel of water-soluble polymeric Schiff base–nickel complex and reported its interaction with DNA.[12] In this paper, a new series of water-soluble polymeric Schiff base metal complexes have been synthesized progressively, and the interaction of these polymeric metal complexes with calf thymus DNA have been studied.

EXPERIMENTAL

Materials and Measurements

Calf thymus DNA and ethidium bromide, the product of Fluka, were purchased from Peking

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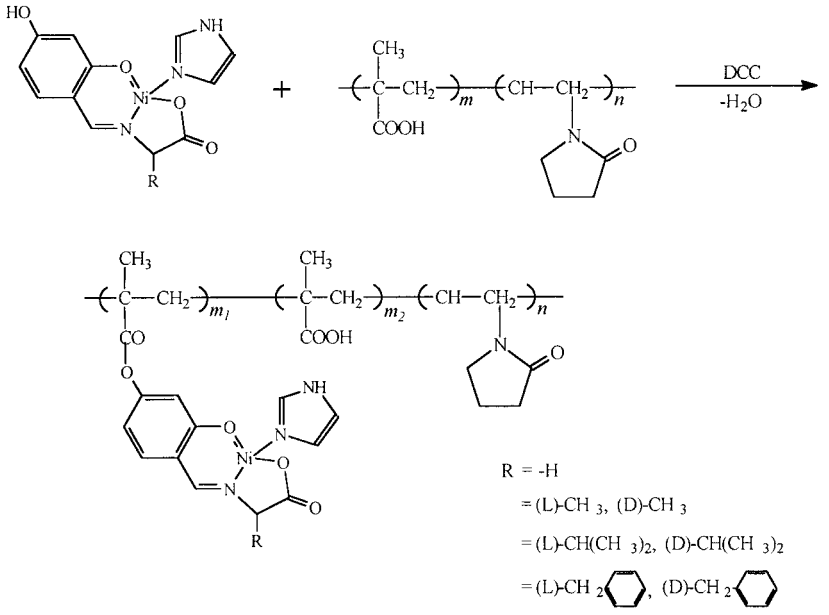

Scheme 1 Characteristics of water-soluble polymeric Schiff base–nickel complexes.

Sino-American Biotechnology Company. DNA concentration per nucleotide was determined spectrophotometrically by ϵ_{260} 6600 $mol^{-1} \cdot L \cdot cm^{-1}$. All amino acids (biochemical reagent) and Tris were purchased from Shanghai Bo-Ao Biotechnology Company. The other chemical reagents were of A.R. grade, and deionized water was used.

Fourier transform infrared (FTIR) spectra were recorded on an Alpha-Centauri FT-IR spectrometer (KBr sheets). The content of C, H, and N, and that of nickel, were determined by using a Vario El106 model elementary analyzer and a WFX-1D atomic absorption spectrophotometer respectively. Absorption spectra were measured on a Hitachi UV-3400 spectrophotometer. The fluorescence spectra were generated using a Shimazu RF-540 spectrofluorimeter. The circular dichroism spectra were performed on a JASCO, J-20C, DP-501N spectropolarimeter.

Preparation of Water-Soluble Polymeric Schiff Base–Nickel Complexes

The water-soluble polymeric Schiff base–nickel complexes were synthesized via the route shown in Scheme 1.

The low molecular weight ternary Schiff base–nickel complexes were synthesized from 2,4-dihydroxy benzaldehyde, amino acids and imidazole (the method of synthesis is similar to that in Ref. 13), and they were only slightly soluble in water. A water-soluble copolymer (N-vinylpyrrolidone-*co*-methacrylic acid) support (PVP-MA) was allowed to swell in dioxane for 1 h. To this a solution of the appropriate low molecular weight Schiff base–nickel complex in dioxane was added dropwise with constant stirring. The equivalent dehydrolyzing agent N,N′-dicyclohexylcarbodiimide (DCC) and a few tetrabutylammonium iodides were separately dissolved in dioxane and introduced successively into the reaction mixture under vigorous stirring. The system was maintained at room temperature for 30 min and then heated to 60°C for 24 h under magnetical stirring. The polymeric Schiff base–nickel complex was suction filtered and washed several times with dioxane, methanol, and trichloromethane. The dicyclohexylurea (DCU) formed by DCC during the reaction was eliminated by extraction in a soxhlet with tetrahydrofuran for at least 24 h. The water-soluble polymeric Schiff base–nickel complex was finally dried in a vacuum desiccator at 50°C and the yields were over 90%.

