# Synthesis, Characterization, Antitumour and Antibacterial Activity of Rare Earth Metal(III) Solid Complexes with Tetraiodofluorescein

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Eight new solid complexes of tetraiodofluorescein ($H_2L$) with rare earth metals have been prepared and characterized by means of elemental analysis, molar conductivity, IR, $^1$H NMR, XPS, and TG-DTA. The general formula for the complexes is $M_2L_3 \cdot nH_2O$, where L = tetraiodofluorescein (2,7-OH group deprotonated and the lactonic ring opened); M = La, Ce, Pr, Nd, Sm, Eu, Gd or Y; $n$ = 8, 9, 10, 11 or 14. *In vitro*, $H_2L$ and its complexes have been studied for their possible antitumour activity against Hep-2 pharynx cancer cells and their possible antibacterial activity against *Shigella flexner* F2a, *Salmonella typhi* H034, and *Staphylococcus aureus*.

Fluorescein and tetraiodofluorescein ($H_2L$) have been used in analysis of metal ions because of their ability to form complexes with metal ions in solution [1—3]. As $H_2L$ itself exhibits antitumour and antibacterial activity [4—7], and rare earth metals exhibit special pharmacological action [8—10], the study of the synthesis of rare earth metal complexes with $H_2L$ and their biological activity is of interest. In this paper, we report on the preparation and characterization of eight new solid complexes of rare earth metals with $H_2L$ and discuss their biological activity for the first time.

## RESULTS AND DISCUSSION

Elemental compositions, decomposition temperature, molar conductance, and molecular formulae of the newly prepared complexes are presented in Table 1. The elemental analyses data show that the complexes have the general formula $M_2L_3 \cdot nH_2O$ (M = La, $n$ = 8; M = Ce, $n$ = 9; M = Nd, $n$ = 10; M = Pr, Sm, Eu, Y, $n$ = 11; M = Gd, $n$ = 14). The complexes are red in colour, air-stable, and soluble in methanol, ethanol, DMSO, THF, and acetone, but not in benzene, ether, and water. The molar conductivity values of the complexes in DMSO at 25 ℃ show that all complexes are nonelectrolytes [11].

The principal IR bands of $H_2L$ and its complexes are listed in Table 2. The $\nu$(OH) frequency of the ligand was not observed in the complexes and the $\nu$(C—O) (phenol) band observed in the ligand at 1251 cm$^{-1}$ shifted to higher frequency in the complexes, indicating coordination of the OH group of the ligand after deprotonation to form the M—O bond in the complexes [12]. The $\nu$(C═O) band of lactone of the ligand disappears upon complexation. However, two new bands corresponding to $\nu_{as}(COO^-)$ and $\nu_s(COO^-)$ vibrations are observed at 1546 cm$^{-1}$ and 1331 cm$^{-1}$. This shows that the carboxylate group of the ligand coordinates to the metal ion after the lactonic ring was opened. Since $\Delta\tilde{\nu}$ ($\Delta\tilde{\nu} = \tilde{\nu}(\nu_{as}(COO^-)) - \tilde{\nu}(\nu_s(COO^-))$) of the complexes are close to that of $Na_2L$ (disodium salt of $H_2L$, $\Delta\tilde{\nu}$ = 200 cm$^{-1}$), the carboxylate group is coordinated to the metal ion with bridging bidentate mode [13]. The complexes display $\nu$(C═O) of the quinoid carbonyl at $\tilde{\nu}$ = 1605 cm$^{-1}$, they are lower than the normal frequency of the quinoid carbonyl, indicating the oxygen atom in the quinoid carbonyl participated in the bond formation with the metal ion. The stretching vibration of the ligand ring C—O—C ($\tilde{\nu}$ = 1207 cm$^{-1}$) shifts to higher frequency in the complexes ($\tilde{\nu}$ = 1235—1240 cm$^{-1}$), excluding the possibility of the coordination of this oxygen to rare earth metals [13]. By comparison of the far-IR spectra of the complexes with that of the ligand, new peaks appear at 435—438 cm$^{-1}$ and

