A FLUORESCENCE SPECTROSCOPY STUDY OF THE INTERACTION OF Tb(III) WITH DEXTRAN

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Abstract – A fluorescence spectroscopy study of Tb(III) with dextran in aqueous solution (pH 2.9 - 6.6) indicated the formation of a complex with the displacement of water from the cation coordination sphere.

Dextran is a polysaccharide formed by monomers of α-D glucopyranose, connected mainly through α 1-6 bonds (figure 1). It is known to interact with Cu(II), Zn(II), Co(II), Mn(II) and Ca(II) in aqueous solution. Dextran complexes with rare earth (RE) elements were suggested in the literature by Selye et al.[1], only. This work reports the intoxication of rats by lethal doses of rare earth chlorides, which could be prevented by pretreatment with dextran complexes of Fe and Cr.

Terbium(III) is a RE ion that presents intense fluorescence in solution. It has been observed that this fluorescence intensity is enhanced when the metallic ion is bonded to polymers[2,3]. We have described fluorescence experiments showing that dextran (molecular weight 60.000 - 90.000) interacts with Tb(III) in aqueous solution.

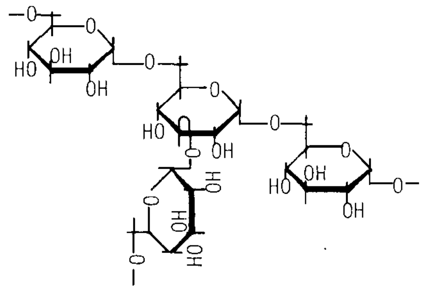

Figure 1. α(1-6) bond type in the main chain and α(1-3) bond type in side branches of dextran.

The emission spectra of the Tb(III) ion in the presence of dextran and in the absence of the polysaccharide are shown in figure 2. The position and shape of the four characteristic bands of the RE ion are unaltered by the presence of the polymer. However, the fluorescence intensity (F) was considerably enhanced. This enhancement was verified under λ_{ex} 250-310 nm (table 1) with a maximum intensification at 290 nm. The F measurements were made in the same experimental conditions, so, no standard was necessary.

Since the intensity of Tb(III) fluorescence is pH dependent, the pH effect in fluorescence enhancement was verified by titration of the dextran solution, at pH 3.0, 4.4 and 6.6, with Tb(III) (figure 3). The first two pHs were reached by addition of HCl and the latter was buffered with 0.03 M tris (tris hydroxy methyl amino methane). Precipitation of terbium hydroxide occurs at a pH higher than 6.6.

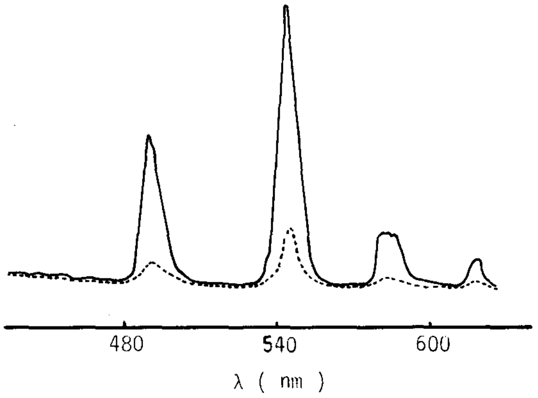

Figure 2. Fluorescence spectrum of Tb(III) in water (----) and dextran-Tb(——); $_{ex}$ = 290 nm; $5x10^{-3}$ M $TbCl_3$ and 0.05 M dextran (in monomer unit).

Table 1. Enhancement of Tb(III) fluorescence in the presence of dextran; $5x10^{-3}$ M $TbCl_3$, 0.05 M dextran (in monomer unit); λ_{em} = 543 nm.

λ_{ex} (nm)	$F^*_{Tb + dex}$	F^*_{Tb}	ΔF^*
250	6.4	4.0	2.4
260	18.1	16.4	1.7
280	19.3	9.6	9.7
290	17.7	5.3	12.4
300	16.2	4.8	11.4
310	6.5	1.6	4.9

* Arbitrary units.