Studies of the Interaction of These Water-Soluble Polymeric Schiff Base–Nickel Complexes with DNA

All the measurements were performed using solutions of these compounds in buffer [Tris-HCl (5 mmol/L) with 20 mmol/L NaCl, pH = 7.4]

Ultraviolet/Visible Spectroscopy

The water-soluble polymeric Schiff base–nickel complex was added to calf thymus DNA [$C_{DNA(P)}$

$= 2.0 \times 10^{-4}$ mol/L] solution and kept in the dark at room temperature for 12 h. The absorbance of the calf thymus DNA bases at 260 nm and maximum absorption were measured, respectively, using a quartz cell with a 1-cm path length. During the measurement, the background of buffer and polymeric Schiff base–nickel complex was electronically subtracted.

Fluorimetry Method

Ethidium bromide (2, 7-diamino, 9-phenylphenanthridinium 10-ethyl bromide) (EB), a fluorescent dye, can intercalate into the double helix chains of DNA and greatly enhance the intensity of fluorescence[14]; so do some metal complexes. Furthermore, when these metal complexes are added to the DNA–EB fluorescent system, the fluorescence intensity will be decreased. So the DNA–EB fluorescent system can be used for investigating the interaction modes of the metal complex with DNA and evaluating the anticancer activities of the metal complex. Moreover, in this measurement system, it will be ensured that the metal complex does not exhibit any measurable fluorescence and quench the fluorescence of free EB under the experimental conditions.

The Fluorescence Spectra of DNA–EB System with Increasing Added Amounts of Polymeric Schiff Base Nickel Complex

The reaction mixture, containing $C_{DNA(P)} = 5 \times 10^{-6}$ mol/L calf thymus DNA, 5×10^{-6} mol/L EB and nickel complex, was kept in the dark at room temperature for 12 h. The emission spectra of the DNA–EB system was measured with excited at 534 nm. The silts of excitation and emission were all 10 nm.

Effects of Ionic Strength

NaCl was used in this experiment to adjust the ionic strength in polymeric Schiff base–nickel complex–DNA–EB reaction system [$C_{DNA(P)} = 1.4 \times 10^{-4}$ mol/L, $C_{EB} = 5 \times 10^{-6}$ mol/L, and $C_{Ni} = 5 \times 10^{-5}$ mol/L]. The emission spectra of reaction system was measured at the concentration of NaCl was 20, 30, 40, 50, and 60 mmol/L, respectively, and the other conditions as above.

Distinguishing the Pattern of Fluorescence Quenching of Polymeric Schiff Base Nickel Complex to DNA–EB System

The reaction mixture of water-soluble polymeric Schiff base–nickel complex with DNA–EB system was incubated in the dark at 40°C for 12 h. The other measure conditions were same as for the flourescence spectra above**.** Then making plot of F_0/F vs C_{Ni}, where F and F_0 are respectively the fluorescence intensity of the DNA–EB system in the presence and the absence of polymeric Schiff base–nickel complex.

Circular Dichroism

The water-soluble polymeric Schiff base–nickel complex and calf thymus DNA [$C_{DNA(P)} = 4.0 \times 10^{-5}$ mol/L] were mixed and kept in the dark at room temperature for 24 h. The circular dichroism (CD) of this reaction system was scanned at the range of 200–350 nm. The background of buffer and polymeric Schiff base–nickel complex was electronically subtracted.

RESULTS AND DISCUSSION

The structure and composition of water-soluble polymeric Schiff base–nickel complexes are as in Scheme 1.

Characteristics of Water-Soluble Polymeric Schiff Base–Nickel Complexes

In the ultraviolet-visible spectra, the water-soluble copolymer (PVP-MA) has no absorption from 200 to 400 nm. Compared with the low molecular weight Schiff base–nickel complexes, the absorption peaks of the polymeric Schiff base–nickel complexes showed a 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 low molecular weight Schiff base–nickel complexes disappeared in the polymeric Schiff base–nickel complexes, and the characteristic absorption peak of the polymeric Schiff base–nickel complexes were presented at 1030 cm^{-1} by $\nu_{S(C—O—C)}$ or 1229 cm^{-1} by $\nu_{as(C—O—C)}$ and appeared wide overlapped peak at 1657 cm^{-1} by ν_{COO}, $\nu_{C=O}$, and $\nu_{C=N}$. The water-soluble polymeric Schiff base–nickel complexes have also been characterized by thermal analysis and low angle laser light scattering (LALLS).