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**Table 1.** Characterization of the Complexes

| Compound | Formula | $w_i$(calc.)/% $w_i$(found)/% | | | Decomp. temp. | Molar conductivity | Yield |
|---|---|---|---|---|---|---|---|
| | | Metal | C | H | °C | $\Omega^{-1}$ cm$^2$ mol$^{-1}$ | % |
| *I* | $La_2(C_{20}H_6I_4O_5)_3 \cdot 8H_2O$ | 9.50 | 24.65 | 1.17 | 340 | 29.69 | 87 |
| | | 9.38 | 24.17 | 1.14 | | | |
| *II* | $Ce_2(C_{20}H_6I_4O_5)_3 \cdot 9H_2O$ | 9.52 | 24.48 | 1.23 | 348 | 44.27 | 85 |
| | | 9.58 | 24.42 | 1.22 | | | |
| *III* | $Pr_2(C_{20}H_6I_4O_5)_3 \cdot 11H_2O$ | 9.45 | 24.17 | 1.35 | 356 | 37.43 | 88 |
| | | 9.66 | 24.16 | 1.31 | | | |
| *IV* | $Nd_2(C_{20}H_6I_4O_5)_3 \cdot 10H_2O$ | 9.76 | 24.26 | 1.29 | 373 | 33.86 | 87 |
| | | 9.81 | 23.98 | 1.29 | | | |
| *V* | $Sm_2(C_{20}H_6I_4O_5)_3 \cdot 11H_2O$ | 10.02 | 24.02 | 1.34 | 345 | 37.85 | 86 |
| | | 10.01 | 24.02 | 1.33 | | | |
| *VI* | $Eu_2(C_{20}H_6I_4O_5)_3 \cdot 11H_2O$ | 10.12 | 23.99 | 1.34 | 353 | 36.02 | 86 |
| | | 10.32 | 23.96 | 1.36 | | | |
| *VII* | $Gd_2(C_{20}H_6I_4O_5)_3 \cdot 14H_2O$ | 10.24 | 23.49 | 1.51 | 385 | 44.44 | 85 |
| | | 10.38 | 23.66 | 1.25 | | | |
| *VIII* | $Y_2(C_{20}H_6I_4O_5)_3 \cdot 11H_2O$ | 6.18 | 25.04 | 1.40 | 360 | 42.50 | 88 |
| | | 6.44 | 24.88 | 1.32 | | | |

**Table 2.** IR Data of $H_2L$ and its Complexes

| Compound | $\tilde{\nu}$/cm$^{-1}$ | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | $\nu$(OH) | $\nu$(C═O)$^a$ | $\nu$(C═O)$^b$ | $\nu_{as}$(COO$^-$) | $\nu_s$(COO$^-$) | $\nu$(C—O) | $\nu$(C—O—C) | $\nu$(M—O) |
| $H_2L$ | 3424 | 1755 | | | | 1251 | 1207 | |
| *I* | | | 1603 | 1544 | 1331 | 1284 | 1235 | 438, 395 |
| *II* | | | 1603 | 1543 | 1331 | 1284 | 1238 | 436, 395 |
| *III* | | | 1605 | 1547 | 1332 | 1282 | 1236 | 438, 394 |
| *IV* | | | 1606 | 1545 | 1331 | 1284 | 1238 | 437, 396 |
| *V* | | | 1605 | 1546 | 1331 | 1284 | 1240 | 435, 393 |
| *VI* | | | 1603 | 1546 | 1330 | 1284 | 1239 | 438, 396 |
| *VII* | | | 1605 | 1546 | 1331 | 1284 | 1240 | 436, 395 |
| *VIII* | | | 1603 | 1545 | 1332 | 1284 | 1236 | 436, 393 |

*a*) $\nu$(C═O) of lactone; *b*) $\nu$(C═O) of the quinoid carbonyl.

393—396 cm$^{-1}$, indicating the formation of the M—O bond.