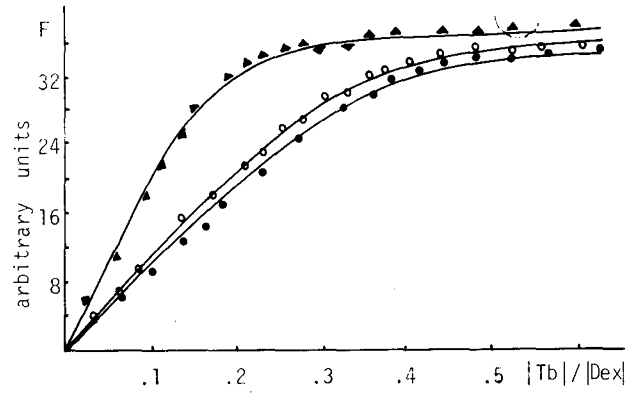

Figure 3. Verification of the pH effect on the spectrofluorimetric titration of 0.05 M dextran with Tb(III); λ_{ex} = 290 nm and λ_{em} = 543 nm; pH 6.6 (Δ), pH 4.4 (o) and pH 3.0 (o).

A saturation of the coordination sites of the polymer, as the Tb(III) concentration increases, was achieved in all experiments. This indicates that Tb(III) ions form a complex with dextran at a pH solution in a range of 2.9-6.6. The evidence of complexation by saturation sites was related in the literature in fluorimetric studies of RE ions with hydrolyzed polyacrylamide[2] and transferrin[3].

A shift of the titration curves to the right, as the pH decreases, suggests that the interaction of dextran-Tb, or the complex stoichiometry (molar ratio dex/Tb), increases with the pH.

Elmgren[4] demonstrated that the dextran deprotonation occurs in a pH superior to 12. Therefore, this effect, as the one responsible for the possible increase in the dextran - Tb interaction with pH, is excluded. As this interaction is of electrostatic character, variation in the ionic strength by pH could be one explanation for such pH effect. Small modifications in dextran macromolecular conformation with pH could also be considered.

As for the variation of the complex stoichiometry, data on figure 3 indicate that Tb(III) coordination is completed when the ratio between dextran monomer molar concentration and Tb(III) ion concentration is > 5.0, > 2.6 and > 2.3 at pHs 6.6, 4.4 and 3.0, respectively. The great difference between the molar ratio dex/Tb of the complex formed at pH 6.6 and the molar ratio obtained at other pHs could be associated with the degree of terbium hydrolysis. Species such as $[Tb(H_2O)_xOH_y]^{+(3-y)}$, $(x + y \simeq 9)$, at this pH, might be bonded to the dextran.

A 1:1 stoichiometry was determined for many RE complexes with cyclic polyhydroxyl ligands[5]. The small number of coordinated dextran monomers is probably due to the electrostatic repulsion between the polysaccharide chain, positively charged by the bonded metallic ion, and Tb(III) species, unbonded, surrounding the complex. Another explanation could be the steric hindrance effect of the dextran side branches.

Lugovaya et al.[6] showed that from 340 glucopyranose units, in 0.1 M dextran solution, only 17 copper ions are combined, a still smaller proportion than that observed for the dextran-Tb system.

The data in figure 3, together with the Tb(III) ion fluorescence intensity in the absence of the polymer, make it possible to estimate the relative increase in the RE ion fluorescence when bonded to the dextran. The complex Tb-dex fluorescence, for 10^{-3} M RE ion, has an intensity three times larger than the one for the pure Tb(III) ions, at the same concentration.

It is well known that high-frequency vibration, such as O-H and C-H, assumes a dominant role in the radiationless deexcitation of RE ions in solution. Deuterated systems have a lower vibrational spacings than that of the hydrogenated systems. An intensification of the fluorescence of RE ions and complexes is observed in these cases. The effect of deuteration, applied to water solvent, has been related to the number of water molecules coordinated to the metal[7].

We determined that the ratio of fluorescence in D_2O and H_2O (F_{D_2O}/F_{H_2O}) for 0.02 M $TbCl_3$ is 16 and for the same solution in the presence of 0.05 M dextran is 11. The rate of this radiationless deexcitation is proportional to the number of OH vibrations in the local RE environment. The substitution of HOH by COH groups of the polysaccharide in the cation coordination sphere could be the explanation for this observed effect. The asymmetry of the formed complex must also contribute to the intensification of the Tb(III) fluorescence.

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