^{1}H NMR spectroscopic characterization of inclusion complex of Desferrioxamine B chelator and β-Cyclodextrin

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Abstract

In this work the complexation between desferioxamine B (DFOB), and β-Cyclodextrin (β-CD) in solution was investigated using ^{1}H NMR spectroscopy. Inclusion compound formation was confirmed by the upfield shifts of H3 and H5 protons, located inside the cavity of β-CD, and downfield shifts of some of the methylene protons of DFOB, observed during NMR titration experiments. Using the continuous variation method, we determined a 1:1 stoichiometry and the association constant was calculated using a nonlinear least-square regression analysis implemented in CONSTEQ, a software developed in our group. A theoretical molecular docking study was additionally performed to ascertain possible conformations of the inclusion complex.

Experimental

The protagonists: Desferrioxamine B mesylate with 99.4% purity purchased from Ciba-Geigy and β-cyclodextrin containing an average of 8 water molecules/molecule, from Sigma Aldrich, are depicted in Fig. 1. DFOB is a siderophore synthesized by several species of actinomycetes and is the only chelator of iron used clinically to treat disorders related to iron overload and pathological iron deposition in man [1-3].

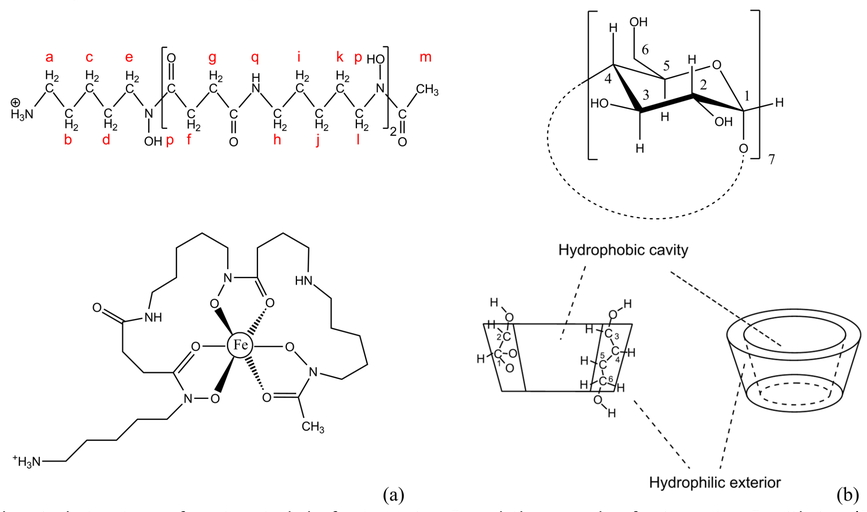


Fig. 1 Chemical structure of protonated desferrioxamine B and the complex ferrioxamine B with iron(3+). (b) β-cyclodextrin with α-D-glucopyranose subunit highlighted and its toroidal structure, with the larger and the smaller openings exposing to the solvent secondary and primary hydroxyl groups. The notation of protons referred to in the NMR study are additionally specified.

NMR spectra

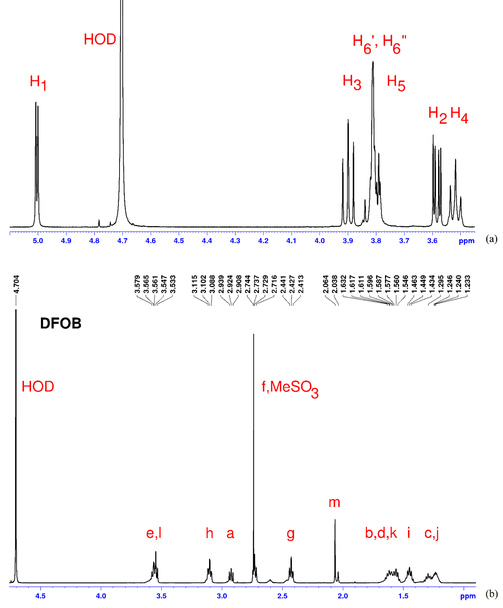


Fig. 2 500 MHz ^{1}H NMR spectra of pure bCD and DFOB samples.

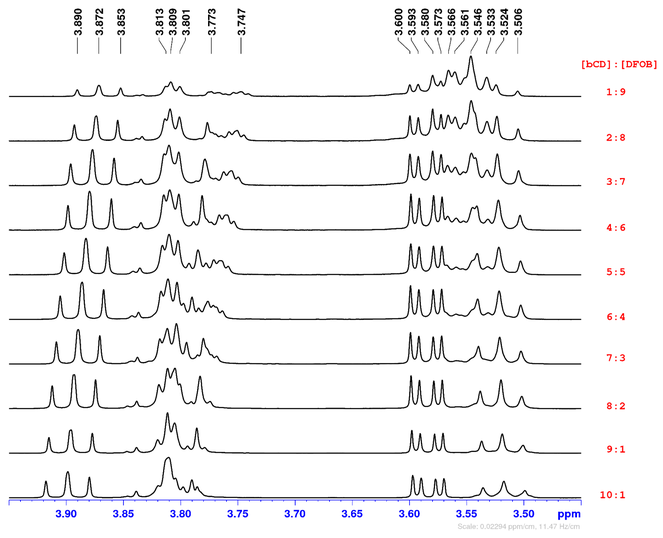


Fig. 3 Expanded ^{1}H NMR spectra on the region containing the β-cyclodextin resonances of all measured samples. Shifts of peaks corresponding to H3 and H5 protons are visible.