The $^1$H NMR spectra of $H_2L$ and complex *VIII* were studied. The chemical shifts for $H_2L$, $\delta$: 7.40 (s, 2H, $H_1$), 7.00 (br, 1H, $H_2$), 7.61 (br, 2H, $H_3$), 7.99 (br, 1H, $H_4$), 10.20 (br, 2H, $H_5$); and for complex *VIII*, $\delta$: 7.17 (s, 2H, $H_1$), 7.34 (br, 1H, $H_2$), 7.72 (br, 2H, $H_3$), 8.02 (br, 1H, $H_4$). There are three apparent differences in their $^1$H NMR spectra. Firstly, there is no $\delta = 10.20$ peak of the ligand OH hydrogen atom in the spectrum of the complex, *i.e.* ligand OH hydrogen atom is replaced by Y(III) on complex formation. Secondly, the resonances of hydrogens 2, 3, and 4 shift towards lower field (0.34, 0.11, 0.03, respectively), these are due to the decreasing of the electron density of the benzene ring where the hydrogens 2, 3, and 4 are located and result from the coordination between the metal ion and the ligand after the lactonic ring was opened. Thirdly, because of the coordination of ligand OH after deprotonation, the electron density of the benzene rings where the hydrogen 1 is located increases, causing that the resonance of hydrogen 1 shifts towards higher field by 0.23.

XPS of complex *VII* and the ligand were studied as well. The FWHM of O1*s* of the ligand is wider, since the lactonic ring contained two kinds of oxygen (C═O, C—O). However, the peak of O1*s* is single in the spectra of the complex, resulting from the charge transfer of carbonyl oxygen to the oxygen of C—O, suggesting that the ligand coordinates with Gd(III) through carboxyl oxygen in a bidentate fashion after the lactonic ring opened. This result is consistent with the IR studies. By comparison with gadolinium chloride, the binding energy of Gd4*d* of the complex shifted lower (0.3 eV), as a result of the transfer of the electron of the ligand oxygen to Gd, which suggested coordination of the metal with the ligand.

The thermal behaviour of the complexes is similar. Data of *IV* and *VII* are given in Table 3. The endothermic peaks of the complexes corresponding to water

**Table 3.** Thermal Data of Complexes *IV* and *VII*

| Compound | Water loss | | Decomp. temp. | Residue | |
|---|---|---|---|---|---|
| | Temp./℃ | $-\Delta w$/% | ℃ | $-\Delta w$/% | Formula |
| *IV* | 74.2 | 5.88 | 373.4 | 33.36 | $NdI_3$ |
| *VII* | 94.1 | 8.52 | 385.4 | 32.40 | $GdI_3$ |

**Table 4.** Data of Inhibitory Activity of the Ligand and its Complexes against Hep-2 Cells

| Compound | Concentration | Inhibitory ratio | $IC_{50}$ |
|---|---|---|---|
| | $\mu$g cm$^{-3}$ | % | $\mu$g cm$^{-3}$ |
| $H_2L$ | 0.1 | 5.5 | 23.10 |
| | 1.0 | 12.0 | |
| | 10 | 30.2 | |
| | 100 | 74.5 | |
| *IV* | 0.1 | 18.7 | 1.07 |
| | 1.0 | 49.1 | |
| | 10 | 86.2 | |
| | 100 | 97.1 | |
| *VIII* | 0.1 | 11.6 | 12.69 |
| | 1.0 | 19.7 | |
| | 10 | 45.5 | |
| | 100 | 82.0 | |

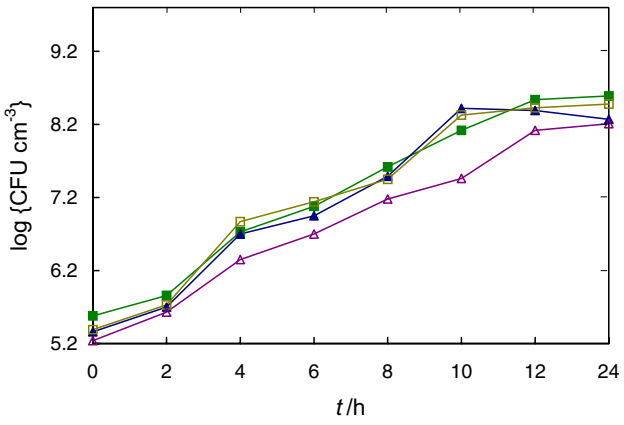


**Fig. 1.** The growth curves of *Shigella flexner* F2a. ■ Ethanol, ▲ tetraiodofluorescein, □ yttrium chloride, △ complex *VIII*.

