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Spectral studies on the interaction of yttrium ion with the ligands of phenolic groups: *N*,*N*′-Ethylenebis[2-(*o*-hydroxyphenolic)glycine] and *N*,*N*′-di(2-hydroxybenzyl)ethylenediamine-*N*,*N*′-diacetic acid

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Abstract

The interactions of yttrium with *N*,*N*′-ethylenebis[2-(*o*-hydroxyphenolic)glycine] (EHPG) and *N*,*N*′-di(2-hydroxybenzyl)ethylenediamine-*N*,*N*′-diacetic acid (HBED) are investigated by using UV difference and fluorescence spectra methods in 0.1 M *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid (Hepes) at pH 7.4. Yttrium binding produces two UV difference peaks near 240 and 294 nm, respectively, that both are the characteristic of phenolic groups binding to yttrium. The molar extinction coefficient of Y-EHPG and Y-HBED are $(15.7 \pm 0.40) \times 10^3$, $(15.8 \pm 0.80) \times 10^3$ cm^{-1} M^{-1} at 240 nm, respectively. Using EDTA as a competitor the obtained conditional equilibrium constants of the complexes are $\log K_{Y\text{-}EHPG} = 15.07 \pm 0.32$ and $\log K_{Y\text{-}HBED} = 15.18 \pm 0.26$, respectively. However, the effects of yttrium binding on the fluorescence intensity of EHPG and HBED are quite different, the former showing a decrease but the latter an increase.

Keywords: HBED; EHPG; Yttrium; Spectra

1. Introduction

Serum transferring (Tf) is a kind of important iron-transport proteins present in the serum of vertebrates at a concentration of 35 μM [1]. Chemistry of transferrin is important for understanding the role of metals in health, disease, therapy and diagnosis. Transferrin has two remarkably similar domains, in which iron is octahedrally coordinated to two oxygens from two tyrosine residues, one nitrogen from an imidazole ring of histidine residues, one oxygen from aspartic acid residue and a carbonate anion (the so-called "synergistic anion") adjacent to arginine residue in an unknown state of protonation [2,3]. Both EHPG and HBED (Fig. 1) can form stable sexadentate complexes with lanthanide ions, similar to transferrin [4,5], which played important roles to determine transferrin how to coordinate with metal ions [6]. Lanthanides have been known for their diversity in biological effects and the application of lanthanides in medicine has high potential [7,8]. Y. Zhao, B. Yang [9] have studied on the interaction of EHPG with lanthanum and obtained the conditional binding constants of the complexes of EHPG with it. Z. Wang, B. Yang [10] have observed the UV difference spectra and fluorescence spectra of the complexes of EHPG and HBED with neodymium ion, and obtained the conditional binding constants of the complexes of EHPG or HBED with it.

In this paper, we have observed that the UV difference spectra and fluorescence spectra of the complexes of EHPG or HBED with yttrium ions and obtained the conditional binding constants of the complexes of EHPG or HBED with it so as to further explain how the transferrin coordinate with metal ions. There are different effects on the fluorescence of HBED during yttrium binding to HBED when it is contrast to neodymium due to the different structures of valence shell between yttrium and neodymium.

2. Experimental

2.1. Materials

N,*N*′-Ethylenebis[2-(*o*-hydroxyphenolic)glycine] (EHPG), *N*,*N*′-di(2-hydroxybenzyl)ethylenedi-amine-*N*,*N*′-diaceticacid

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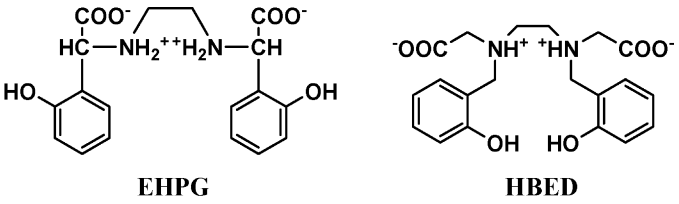

Fig. 1. Molecular structure of EHPG, HBED.