The composition of these water-soluble polymeric Schiff base–nickel complexes was estimated by analysis of nickel and nitrogen contents. The results are shown in Table I.

Table I The Compositions and Abbreviation of the Polymeric Schiff Base–Nickel Complexes

R	Polymeric Schiff Base–Nickel Complexes Abbreviation	Nickel Content (wt %)	Compositions		
			m_1	m_2	n
-H	Poly-NiSGI	2.90	0.057	0.399	0.544
(L)-CH_3	Poly-L-NiSAI	7.43	0.213	0.188	0.599
(D)-CH_3	Poly-D-NiSAI	6.50	0.172	0.229	0.599
(L)-$CH(CH_3)_2$	Poly-L-NiSVI	1.89	0.037	0.364	0.599
(D)-$CH(CH_3)_2$	Poly-D-NiSVI	4.66	0.110	0.291	0.599
(L)-CH_2Ph	Poly-L-NiSPI	2.35	0.048	0.353	0.599
(D)-CH_2Ph	Poly-D-NiSPI	2.58	0.054	0.347	0.599

Studies on the Interaction of Water-Soluble Polymeric Schiff Base Nickel Complexes with DNA

Absorption Study

The obvious change of absorption spectra, before and after adding Poly-NiSGI to Calf thymus DNA solution is shown in Table II.

When the molar ratio of $C_{Ni}/C_{DNA(P)}$ is low, the electronic absorption intensity of calf thymus DNA in the presence of increasing amounts of poly-NiSGI showed strong decrease at 260 nm and maximum absorption. But when the ratio is relatively high, the electronic absorption intensity began to increase. In addition to the change in intensity, an obvious red shift at maximum absorption was also observed in the spectra. These various spectral changes are consistent with the intercalation of poly-NiSGI into the calf thymus DNA base stack. These results showed that there is a strong interaction of poly-NiSGI with calf thymus DNA.

The characteristic spectrum of hypochromism and hyperchromism are closely related to the double helix structure of DNA. Hypochromism was suggested to be due to the positively charged polymeric Schiff base–nickel complex electrostatic binding to negatively charged phosphate backbone at the periphery of the double helix calf thymus DNA, which resulted in calf thymus DNA molecular contracted and its conformation changed. After that, further interaction of polymeric Schiff base–nickel complex with Calf thymus DNA caused the secondary structure of Calf thymus DNA disnatured and destroyed, which bought about a hyperchromic effect.

Fluorescence Studies

Effects of the Polymeric Schiff Base–Nickel Complexes on the Fluorescence of DNA–EB System. The fluorescence intensity of the DNA–EB system was found to be decreased strongly when water-soluble polymeric Schiff base–nickel complex was added, as shown in Figure 1.

When low molecular weight Schiff base–nickel complex and polymeric Schiff base–nickel complexes were added into the DNA–EB system, respectively, the fluorescence intensity of the DNA–EB system decreased more quickly with increasing amounts of polymeric Schiff base–nickel complexes than that of low molecular weight Schiff base–nickel complex (Fig. 1), where F and F_0 are respectively the fluorescence intensity of DNA–EB system in the presence and the absence of nickel complexes. $C_{Ni}/C_{DNA(P)}$ represent ratio of concentration of nickel to that of DNA(P) in system. It suggested that there is a strong interaction of polymeric Schiff base–nickel complexes with calf thymus DNA. It can be explained that a portion of the metal complex, possessing a planar

Table II Absorption Spectra Data of the Interaction of Poly-NiSGI with DNA

$C_{Ni}/C_{DNA(P)}$	0	0.248	0.497	0.745	0.993
$A_{\lambda=260\ nm}$	1.3291	1.3123	1.0772	1.2953	1.4160
$A_{\lambda=max}$	1.3532	1.3190	1.0772	1.3033	1.5059
λ_{max} (nm)	256.1	256.2	259.2	263.9	268.4

