

The Interaction of DNA and Water-Soluble Polymeric Schiff-Base Nickel Complexes

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The modes and activities of the interaction of DNA and water-soluble polymeric Schiff-base nickel complexes, prepared by polymeric analogous reaction, have been discussed according to the absorption spectra, circular dichroism spectra, and fluorescent probe results. The polymeric matrix effect and increasing solubility in water can increase the interaction of these water-soluble polymeric metal complexes with DNA.

The effective clinical use of *cis*-diammine-dichloro-platinum(II) complex (DDP) and other metal complexes[1] in the treatment of cancer has stimulated some 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 difficult to measure because of their low solubility in both aqueous and organic media, moreover, since they are administered as suspensions, 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 drugs) which may be active as drugs themselves or may serve as carries alternatively 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 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 interactions of these 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 Sino-American Biotechnology Company. DNA concentration per nucleotide was determined spectrophotometrically by ε_{260} 6600 mol^{-1} L 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. Deionized water was used.

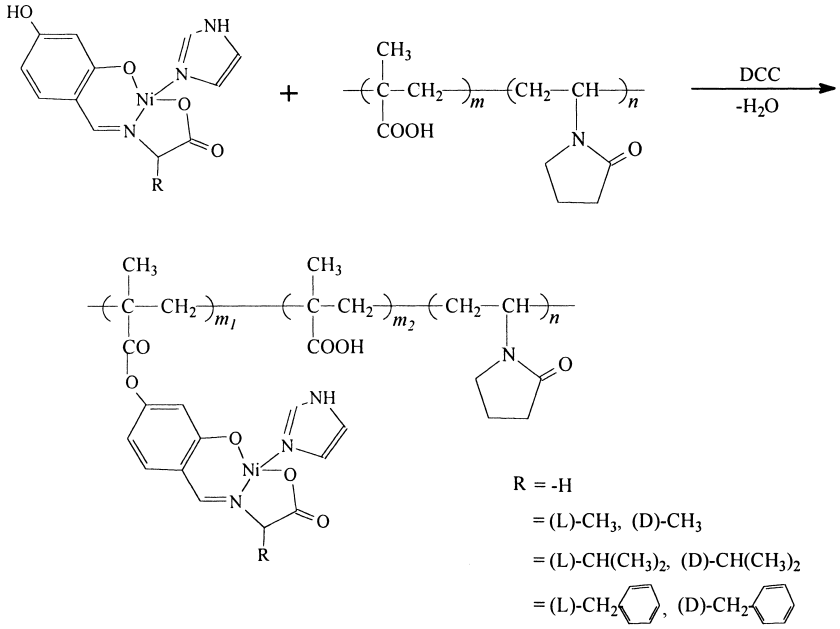
Fourier transform infrared (FT-IR) spectra were recorded on an Alpha-Centauri FT-IR spectrometer (KBr sheets). The content of C, H, and N and that of nickel were determined by using a Vario El106 model elementary analyzer and a WFX-1D atomic absorption spectrophotometer, respectively. Absorption spectra were measured on a Hitachi UV-3400 spectrophotometer. The fluorescence spectra were generated using a Shimadzu 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 as shown in Scheme 1.

The low molecular weight ternary Schiff-base nickel complexes were synthesized from 2,4-dihydroxybenzaldehyde, amino acids, and imidazole, [13] and they were only slightly soluble in water. A water-soluble copolymer (*N*-vinyl-2-pyrrolidone-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 dicyclohexylcarbodiimide (DCC) and a few tetrabutylammonium iodide 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 magnetic stirring. The polymeric Schiff-base nickel complex was filtered by suction and washed several times with dioxane, methanol, and trichloromethane. The *N*, *N*′-dicyclohexylurea (DCU) formed by DCC during the reaction was eliminated by extraction in a Soxhlet extractor 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. 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 into calf thymus DNA ($C_{DNA(P)} = 2.0 \times 10^{-4}$ mol/L) solution and kept in the dark



Scheme 1.

at room temperature for 12 h. The absorbance of the calf thymus DNA bases at 260 nm and maximum absorption were then measured using a quartz cell with a 1 cm path length. During the measurements, the background of buffer and polymeric Schiff-base nickel complex was electronically subtracted.

Fluorimetry Method. Ethidium bromide (3,8-diamino-5-ethyl-6-phenylphenanthridinium 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 decrease, so DNA-EB fluorescent system can be used to investigate the interaction modes of metal complex with DNA and evaluate the anticancer activities. Moreover, in this measurement system, it can be ensured that the metal complex does not exhibit any measurable fluorescence and does not quench the fluorescence of free EB under the experimental conditions.