(HBED), *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid (Hepes), and ethylenediaminetetraacetate (EDTA) were all analytical grade reagents and used without further purification. Yttrium oxide is 99.99%.

2.2. Stock solutions

Yttrium solution was prepared by dissolving a certain weight of the yttrium oxide in a small volume of diluted hydrochloric acid. The solution was diluted to the mark with distilled water. The yttrium oxide stock solution was standardized by complexiметric titration with EDTA and xylenol orange as the metal indicator in acetate buffer at pH 5.5. The stock solutions of EHPG or HBED were prepared by dissolving weighed samples. Each solution was diluted to a definite volume with distilled water. The stock solutions were standardized by complexiметric titration with standardized zinc by using xylenol orange indicator in HAc/NaAc buffer at pH 5.5.

2.3. Methods

UV difference spectra were recorded on a Hewlett Packard 8453 spectrometer. The sample cuvette containing EHPG and HBED was titrated with a solution of yttrium to determine a value of the molar absorptivity ($\Delta\varepsilon_Y$) of the Y-EHPG or Y-HBED complex to be used in the calculation. The sample was also titrated with a solution of yttrium that contained various concentrations of EDTA as a competing ligand to measure the equilibrium constants. During the titration the sample was maintained at 25 °C by using a jacketed cell holder connected to an external circulating water bath (Huber). To correct dilution during each titration and to normalize the results from different titrations, the absorbance data were converted to absorptivities ($\Delta\varepsilon$) by dividing the absorbance by the analytical concentration of EHPG or HBED.

The series of determined solution during the fluorescence spectra experiment were accorded with the difference UV–vis.

3. Results

3.1. UV difference spectra

UV difference absorption can be used to measure the binding of metal ions with EHPG or HBED [6,11,12]. A set of UV difference spectra caused by the addition of aliquots of yttrium to 2 mL of EHPG in 0.1 M Hepes at pH 7.4 are shown in Fig. 2. Three extremes can be found: two maxima at 240 and 294 nm and a single minimum at 272 nm.

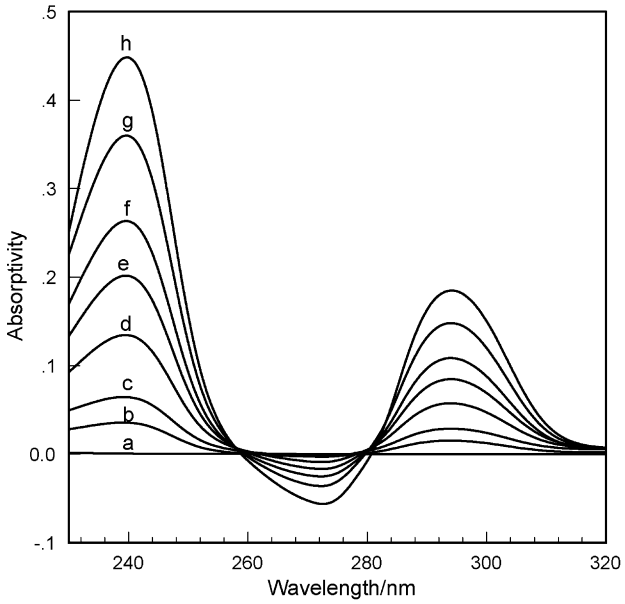

Fig. 2. UV spectra produced by the addition of yttrium to EHPG in 0.1 M Hepes at pH 7.4 and 25 °C. EHPG (2.62×10^{-5} M) 2.0 mL; the volume (μL) of yttrium (1.31 mM): (a) 0; (b) 5; (c) 10; (d) 15; (e) 20; (f) 25; (g) 30; (h) 40.