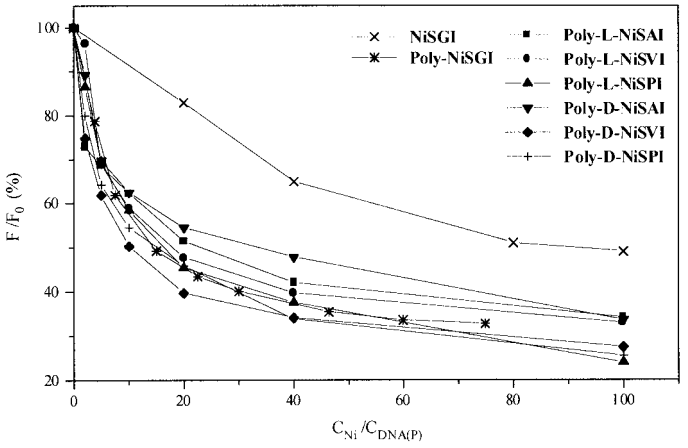

Figure 1 Relative fluorescent intensity of DNA–EB system in the presence of increasing metal concentration. Samples excited at 534 nm.

geometry, intercalates to adjacent base pairs of calf thymus DNA, which inhibit EB binding to calf thymus DNA competitively. After decreasing the binding ability between EB and calf thymus DNA, the fluorescence intensity of the DNA–EB system decreases quickly.

Effects of Ionic Strength on the Fluorescence Intensity of the Polymeric Schiff Base–Nickel Complex–DNA–EB System. By adding NaCl with various concentrations to adjust the ionic strength in rthe poly-NiSGI/DNA–EB system, we measured the fluorescence intensity to study the effects of ionic strength. Results are shown in Table III.

It was found, with supposed the fluorescence intensity of pure DNA–EB system was 100% [$C_{NaCl} = 2 \times 10^{-2}$ mol/L], the fluorescence relative intensity of poly-NiSGI/DNA–EB system increased with increasing of the ionic strength (C_{NaCl}) in the reaction system. It indicated that the intercalary degree of the polymeric Schiff base–nickel complex in DNA weakened with increasing of the ionic strength, because Na^+ atmosphere inhibits the positively charged polymeric Schiff base–nickel complex electrostatic binding to negatively charged phosphate backbone of calf thymus DNA. This result agrees with that of absorption studies and suggests that polymeric Schiff base–nickel complexes interact with DNA by outer electrostatic attraction at first.

Effects of the Polymeric Schiff Base–Nickel Complex on the Fluorescence Spectra of DNA–EB System at different Temperature. We studied the pattern of fluorescence quenching in poly-NiSGI to DNA–EB system by measuring fluorescence intensity at room temperature and 40°C respectively.

From Figure 2, Plot of F_0/F vs C_{Ni} was not straight line and irrelevant to reaction temperature. It indicated that the fluorescence of DNA–EB system was not being quenched by classical mechanism, so it can be concluded that the pattern of fluorescence quenching of poly-NiSGI to DNA–EB system was neither simply dynamic nor simply static quenching.[15] We think, perhaps, there are two interaction modes in polymeric Schiff base–nickel complexes/DNA reaction system.

CD Study

More strong evidence for the interaction of polymeric Schiff base–nickel complexes with DNA was obtained from the DNA CD study. Calf thymus DNA is a B-form helical conformation and

Table III 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

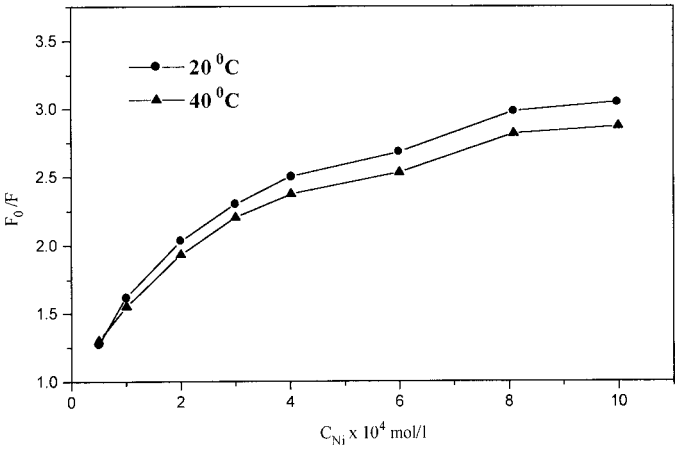

Figure 2 Fluorescence quenching of poly-NiSGI to DNA–EB system at different temperatures.

polymeric Schiff base–nickel complexes have no characteristic of circular dichroism display in the experimental conditions. The calf thymus DNA CD in the absence and in the presence of poly-NiSGI is presented in Figure 3, in which the concentration of poly-NiSGI added to calf thymus DNA buffer solution increasing with 1~3 successively.

