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Spectroscopic Study of the Interaction of Bu_2SnCl_2 with EHPG

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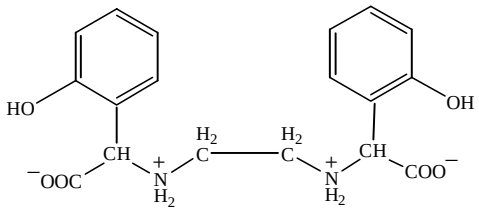
Abstract: The difference UV spectra and fluorescence of Bu_2SnCl_2 and ethylene bis (o-hydroxyphenylglycine) (EHPG), a ligand used previously for mimicking Fe^{3+} binding of transferrins, was first studied in Tris-HCl buffer solution (pH7.4). Difference UV spectral studies show that the binding of Bu_2SnCl_2 to EHPG takes twelve hours and gives rise to a 1:1 complex. The binding constant for Bu_2SnCl_2-EHPG complex is estimated to be 1.65×10^3 M^{-1}. Fluorescence studies also show an increase in intensity of EHPG after interaction with Bu_2SnCl_2.

Keywords: EHPG, spectroscopy, transferrin.

A number of Sn (Ⅳ)complexes have been synthesized since 1980 and tested its anticancer activity[1]. The simplest complex Bu_2SnCl_2 has activity to P388 tumor cells. Several metal complexes such as Ru-ind (trans-indazolium (bisindazole) tetrachloro-ruthenate (Ⅲ)) had used transferrin as a drug delivery system, the complexes still exhibit high antitumor activity and lower toxicity[2]. We use EHPG as a model ligand[3] to study the interaction between Bu_2SnCl_2 and EHPG.



EHPG

At first, we study the difference UV spectra of reaction of Bu_2SnCl_2 with EHPG. When Bu_2SnCl_2 was added to an aqueous solution of EHPG (Tris-HCl buffer, pH 7.4), three new bands gradually appeared and increased in intensity over a period of 12 hours: two maxima at 293 and 238 nm and a single minimum at 276 nm. The wavelengths of two sharp bands at 238 and 293 nm are typical of phenolate groups generated by the binding of metal ions to tyr residues[4]. A single minimum at 276 nm is due to a configurational change of EHPG, and the result is consistent with the binding of Th^{4+} to phenolate groups on the ligand[3]. Analysis of the titration curve for reaction of EHPG with Bu_2SnCl_2 suggests the presumed 1:1 ligand stoichiometry of the Sn-EHPG complex.

The value of the extinction coefficient for the first step of Sn (Ⅳ) binding to EHPG is 2672 M^{-1} cm^{-1} at 238 nm, since this total value represents the coordination of two phenols, the molar extinction coefficient for per phenolic group is 1336M^{-1} cm^{-1}. The resulting EHPG-Sn (Ⅳ) solution was stable at ambient temperature.

The EHPG-Bu_2SnCl_2 binding constants were determined as reported for several drug-protein complexes[5]. The value of K was obtained from the optical absorption at an appropriate wavelength:

$$1/(A-A_0)=1/(A_\infty -A_0) +1/K(A_\infty -A_0)\times 1/[L_0]$$

Where A_0 is the initial absorption of the free EHPG at 238 nm and A is the recorded absorption at different EHPG-Bu_2SnCl_2 concentrations (L_0). A_∞ is the final absorption of the ligated EHPG. The double reciprocal plot of $1/[A-A_0]$ *vs.* $1/[L_0]$ is linear and binding constant can be estimated from the ratio of the intercept to the slope. The overall binding constant for EHPG-Bu_2SnCl_2 complexes is estimated to be 1.651×10^3 M^{-1}.

The same reaction was monitored through fluorescence spectroscopy. Excitation of EHPG at 280 nm stimulates fluorescence of tyrosine[6], there is a shoulder band after 280 nm excitation at near 310 nm, and it is concluded that two tyrosines are fluorescing. This fluorescence intensity is enhanced upon addition of Bu_2SnCl_2. Fluorescence excitation spectra of EHPG in the presence and absence of Bu_2SnCl_2 are investigated at fixed emission wavelength of 330 nm. The excitation spectrum of 275 nm at a fixed emission wavelength of 300 nm is produced by tyrosine[6]. The relative fluorescence intensity at 300 nm is enhanced upon addition of Bu_2SnCl_2 to EHPG and also has a shoulder band at 275 nm. These fluorescent results further suggest that Bu_2SnCl_2 can bind to EHPG and agree with the UV results.

The apparently strong binding of Sn (Ⅳ) ion to EHPG is consistent with predictions based on metal ion acidity[7]. Though preliminary, results may be of interest for studies of the organotin complexes using transferrin as a drug delivery system.

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