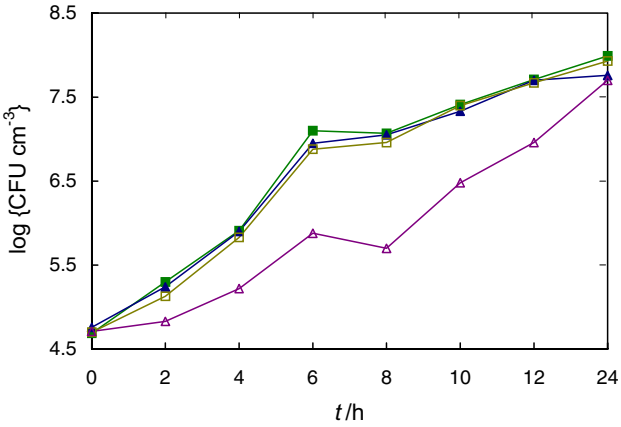


**Fig. 2.** The growth curves of *Salmonella typhi* H034. Denotation as in Fig. 1.

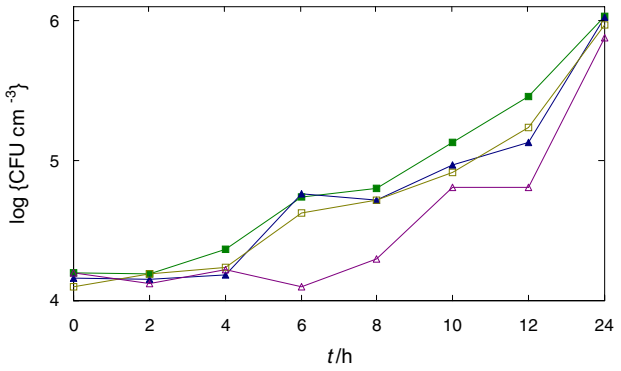


**Fig. 3.** The growth curves of *Staphylococcus aureus*. Denotation as in Fig. 1.

loss in the DTA curve (at 74.2℃ and 94.1℃, respectively) suggest that the water molecules are present as crystal water. The exothermic peaks of the complexes show the higher decomposition temperature (around 373—385℃) than that of the ligand (303℃), indicating the former are more stable than the latter. The complexes decompose completely at around 520℃. The residues are metal iodides, consistent with that of disodium salt of $H_2L$ [14].

Data on effects of $H_2L$, complex *IV*, and complex *VIII* against Hep-2 cells are listed in Table 4. It can be seen that both $H_2L$ and its complexes have dose-dependent inhibitory activity against Hep-2 cells. Their inhibitory ratios are greatly enhanced at higher concentration. Also, it has been observed that the complexes show obviously stronger effect than the ligand.

Calculating the log{CFU cm$^{-3}$} at different moment in each group studied ($T$ test was used in the statistics), we found that only the complex *VIII* shows obviously stronger inhibitory activity than the ligand and the rest of the complexes show similar inhibitory activity to the ligand. The growth curves of different bacteria in group of complex *VIII* are shown in Figs. 1—3. From the results it can be seen that the complex *VIII* with the concentration 32 $\mu$g cm$^{-3}$ has stronger inhibition on the growth of *Shigella flexner* F2a, *Salmonella typhi* H034, and *Staphylococcus au-*

*reus* than $H_2L$ itself. So we conclude that the antibacterial effect of $H_2L$ could be enhanced by rare earth Y(III) after complexation.

