Antibacterial Activities and Interactions Study of Ternary Lanthanide Schiff Base and Nitrogen-heterocyclic Complexes Bind to DNA

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Abstract. In this work, a series of ternary rare earth complexes were synthesized with salicylidene glycine schiff base (Sal-GlyK) as anion ligand and 2,2'-bipyridine (bipy), l, 10-phenanthroline (phen) as neutral ligand. The antibacterial activities and interactions between calf thymus DNA (ct-DNA) in Tris-HCl (pH=7.1) and the complexes were investigated by UV-Vis spectrophotometry, fluorescence quench experiment and viscosity measurement. The antibacterial activities of the complexes are all better than the ligands, and the complexes with the bigger planarity ligands are better than the small one.

1 Introduction

The interactions between lanthanide complexes and nucleic acids have aroused tremendous interests due to the potential applications of the metal complexes as anticancer drugs with other biological functions in recent years[1]. A lot of amino acid Schiff Base lanthanide complexes have been synthesized[2][3], but the interaction with DNA and antibacterial activity of salicylidene glycine Schiff Base (Sal-GlyK) lanthanide complexes has not been reported.

In the synthesis of complexes, we choose 1, 10-phenanthroline (phen) and 2,2'-dipyridyl(bipy) which have a plane conjugate structure molecular as the second ligands. The results indicate that good planarity and large surface area of the ligand have a positive effect in the interaction between DNA and complexes. Antibacterial activities of those complexes were also investigated.

2 Experimental sections

2.1 Materials and instrumentation

Calf thymus DNA (ct-DNA, Sigma Chem. Co., USA), Ethidium bromide (EB) and tris (hydroxymethyl) aminomethane hydrochloride (Tris-HCl) are used as received. Purity of rare earth oxides is 99.99%. Other reactants and solvents are all of analytical grade.

IR spectra were obtained on a Nicolet Nexus 670 FT-IR spectrophotometer within the range of 4000-400 cm-1. Absorption spectra were recorded on TU1901 spectrophotometer, using DMF as solvent. Fluorescence spectra were carried out using Shimadzu RF-5301PC Spectrophotometer with excitation and emission slits of 10.0 nm at room temperature. Viscosity experiments were conducted on Ubbelohde Viscometer at 25.0 ± 0.1 °C.

2.2 Synthesis of Sal-GlyK

Dissolved 10mmol glycine and 10mmol KOH in ethanol solution (20ml), respectively. After the solution under stirring at 50 °C for 1 h, we got clarification transparent solution by filtering. The solution was added dropwise to another ethanol solution (20ml) containing salicylide (10mmol) and stirring at 65 °C for 2 h. The precipitate was filtered out, washed twice with 20ml ethanol and recrystallized by the solution of 20 ml ethanol and n-propanol (V:V=60:40) to get a yellow needle product, yield: 63%.

2.3 Synthesis of rare earth complexes

The solution of rare earth nitrate was prepared according to literature[4]. In a typical synthesis, Sal-GlyK (0.5 mmol) was dissolved in 20 ml methanol, and then $RE(NO_3)_3$ (0.5mmol) was added

dropwise into this solution with continuous stirring, then KOH was added into the solution to adjust the pH to 6 at 50 °C for 3 h. the precipitate was filtered out, washed twice with 20ml methanol and dried at 50 °C for several hours to get the product of RE(Sal-Gly)(NO_3).2H_2O.

0.5mmol phen was dissolved in 10ml methanol, then a RE(NO_3)$_3$ (0.5 mmol) solution was added dropwise into the solution under stirring at 50 °C for 1 h. 0.5 mmol Sal-GlyK dissolved in 10ml methanol was added dropwise into this mixture under stirring, then the pH of solution was adjusted to 6 by KOH, After reaction for another 2 h at 50 °C, the precipitate was filtered out, washed twice with 20ml methanol, and dried at 50 °C for several hours to get the product of RE(Sal-Gly)(phen)(NO_3).H_2O. The synthesis procedure for RE(Sal-Gly)(bipy)(NO_3)-H_2O is similar to that of RE(Sal-Gly)(phen)(NO_3).H_2O except for phen was replaced by bipy.