1. 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 DNA–EB system were measured with excitation at 534 nm. The slits of excitation and emission were all 10 nm wide.

2. 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 were measured at each concentration of NaCl: 20, 30, 40, 50, and 60 mmol/L, respectively, and the other conditions were as above.

3. 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 conditions were the same as **1**. Then we made a plot of F_0/F versus C_{Ni}, where F and F_0 are the fluorescence intensity of DNA-EB system in the presence and the absence of polymeric Schiff-base nickel complex, respectively.

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 over 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 of 40–50 nm or so, as shown in Table 1.

In the IR spectra of the polymeric Schiff-base nickel complexes, the characteristic absorption bands of ν_{OH} (3067 cm^{-1}) and δ_{OH} (770 cm^{-1}) of the low molecular weight analogues disappeared, while the characteristic absorption peak of the polymeric Schiff-base nickel complexes was present at 1030 cm^{-1} by $\nu_{S(C\text{-}O\text{-}C)}$ or 1229 cm^{-1} by $\nu_{as(C\text{-}O\text{-}C)}$ and a wide overlapped peak appeared 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 2.

Studies on The Interaction of Water-Soluble Polymeric Schiff-Base Nickel Complexes With DNA. Absorption

Table 1. The Absorption Spectra Data of SG, NiSGI, and Poly-NiSGI*

Compounds	λ_{max}/nm			
SG	205.4	248.3	286.4	325.1
NiSGI	211.0	243.0	284.8	337.3
Poly-NiSGI	265.2	280.0	326.1	392.0

* SG: Schiff-base glycine ligand.
NiSGI: the ternary Schiff-base complexes of nickel, SG, and imidazole.
Poly-NiSGI: polymer supported NiSGI.

Table 2. The Compositions and Abbreviation of the Polymeric Schiff-Base Nickel Complexes

R	Polymeric Schiff-base nickel complexes abbreviation	Nickel content	Compositions		
		wt%	m_1	m_2	n
-H	Poly-NiSGI	2.90	0.057	0.399	0.544
-H	Poly-NiSGI1	0.38	0.007	0.328	0.665
-H	Poly-NiSGI2	0.82	0.014	0.321	0.665
-H	Poly-NiSGI3	1.93	0.036	0.299	0.665
-H	Poly-NiSGI4	9.38	0.306	0.029	0.665
(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

Table 3. 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

Study. The obvious change of absorption spectra before and after adding Poly-NiSGI to calf thymus DNA solution is shown in Table 3.

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 a 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 between Poly-NiSGI and calf thymus DNA.

The characteristic spectra of hypochromism and hyperchromism are closely related to the double helix structure of DNA. Hypochromism was suggested to be due to the positive-charged polymeric Schiff-base nickel complex electrostatic binding to negative-charged phosphate backbone at the periphery of the double helix calf thymus DNA, which resulted in a contraction of calf thymus DNA molecule and a change of its conformation. After that, further interaction of polymeric Schiff-base nickel complex with calf thymus DNA caused the secondary structure of calf thymus DNA to denature and destroyed it, which bought about a hyperchromic effect.

Fluorescence Studies. 1. Effects of the Polymeric Schiff-Base Nickel Complexes on the Fluorescence of DNA–EB System. The fluorescence intensity of DNA–EB system was found to decrease strongly when water-soluble polymeric Schiff-base nickel complex was added, as shown in Fig. 1 and Fig. 2.

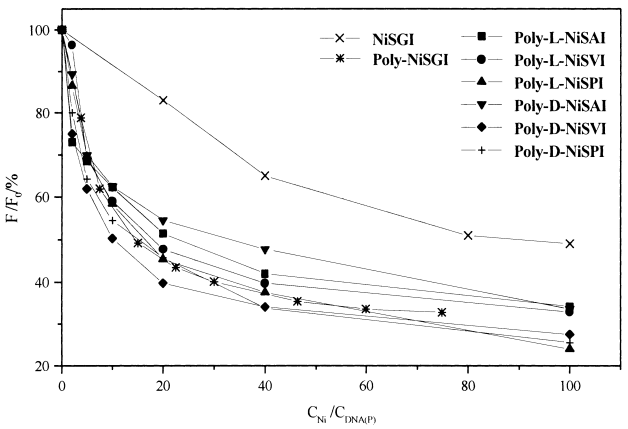


Fig. 1. Relative fluorescent intensity of EB/DNA system in the presence of increasing metal concentration.
Sample excitated 534 nm.