At pH 7.4, the phenolic oxygens of the free ligand are completely protonated [13]. Yttrium coordination displaces these protons, so the absorption spectrum reflects the differences in absorptivity between the yttrium complex and the protonated form of the ligand. The absorbance at 240 nm at each point in the titration was divided by the analytical concentration of EHPG to give a value of $\Delta\varepsilon$. Titration curves were prepared by plotting $\Delta\varepsilon$ versus r, which was defined as the ratio of total yttrium to the analytical concentration of EHPG. Titration of EHPG by adding the solutions of yttrium is shown in Fig. 3a. The plot has

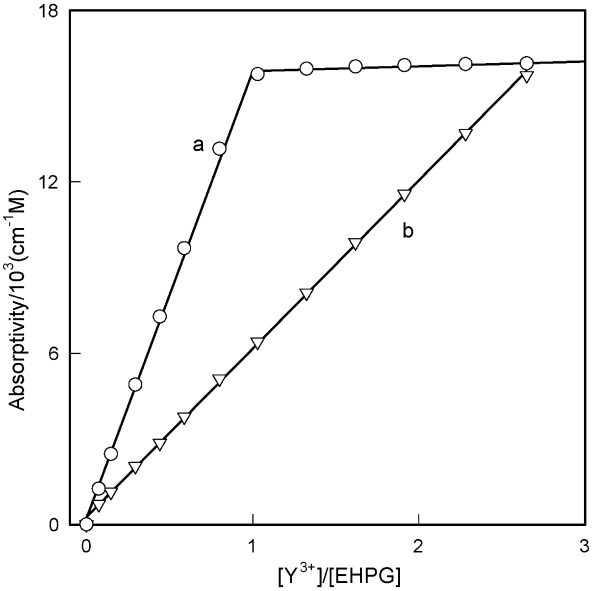

Fig. 3. Titration curve of EHPG in 0.1 M Hepes at 25 °C, and pH 7.4 with the solution of yttrium that contained different [EDTA]/[Y] ratios. EHPG (2.62×10^{-5} M) 2 mL; yttrium (1.31 mM) R_t = [EDTA]/[Y(III)]. (a) $R_t = 0$; (b) $R_t = 0.5$.

a sharp break near $r = 1$, showing the 1:1 ligand stoichiometry of the Y-EHPG complex. From the slope of the line at $r \leq 1$ the molar extinction absorptivity ($\Delta\varepsilon_Y$) of Y-EHPG complex is $(15.7 \pm 0.40) \times 10^3\ cm^{-1}\ M^{-1}$. To determine the binding constant of Y-EHPG, the solutions of EHPG were titrated with titrant containing EDTA. Fig. 3b shows the titration curves. EDTA serves as a competitive ligand, thus the observed absorptivity decreases as the EDTA:Y ratio increase.

Assuming that the decrease of absorptivity at a given r value is attributed to the change of Y-EHPG to Y-EDTA, the system being described by mass balance equations for yttrium, EHPG, and EDTA, the concentrations of species for EDTA, EHPG and yttrium can be calculated as follows:

$$[\text{Y–EHPG}] = \frac{\Delta\varepsilon_{(b)}}{\Delta\varepsilon_Y} \times [\text{EHPG}]_t \quad (1)$$

$$[\text{Y–EDTA}] = [Y^{3+}]_t - [\text{Y–EHPG}] \quad (2)$$

$$[\text{EDTA}]_f = [\text{EDTA}]_t - [\text{Y–EDTA}] \quad (3)$$

$$[\text{EHPG}]_f = [\text{EHPG}]_t - [\text{Y–EHPG}] \quad (4)$$

$$[Y^{3+}]_f = \frac{[\text{Y–EDTA}]}{[\text{EDTA}]_f \times K_{\text{Y–EDTA}}} \quad (5)$$

The conditional binding constant, $K_{\text{Y-EDTA}}$ of Y-EDTA complex is $10^{15.21}$ at pH 7.4 [14]. Thus from Fig. 3 we can calculate the binding constant, $\log K_{\text{Y-EHPG}} = 15.07 \pm 0.32$, in 0.1 M Hepes at pH 7.4 by using Eq. (6). Data were taken for Y:EHPG ratios less than 1.0 for the calculation.

$$K_{\text{Y–EHPG}} = \frac{[\text{Y–EHPG}]}{[Y^{3+}]_f \times [\text{EHPG}]_f} \quad (6)$$

In a similar way $\log K_{\text{Y-HBED}} = 15.18 \pm 0.26$ can be obtained for Y-HBED complex, with a molar absorptivity ($\Delta\varepsilon_Y$) of $(15.8 \pm 0.80) \times 10^3\ cm^{-1}\ M^{-1}$ in 0.1 M Hepes at pH 7.4.