3 Results and discussions

3.1 IR spectra analysis

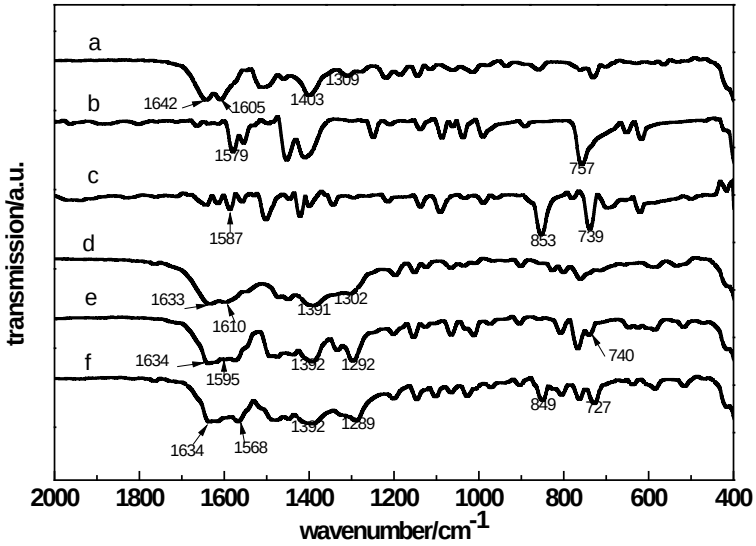

Fig. 1. IR spectra of ligands and some complexes. a, Sal-GlyK; b, Bipy; c, Phen; d, Tb(Sal-Gly)(NO_3)•2H_2O; e, Tb(Sal-Gly)(Bipy)(NO_3)•H_2O; f,Tb(Sal-Gly)(phen)(NO_3)•H_2O

As shown in Fig. 1, compared with the ligands, the IR band position and relative intensity of the complexes have significant changes, which indicated that the ligands coordinated with the rare earth ions.

3.2 UV-vis absorption spectra

As shown in Fig. 2, after the formation of complexes, the two characteristic absorption peaks of ligand Sal-GlyK at 259nm shifted to 258nm, and the peak at 379nm disappeared, indicating the coordination of Sal-GlyK and RE^{3+}.

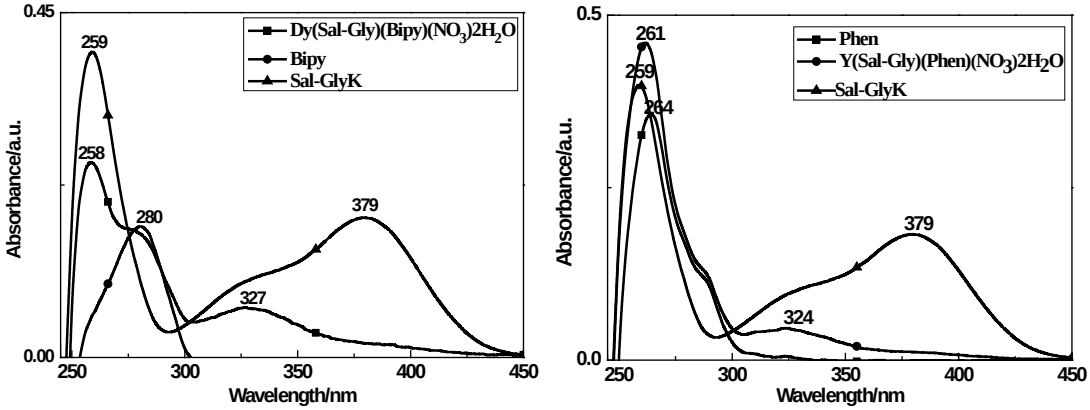

Fig. 2. (a) UV spectra of the Sal-Glyk, Bipy and Dy(Sal-Gly)(Bipy)(NO_3)•H_2O.
(b) UV spectra of the Sal-Glyk, Phen and Y(Sal-Gly)(Phen)(NO_3)•H_2O.

3.3 UV-Vis absorption spectral studies of the DNA binding

The spectra of Dy(Sal-Gly)(Phen)(NO_3)•H_2O with increasing the concentration of DNA are shown in Fig. 3a . The hypochromicity and red shift indicated a intercalative binding between complexes and DNA bases.

The binding constants of some complexes are obtained in the similar way and shown in Table 1. As show in Table 1, the sequence of binding ability of the complex to DNA is RE(Sal-Gly)(phen)(NO_3)•H_2O>RE(Sal-Gly)(bipy)(NO_3)•H_2O>RE(Sal-Gly)(NO_3)•$2H_2O$.