When low molecular weight Schiff-base nickel complex and polymeric Schiff-base nickel complexes were added into

Table 4. Effects of Ionic Strength on the Fluorescence Intensity of the Poly-NiSGI/DNA–EB Systems

$C_{NaCl} \times 10^3$/mol L^{-1}	20	30	40	50	60
Relative fluorescence intensity/%	63.13	64.04	71.47	74.20	71.62

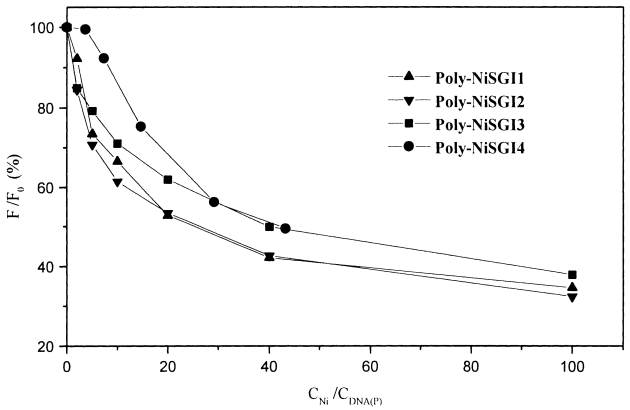


Fig. 2. Relative fluorescent intensity of EB/DNA system in the presence of increasing metal concentration, Sample excitated 534 nm.
The content of nickel supported in polymer increased with the Poly-NiSGI1 to Poly-NiSGI4.

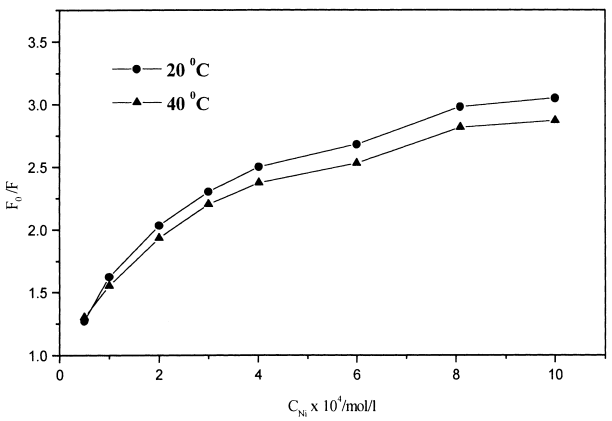


Fig. 3. Fluorescence quenching of Poly-NiSGI to DNA–EB system at different temperatures.

DNA–EB system, respectively, the fluorescence intensity of DNA–EB system decreased more quickly with the increasing amounts of polymeric Schiff-base nickel complexes than with the increasing amounts of low molecular weight Schiff-base nickel complex (Fig. 1). F and F_0 are the fluorescence intensity of DNA–EB system in the presence and the absence of nickel complexes, respectively. $C_{Ni}/C_{DNA(P)}$ represented the ratio of nickel's concentration to DNA(P)'s in the system. Results suggested that there is a strong interaction of polymeric Schiff-base nickel complexes with calf thymus DNA. A portion of the metal complex, possessing a planar geometry, intercalates to adjacent base pairs of calf thymus DNA, which inhibit EB binding to calf thymus DNA competitively. After the binding ability between EB and calf thymus DNA was decreased, the fluorescence intensity of DNA-EB system decreased quickly.

Figure 2 shows that the effect of the content of the low molecular weight nickel complex supported in polymer. At the range of low content, the low molecular weight nickel complexes have good dispersity and the interaction effect is much less, thus the interacting activity of polymeric Schiff-base nickel complexes and calf thymus DNA had no obvious change. However, on increasing the content of low molecular weight nickel complexes supported in polymer much more, the interacting activity also decreased. This could be due to the stereoscopic effect turning into a major factor. The steric hindrance inhibits polymeric Schiff-base nickel complex from interacting with calf thymus DNA.

2. Effects of Ionic Strength on the Fluorescence Intensity of the Polymeric Schiff-Base Nickel Complex-DNA–EB System. By adding NaCl of various concentrations to adjust the ionic strength in Poly-NiSGI/DNA–EB system, we measured the fluorescence intensity to learn the effects of ionic strength. Results are shown in Table 4.