3.2. Fluorescence spectra

A solution of HBED in 0.1 M Hepes buffer (pH 7.4) at room temperature was added to a dry fluorescence cuvette. The addition of sequential aliquots yttrium to the cuvette produced a fluorescence spectra family with excitation at 285 nm as shown in Fig. 4, where peak towers near 318 nm, which is enhanced by the addition of yttrium. To correct the dilution effect, the fluorescence intensity at 318 nm was converted to molar fluorescence intensity (F_M) via dividing the fluorescence intensity by the analytical concentration of HBED. The plot F_M versus r, stands for the ratio of total yttrium to the analytical HBED concentration, as shown in Fig. 5 (HBED). A sharp break occurred near $r = 1$, confirming the 1:1 ligand stoichiometry of the Y-HBED complex. As indicated in Fig. 4, the binding of yttrium leads to an increase in the molar fluorescence intensity as large as 320%. At pH 7.4, the phenolic oxygens of the free HBED are completely protonated [15]. The increase of fluorescence intensity at 318 nm is attributed mainly to the displacement of phenolic oxygens protons by the yttrium coordination. The results are different from adding neodymium to the solution of HBED.

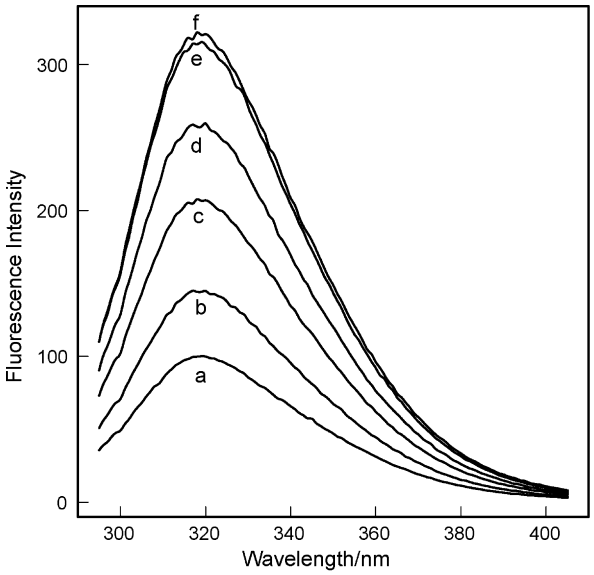

Fig. 4. The fluorescence emission spectra for the addition of yttrium to an aqueous solution of HBED in 0.01 M Hepes at pH 7.4 and room temperature with excitation at 285 nm. HBED (4.43×10^{-5} M) 2.0 mL; slit width, 5 nm; the volume (μL) of yttrium (1.11 mM) is (a) 0; (b) 20; (c) 40; (d) 60; (e) 80; (f) 120.

Z. Wang, B. Yang [10] have studied on the fluorescence spectral changes induced by neodymium binding of HBED, and the results demonstrate that there is no obvious change of fluorescence intensity at 318 nm during the titration of neodymium in 0.01 M Hepes at pH 7.4 and room temperature. It may be attributed to the different structure of valence shell between neodymium and yttrium. The valence shell of neodymium contains f electrons, and the f–f transition results in the quenching of the fluorescence of HBED at 318 nm.