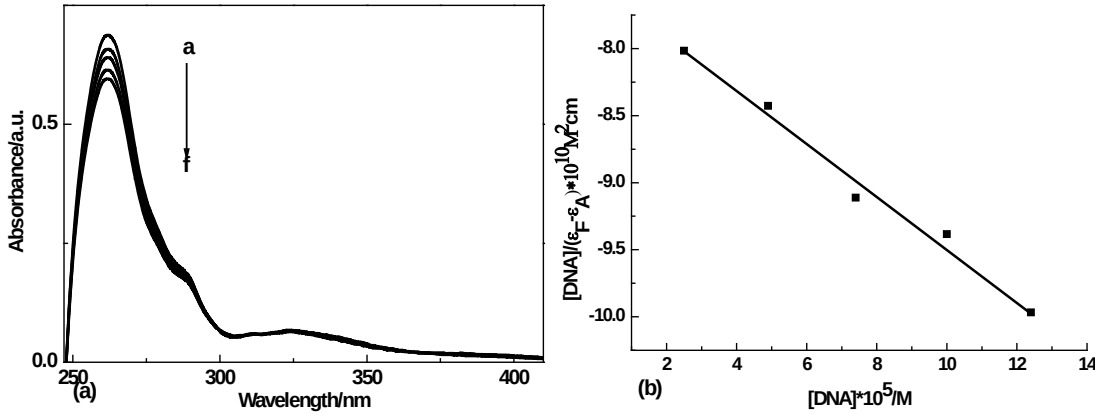

Fig.3. (a) Absorption spectra of Dy(Sal-Gly)(Phen)(NO_3)•H_2O in the presence of increasing amounts of ct-DNA ([DNA]=0-19.6 μM), 10 μl each time. Arrow a → f shows the absorbance changes upon increasing ct-DNA concentration. (b) The pattern of Dy(Sal-Gly)(Phen)(NO_3)•H_2O of [DNA]/(ε_f-ε_a) linear relation with [DNA] .

3.4 Fluorescence spectral

The fluorescence quenching of EB-DNA by complexes indicated that the complexes had a strong interaction with DNA, and the interaction mode the complex and DNA are intercalation binding. In addition, we can see from Fig. 4, complexes with same rare earth Y^{3+} and different neutral ligand have different binding ability to DNA. The interaction ability between complexes and DNA is Y(Sal-Gly)(phen)(NO_3).H_2O > Y(Sal-Gly)(bipy)(NO_3).H_2O > Y(Sal-Gly)(NO_3).$2H_2O$. Meanwhile, by numerous experiments, we found a regular pattern that RE(Sal-Gly)(phen)(NO_3)•H_2O > RE(Sal-Gly)(bipy)(NO_3)•H_2O > RE(Sal-Gly)(NO_3)•$2H_2O$ which is consistent with the result of UV-vis absorption spectra.

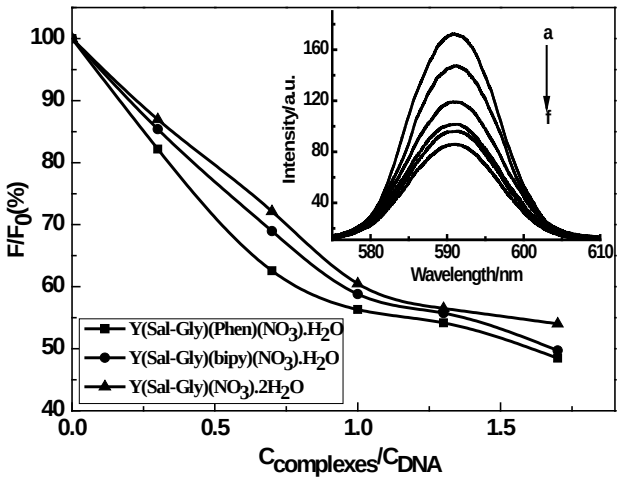

Fig. 4. Relative fluorescent intensity curve of three complexes interaction with EB-DNA. The inset plot shows the effect of Y(Sal-Gly)(Bipy)(NO_3)•H_2O on the fluorescence spectra of EB-DNA system, λ_{ex}=537 nm, $\lambda_{max\text{-}em}$=591 nm. Arrow a→f shows the intensity changes upon increasing concentration of the complexes.

Table 1. The binding constants of rare earth complexes interaction with DNA (mol•L^{-1})

Complex	$K_b/10^4$(mol^{-1}·L)	r
Y(Sal-Gly)(NO_3)•2H_2O	1.53	0.9938
Y(Sal-Gly)(bipy)(NO_3)•H_2O	2.47	0.9926
Y(Sal-Gly)(phen)(NO_3)•H_2O	3.12	0.9877
Dy(Sal-Gly)(NO_3)•2H_2O	1.14	0.9941
Dy(Sal-Gly)(bipy)(NO_3)•H_2O	2.11	0.9815
Dy(Sal-Gly)(phen)(NO_3)•H_2O	2.86	0.9886

3.5 Viscosity measurements

Viscosity studies of DNA is a classical technique used to analyze DNA binding mode in solution[5].