Method of Continous Variation (Job's Plot [4])

· Equimolar 10mM stock solutions of the host and guest were prepared in D_2O.
· The stock solutions are mixed together to constant volume (1.5 ml), at varying proportions, so that a complete range (0 < r <1) of ratios:

$$r = [X]/([H]_t + [G]_t)$$

$[X] = [H]_t$ or $[G]_t$ - the concentration of the host or guest in the sample, where $[H]_t$ and $[G]_t$ are the initial total concentrations of the host and guest
· From the NMR data the quantity $\Delta\delta_{obs}$ is obtained by subtracting the observed chemical shift value for a given sample from the chemical shift of the free X.
· $\Delta\delta_{obs}[X]$ is plotted against r.
· The maximum of the curve corresponds to stoichiometry.

In our case the maximum is at r = **0.5**, indicating **1 : 1 stoichiometry**.

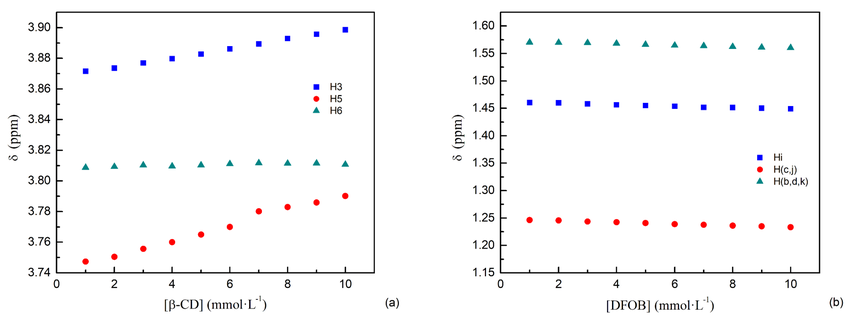


Fig. 4 Chemical shifts variation of some representative β-CD (a) and DFOB (b) protons as a function of their concentration.

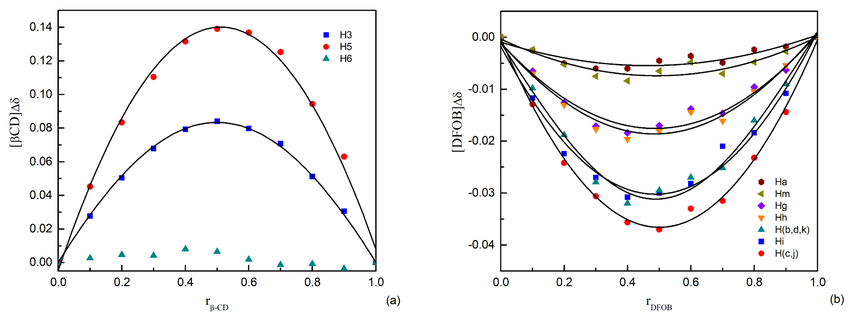
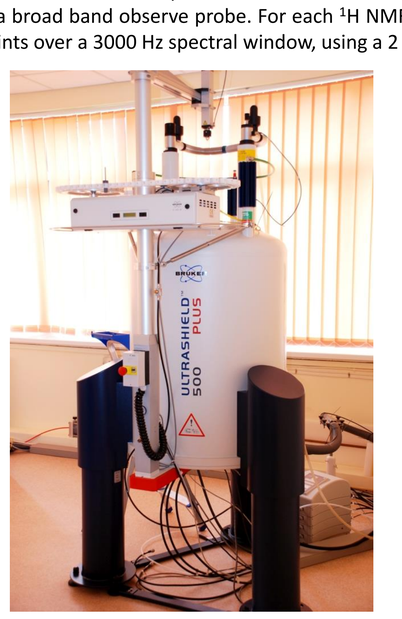


Fig. 5 Job's plots corresponding to the induced chemical variation of some β-CD (a) and DFOB (b) protons for the β-CD-DFOB system.

The Instrument

Nuclear magnetic resonance measurements were performed on a Bruker Advance III spectrometer operating at 500.13 MHz for proton, equipped with a broad band observe probe. For each ^{1}H NMR experiment recorded at 298K, 64 transients were collected into 32 K data points over a 3000 Hz spectral window, using a 2 s relaxation delay.