CD spectra reflected the change of the conformation of calf thymus DNA. It showed that the shape of CD spectra was strongly dependent on the concentration of the polymeric Schiff base–nickel complex added in the reaction system. When Poly-NiSGI interacted with calf thymus DNA at low concentration [$C_{Ni}/C_{DNA(P)}$ 1], the shape of CD spectra had no obvious change. However, when $C_{Ni}/C_{DNA(P)}$ = 2 and 5, the positive band at 273 nm of calf thymus DNA increased a little and appeared to purple shift slightly; at the same time the negative band at 239 nm decreased a little contrarily, which indicated that the conformation of calf thymus DNA transited to A from B. This result is closely agreement with those previous studies, that is polymeric Schiff base–nickel complex electrostatic attracted with Calf thymus DNA at outer phosphate backbone firstly and caused calf thymus DNA molecular contracted and its conformation changed.

When $C_{Ni}/C_{DNA(P)}$ =10 and 20, the shape of CD spectra changed obviously. There appeared a red

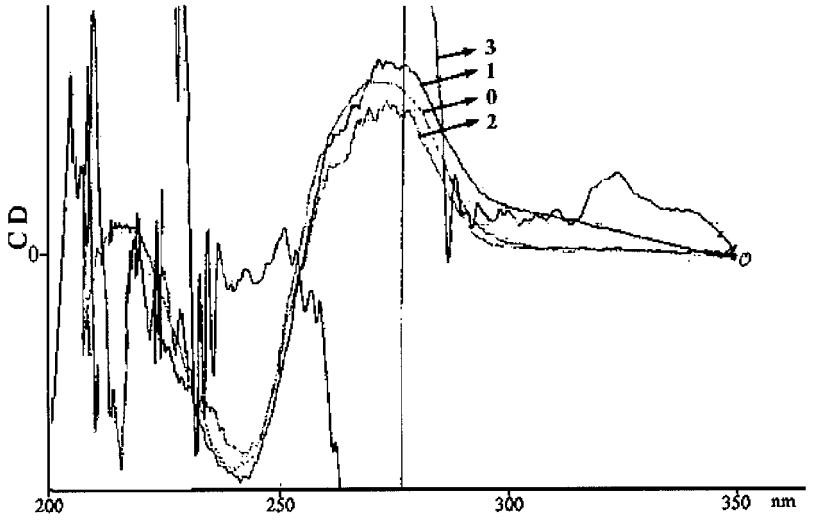

Figure 3 CD spectra of system of poly-NiSGI/DNA. (0. $C_{Ni}/C_{DNA(P)}$ = 0; 1. $C_{Ni}/C_{DNA(P)}$ = 5; 2. $C_{Ni}/C_{DNA(P)}$ = 20; 3. $C_{Ni}/C_{DNA(P)}$ = 40)

shift from 273 to 279 nm for the positive band and that from 239 to 246 nm for the negative band, and the two bands all lost intensity seriously, which suggested helicity decrease and denaturation in the structure of calf thymus DNA.[16] Moreover, when $C_{Ni}/C_{DNA(P)}$ 40, the CD spectra of calf thymus DNA changed violently and the shape of peak cannot be distinguished, which indicated that the polymeric Schiff base–nickel complex further destroy the secondary structure of DNA under high concentration.

The above results showed that the polymeric Schiff base–nickel complexes 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, and then a portion of ligands intercalates between the base pairs on the DNA duplex strand.

CONCLUSIONS

In this work, the water solubility of polymeric Schiff base–nickel complexes was greatly improved by introducing hydrophilic groups. This water solubility and polymeric matrix effect jointly made DNA interact with polymeric Schiff base–nickel complexes stronger than the low molecular weight analogues. The fluorescence intensity of the DNAndash;EB system decreased about 55% in low ratio of concentration of metal to DNA. So it is possible[17] to design this kind of water-soluble polymeric metal complex as polymer anticancer drug.

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