## EXPERIMENTAL

The starting compounds included rare earth chlorides which were transformed from respective oxides (Shanghai Yuelong Chemical Works, China), $H_2L$ (Merck, USA), RPMI1640 medium (Gibco, USA), inactive fetal calf serum (FCS, Lanzhou Institute of Biological Products, Ministry of Health, China), Hep-2 human pharynx cancer cells (The Fourth Military Medical University, China), *Shigella flexner* F2a, *Salmonella typhi* H034, *Staphylococcus aureus* (Lanzhou Institute of Biological Products, Ministry of Health, China). Solvents and reagents used were of anal. grade.

The elemental analyses of the complexes were obtained using a Varian EL analyzer. The metal content was determined by titration with EDTA. Electrolytic conductance measurements of compounds ($c \approx 10^{-3}$ mol dm$^{-3}$) were made with a DDS-11A digital conductometer with DMSO as solvent at 25 ℃. IR spectra were recorded on a Nicolet 170SX FTIR spectrophotometer using KBr discs in the range $\tilde{\nu} = 200$—4000 cm$^{-1}$. $^1$H NMR spectra were measured with an FT-80A nuclear magnetic resonance instrument using DMSO-$d_6$ as solvent and TMS as internal reference. The XPS were recorded on PHI550 multifunction X-ray photoelectron spectrometers and analyzed by the ESCALAB-210 processor, MGK$\alpha$ ($E_{h\nu} = 1253.6$ eV) as the X-ray source and the C1*s* binding energy of the ring—C and hydrocarbon—C as the standard ($E_b = 284.6$ eV, CAE = 30 eV) were used. TG-DTA measurements were made in a nitrogen atmosphere between room temperature and 800 ℃ using a Dupont 1090-B thermal analyzer.

Antitumour activity tests of $H_2L$, complex *IV*, and complex *VIII* were performed as in Ref. [3].

### Complexes *I*—*VIII*

$H_2L$ (2 g; 2.4 mmol) was dissolved in 20 cm$^3$ of absolute ethanol and mixed with 10 cm$^3$ of aqueous solution containing NaOH (0.072 g; 1.8 mmol), then with stirring the solution was added dropwise to 20 cm$^3$ of an aqueous solution containing $RECl_3$(1 mmol). Along with quick formation of precipitates the solution was stirred continuously for 2 h. The precipitates were isolated by filtration, washed several times with 95 % ethanol, dried in vacuum to constant mass. Yield: $\geq$ 85 %.

### Antibacterial Activity Tests

*In vitro*, the tests of $H_2L$ and complexes were carried out by cultivating bacteria in liquid culture medium and counting the bacteria using the method of dropping plate after diluting [15]. The chosen strains included *Shigella flexner* F2a, *Salmonella typhi* H034, and *Staphylococcus aureus*, since $H_2L$ was reported to have antibacterial activity against these strains [6, 7]. The procedures of the tests with different bacteria were similar. We demonstrate the procedure of *Shigella flexner* F2a as an example:

$H_2L$, $RECl_3$, and the new complexes were dissolved in ethanol to make stock solutions ($\rho = 1280$ $\mu$g cm$^{-3}$, respectively) which were kept in a refrigerator at 4 ℃. After *Shigella flexner* F2a was inoculated to log growth stage, the bacterial culture (50 mm$^3$) was inoculated into a tube containing 9 cm$^3$ of culture medium and the complex stock solution (0.23 cm$^3$) was added to obtain the concentration of 32 $\mu$g cm$^{-3}$. At the same time, we used the stock solutions of $RECl_3$ and $H_2L$, as well as ethanol as controls. All groups were cultivated at 37 ℃. At the start (0 h) and after 2 h, 4 h, 6 h, 8 h, 10 h, 12 h, and 24 h, some bacterial cultures were taken out and dropped on different agar plate after diluting, then cultivated for 24 h at 37 ℃, the CFUs (Colony Forming Unit) of different plate were counted, and the CFU cm$^{-3}$ of each group at different moment was calculated.

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