As shown in Fig. 5, the relative viscosity of the DNA solution enhance with the increasement of the amount of complexes and the degree of enhancement is Y(Sal-Gly)(phen)(NO_3)•H_2O>Y(Sal-Gly)(bipy)(NO_3)•H_2O>Y(Sal-Gly)(NO_3)•2H_2O, which is further illustrate the planarity and surface area of the ligand is a key influencing factors in the interaction between DNA and complexes. Such behaviorfurther suggests that the major interaction mode between complexes and DNA should be intercalation binding.

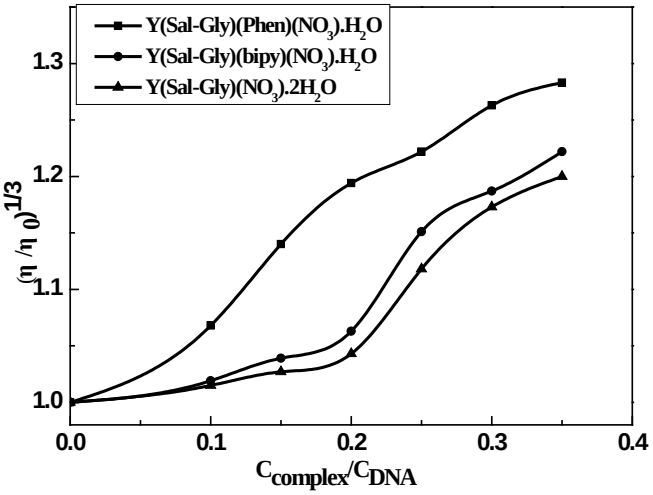

Fig. 5. Influence on DNA viscosity with different concentrations of complexes. η^0 and η are the relative viscosity of DNA solution before and after the complexes added, respectively. C_{DNA} = 1.00×10^{-4} mol/L. a, Y(Sal-Gly)(Phen)(NO_3)•H_2O; b,Y(Sal-Gly)(Bipy)(NO_3)•H_2O; c, Y(Sal-Gly)(NO_3)•2H_2O.

3.6 Antibacterial activities of ligands and complexes

As shown in Table 2, all the complexes exhibit stronger inhibitory effect on the *E. coli* than the ligands. The antibacterial activities of the rare earth complexes increased with the increasing concentration of the complexes in the range of 0.004-0.012 mol/L. we can see from table 2, the antibacterial activities of complexes is RE(Sal-Gly)(phen)(NO_3)•H_2O>RE(Sal-Gly)(bipy)(NO_3)•H_2O. The enhanced antibacterial activities of the complexes presumably result from the increasement of liposolubility of the rare earth complexes by chelate effect[6].

Table 2. Diameters of antibacterial rings of compounds with different concentrations on E. coli /mm

Concentration compounds	0.004 mol/L	0.008 mol/L	0.012 mol/L
DMF	＜10.0	＜10.0	＜10.0
Sal-GlyK	＜10.0	11.0	11.0
phen	14.0	16.0	19.0
bipy	＜10.0	＜10.0	＜10.0
Tb(Sal-Gly)(phen)(NO_3)•H_2O	20	25	29
Sm(Sal-Gly)(phen)(NO_3)•H_2O	19	23	25
Dy(Sal-Gly)(phen)(NO_3)•H_2O	23	24	28
Y(Sal-Gly)(phen)(NO_3)•H_2O	21	23	26
Gd(Sal-Gly)(phen)(NO_3)•H_2O	20	25	27
Y(Sal-Gly)(bipy)(NO_3)•H_2O	16	18	22
Tb(Sal-Gly)(bipy)(NO_3)•H_2O	16	19	24
Sm(Sal-Gly)(bipy)(NO_3)•H_2O	15	18	21

4 Conclusion

In this work, a new series of Schiff base ternary rare earth complexes: RE(Sal-Gly)(phen)(NO_3)•H_2O, RE(Sal-Gly)(bipy)(NO_3)•H_2O and RE(Sal-Gly)(NO_3)•$2H_2O$ (RE=Tb, Sm, Dy, Y, Gd) were synthesized. Fluorescence quench, hypochromism studies and viscosity measurements of them suggest that the complexes exhibit a strong interaction towards DNA via intercalative binding. Especially, we found the sequence of binding ability of the complexes to DNA is RE(Sal-Gly)(phen)(NO_3)•H_2O>RE(Sal-Gly)(bipy)(NO_3)•H_2O>RE(Sal-Gly)(NO_3)•$2H_2O$. In addition, all these complexes exhibit excellent antibacterial ability against E. coli, and the antibacterial activities of complexes with big planarity ligand are stronger than that of the small one.

Acknowledgements

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