Supposing the fluorescence intensity of pure DNA–EB system was 100% ($C_{NaCl} = 2 \times 10^{-2}$ mol/L), we found that the fluorescence relative intensity of Poly-NiSGI/DNA–EB system went up with the increase of the ionic strength (C_{NaCl}) in the reaction system. Such dependence indicated that the intercalary degree of the polymeric Schiff-base nickel complex in DNA is weakened when the ionic strength increases, because Na^+ atmosphere inhibits positive-charged polymeric Schiff-base nickel complex from binding electrostatically to negative-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.

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

In Fig. 3, the plot of F_0/F versus C_{Ni} was not a straight line and was not related to reaction temperature. But according to classical fluorescence quenching theory, a plot of F_0/F versus C_{Ni} should obviously depend on temperature for either dynamic quenching or static quenching and present linear relationship. So it could be concluded that the pattern of fluorescence quenching of Poly-NiSGI to DNA–EB system was neither simply dynamic nor simply static.[15,16] We think there may be two interaction modes in polymeric Schiff-base nickel complexes/DNA reaction system. The results mentioned above also indicated that the pattern strongly depended on the mole ratio of Poly-NiSGI to DNA. DNA structure would be destroyed only when the ratio reached to a threshold value.

Circular Dichroism Study. Another strong piece of evidence for the interaction of polymeric Schiff-base nickel complexes with DNA was obtained from the DNA circular dichroism study. Calf thymus DNA is a B-form helical conformation

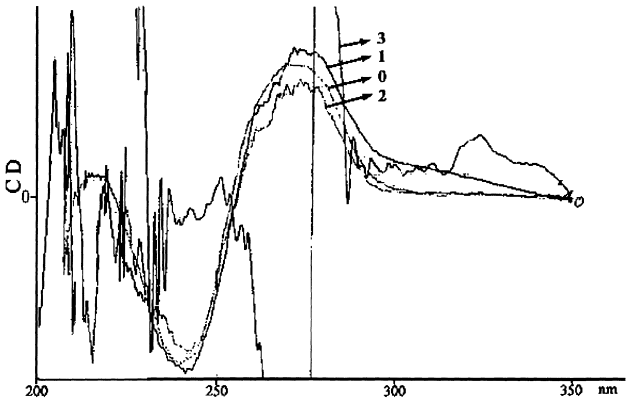


Fig. 4. Circular dichroism spectra of system of Poly-NiSGI/DNA.
0. $C_{Ni}/C_{DNA(P)} = 0$; 1. $C_{Ni}/C_{DNA(P)} = 5$; 2. $C_{Ni}/C_{DNA(P)} = 20$; 3. $C_{Ni}/C_{DNA(P)} = 40$.

and polymeric Schiff-base nickel complexes have no characteristic display of circular dichroism under the experimental conditions. The calf thymus DNA circular dichroism in the absence and in the presence of Poly-NiSGI are presented in Fig. 4, in which the concentration of Poly-NiSGI added to calf thymus DNA buffer solution increased from 1 to 3 successively.

CD spectra reflected the change of the conformation of calf thymus DNA. They showed that the shape of CD spectra greatly depended 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 ($C_{Ni}/C_{DNA(P)} \leq 1$), 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 slightly and a minute purple shift emerged. On the contrary, the negative band at 239 nm decreased slightly at the same time, which indicated that the conformation of calf thymus DNA transited from B to A. This result closely agrees with those previous studies, that is polymeric Schiff-base nickel complex electrostatically attracted calf thymus DNA at outer phosphate backbone firstly and caused a contraction of calf thymus DNA molecule and a change of its conformation.

When $C_{Ni}/C_{DNA(P)} = 10$ and 20, the shape of CD spectra changed obviously. There appeared a red shift from 273 nm to 279 nm for the positive band and that from 239 nm to 246 nm for the negative band. Both of the two bands lost intensity seriously, which suggested helicity decrease and denaturation in the structure of calf thymus DNA.[17] Moreover, when $C_{Ni}/C_{DNA(P)} \geq 40$, the CD spectra of calf thymus DNA changed violently and the shape of peak could not be distinguished, which indicated that the polymeric Schiff-base nickel complex further destroyed 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 electrostatically binds to negative-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 in combination with polymeric matrix effect made DNA interact with polymeric Schiff-base nickel complexes more strongly than the low molecular weight analogues. The fluorescence intensity of DNA–EB system decreased by about 55% in low ratio of concentration of metal to DNA. So it is possible[18] to design this kind of water-soluble polymeric metal complex as a polymer anticancer drug.

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