The Association constant

The analysis of host guest complexation is based on a simple equilibrium model:

$$a \cdot H + b \cdot G \leftrightarrow H_a G_b = C$$

The association constant may be then defined as:

$$K = \frac{[C]}{[H]^a[G]^b}$$
$$[H]_t = [H] + a \cdot [C]$$
$$[G]_t = [G] + b \cdot [C]$$

where:
· H the host, G the guest, C the complex
· a and b - the stoichiometry
· $[H]_t$ and $[G]_t$ - initial total concentrations of host and guest
· [H], [G] and [C] - concentrations at equilibrium
· [M] = $[H]_t$ + $[G]_t$

After some substitutions (see [5-8]) we arrive at the equation:

$$\Delta\delta^{(i,j)} = \frac{\Delta\delta_c^{(j)}}{2[X]_t}\left\{[M] + \frac{1}{K} - \sqrt{\left([M] + \frac{1}{K}\right)^2 - 4[H]_t^{(i)}[G]_t^{(i)}}\right\}$$

where *i* counts the sample number and *j* the studied proton, $\Delta\delta_c^{(j)}$representing the chemical shift difference for a given proton, between the free, uncomplexed molecule, and the pure inclusion complex.
This equation involves no approximation and correlates the initial total concentrations $[H]_t$ and $[G]_t$ with the observed difference in the chemical shift $\Delta\delta_{obs}$:

$$\Delta\delta_{obs}^{(i,j)} = \delta_{free}^{(j)} - \delta_{obs}^{(i,j)}$$
$$\Delta\delta_c^{(j)} = \delta_{free}^{(j)} - \delta_c^{(j)}$$

CONSTEQ software, developed in our group [8], adjusts the parameters (K and $\Delta\delta_c$) in the equation to obtain the best fit to the experimental values $\Delta\delta_{obs}$. Each iteration sets up a quadratic procedure to determine the direction of search and calculates the error function:

$$E = \sum_{i,j}\left(\Delta\delta^{(i,j)} - \Delta\delta_{obs}^{(i,j)}\right)$$

RESULTS

Taking into account all experimental values of proton resonances presenting the highest chemical shift variations, **H3** and **H5** protons of β-CD and **(c,j)**, **i**, **(b,d,k)**, **g** and **h** protons of DFOB, the fitting procedure implemented in CONSTEQ produced a single K value and a set of $\Delta\delta_c$ values. Using this procedure, the obtained association constant was **K = 127 M^{-1}** with an error function E = 3.1×10^{-5} and correlation factor r = 0.99.
The complete set of chemical shifts in the free state and in the pure complex are presented in Table 1.

Table 1 Chemical shifts of the β-CD and DFOB protons in the free and complexed states.

Proton	δ_{free} (ppm)	δ_c^* (ppm)
H3	3.899	3.816
H5	3.791	3.737
H(c,j)	1.233	1.258
Hi	1.449	1.471
H(b,d,k)	1560	1.580
Hg	3.102	3.115
Hh	2.427	2.440

*$\delta_c = \delta_{free} - \Delta\delta_c$ were $\Delta\delta_c$was obtained as a result of the fitting procedure.

Molecular docking

with the aim to obtain the most probable inclusion complex of DFOB in the cavity of β-cyclodextrin was performed using Monte Carlo simulated annealing search implemented in Autodock v4.2. The simulations were done not only for protonated conformer but also for neutral one and for hexadenate ligand, which has the hydroxamic acid groups deprotonated. The lowest binding energy conformations for the protonated conformer and hexadenate ligand are represented in Figure 6.

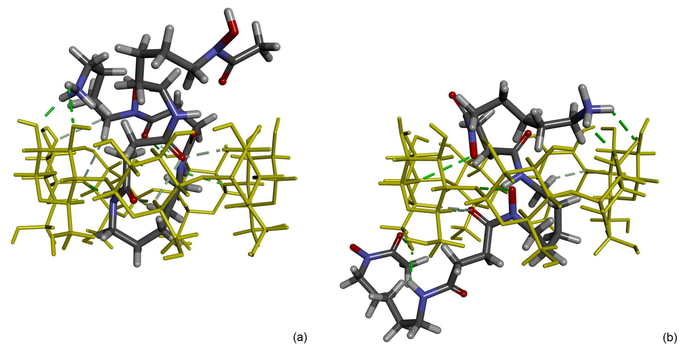


Fig. 6 The lowest binding energy conformation of the inclusion complex of DFOB into β-CD cavity as resulted from molecular docking simulations for protonated conformer (a) and hexadenate ligand (b).

Conclusions

· The resonances due to the H5 protons of β-CD, located inside the cavity, partially overlapped by the H6' and H6" signal in the free state, shown a visible upfield shift and become well solved when the mol fraction of the ligand molecule increases.
· We determined the stoichiometry of the complex to be 1:1 and an association constant K of 127 M^{-1}.
· The initial assumption that a possible conformation for an inclusion compound between β-CD and DFOB could be with the β-CD cavity protecting the hydrophobic pentane part of DFOB is confirmed experimentally.
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