Under the conditions above, we could get the fluorescence spectra family of adding yttrium to EHPG, whose shape is similar as that to EHPG but the peak tower is near 310 nm, which is

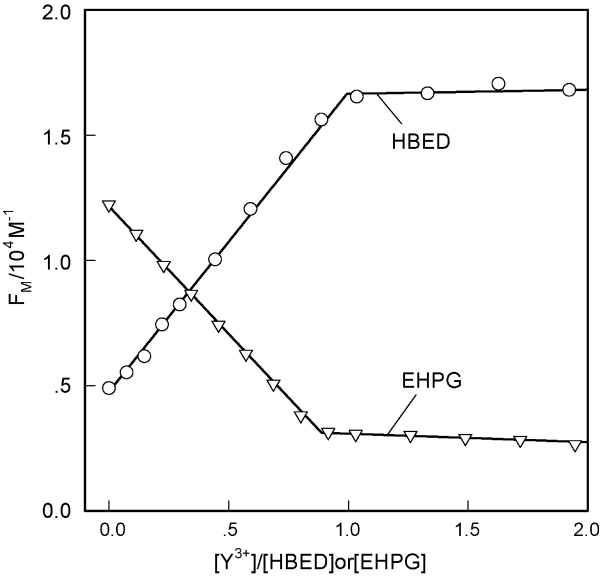

Fig. 5. Fluorescence titration curves for the addition of yttrium(III) to EHPG or HBED in 0.01 M Hepes, respectively.

lowered (Fig is not shown) by the addition of yttrium. Similarly, we correct the dilution effect as above. A sharp break near $r = 1.0$ Fig. 5 (EHPG), confirming the 1:1 ligand stoichiometry of the Y-EHPG complex. At pH 7.4, the phenolic oxygens of the free EHPG are completely protonated [15]. The reduction of fluorescence intensity at 310 nm may also due to the displacement of phenolic oxygens protons by the yttrium coordination.

4. Discussion

At neutral or acidic pH, the phenolic oxygens of EHPG or HBED are completely protonated. Due to the metal coordination displaces these protons, the absorption spectra reflects the differences in absorption of the metal complex and the protonated form of the ligand. From fluorescence spectra it can also be confirmed that EHPG or HBED and yttrium can form a very stable complex. However, the effect of yttrium binding on the fluorescence intensity of EHPG and HBED is disparate during the titration of yttrium in 0.1 M Hepes at pH 7.4. With the addition of yttrium, the fluorescence intensity of EHPG decreases gradually, while that of HBED increases smoothly. The difference of fluorescence property may reflect the differences between the two free ligands in 0.1 M Hepes at pH 7.4. Under that condition, the hydrogen-bonded ring structures, which belongs to N···H–O type hydrogen bond, can be kept partially in HBED, while EHPG can exist in the partially hydrogen-bonded ring structure, which is O···H–O type. When N···H–O type intramolecular hydrogen bond is broken, the fluorescence intensity of HBED increases, while the O···H–O type intramolecular hydrogen bond is destroyed, the fluorescence intensity of EHPG, decreases.

5. Conclusion

We studied the interaction of yttrium with HBED and EHPG by the method of UV difference spectra and fluorescence spectra at pH 7.4, 25 °C. It can be drawn that yttrium can form stable complex with EHPG or HBED by 1:1 ratio. The molar extinction coefficient of Y-EHPG and Y-HBED are $(15.7 \pm 0.40) \times 10^3$, $(15.8 \pm 0.80) \times 10^3\ cm^{-1}\ M^{-1}$ at 240 nm, respectively. The conditional binding constants of the complex are obtained through calculation, $\log K_{\text{Y-EHPG}} = 15.07 \pm 0.32$ and $\log K_{\text{Y-HBED}} = 15.18 \pm 0.26$. From fluorescence spectra, it can be confirmed that EHPG or HBED and yttrium can form stable complex by 1:1 ratio. Yttrium binding to EHPG leads to a quenching of the fluorescence of EHPG at near 310 nm, but an increase of the fluorescence of HBED at near 318 nm is observed with the binding of yttrium to HBED.

Acknowledgements

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