Cyclopeptide Functionalized β-Cyclodextrin. A New Class of Potentially Enzyme Mimicking Compounds with Two Recognition Sites

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The two different recognition sites of the cyclodextrin (CD) and of the cyclopeptide are present together in the *cyclo*-(L-histidyl-L-histidyl) capped β-CD **1**, the synthesis of which is now reported; compound **1** is able to coordinate metal ions.

The problem of designing new molecules with improved catalytic activity at physiological pH and increased selectivities towards the substrates, is attracting more and more interest in biomimetic chemistry. In this context, cyclodextrins (CDs) have emerged as a class of promising enzyme models[1] and their functionalization with metal ion-coordinating moieties appears as a promising approach aiming at mimicking metallo-enzymes.[2–4]

Naturally occurring and synthetic cyclic peptides have been the subject of intensive study in recent years[5,6] for enzyme modelling, membrane transport and other biological processes associated with ion binding.[7,8] The advantages of these model ligands over linear peptides are their 'constrained' geometry and the absence of free $-CO_2^-$ and $-NH_3^+$ terminal groups. Furthermore, cyclic peptides with amino acid residues containing complexing side-chain substituents such as imidazole can coordinate to metal ions in a way that mimics the coordination sites in enzymes.[9]

Copper(II) complexes of *cyclo*-(L-histidyl-L-histidyl) have been characterized in aqueous solution by means of potentiometric, calorimetric and EPR measurements[10] in order to correlate the significant scavenger activity towards the oxygen radicals[11] with the species formed in the experimental conditions and with the structure of the species itself. Coupling of the 'hydrophobic' cavity of cyclodextrins with a cyclic peptide, a cation receptor itself, may afford a molecule with two different recognition sites and also able to coordinate a metal ion that can work as a third recognition site.

Here we report the synthesis of the *cyclo*-(L-histidyl-L-histidyl) (cyhis) capped β-CD **1**, as the first example of this new class of compounds.

The compound **1** was prepared according to the following procedure. Cyhis[10] (0.4 mmol) was added to a solution, in dry dimethylformamide (DMF) (4 ml), of 6-deoxy-6-iodo-β-CD (0.08 mmol), prepared as reported elsewhere.[12] The reaction mixture was stirred vigorously at 135 °C for 5 h. After cooling, the insoluble unreacted cyhis was removed by filtration and the solution evaporated to dryness *in vacuo*. The solid thus obtained was purified by column [CM-Sephadex C-25 (NH_4^+ form)] chromatography using a linear gradient of NH_4HCO_3 (0–0.1 mol dm^{-3}). The purity of the product was checked by HPLC.

The ^1H NMR spectrum at 250 MHz in D_2O is reported in Fig. 1. For peak assignment, a COSY experiment was also carried out. Only the 5- and 6-protons of cyclodextrin are markedly influenced by the substitution (see Fig. 1). In particular, the A ring protons are shifted downfield, mainly by the inductive effect due to the imidazole substitution, whereas another set of protons are shifted upfield. By comparison with the spectrum of 6-deoxy-6-(imidazol-1-yl)-β-CD (CDIm) (obtained according to procedures reported elsewhere[12]), we can attribute this latter set of protons to the B glucopyranosyl ring. The shortness of the CDIm chain requires that the upfield shifted protons belong to a glucopyranosyl ring adjacent to the substituted ring; Corey–Pauling–Koltun (CPK) models show that the substituted imidazole is almost perpendicular to the cavity, near to the B-ring, and thus its ring current shifts upfield the 5-B and the 6-B protons, located perpendicularly to its plane. Thus, by analogy, we can

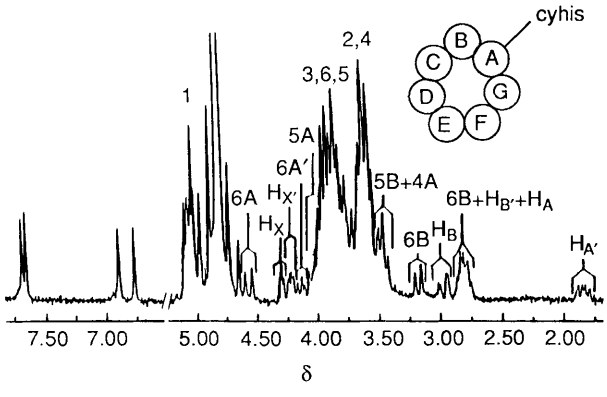
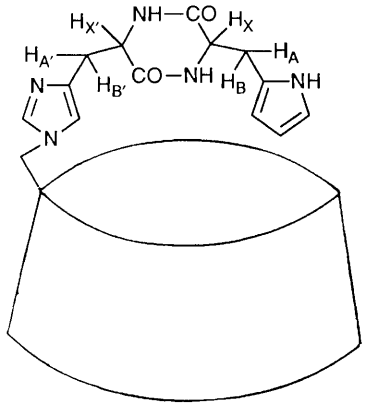


Fig. 1 ^1H NMR spectrum of **1**



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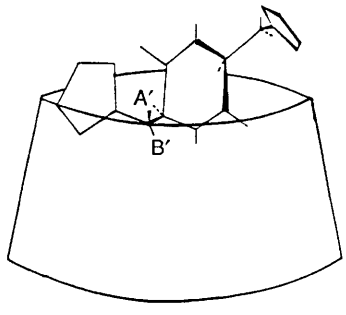

Fig. 2 Proposed conformation of the chain in **1**

conclude that also in **1** the *N*-substituted imidazole ring is almost perpendicular to the cavity. The NMR spectrum of **1** also shows a strong upfield shift of one of the four methylene protons of the cyclopeptide residue [$H_{A'}$ = δ 1.85, compared to δ 3.2 of the corresponding proton of *cyclo*(-glycyl-L-histidyl)[13]]. This shift, together with the coupling constant values (J_{AX} 5.2 Hz; J_{BX} 5.2 Hz; $J_{A'X'}$ 8.0 Hz; $J_{B'X'}$ 3.9 Hz; $J_{5A,6A}$ 0 Hz; $J_{5A,6'A}$ 4.5 Hz) and the resulting preferred rotational conformers, also suggest that the diketopiperazine (DKP) ring is placed almost perpendicular to the cavity. The *N*-unsubstituted imidazole ring is located above the DKP ring and thus perpendicularly to the $H_{A'}$ in agreement with its strong upfield shift. The proposed overall structure is sketched in Fig. 2. In this arrangement both the imidazole and the DKP ring may give rise to hydrogen bonds with 6-OH moieties of cyclodextrin. Preliminary ^{13}C NMR data seem to confirm such hypothesis.

EPR spectra in frozen solution indicate that the copper(II) ion interacts with compound **1**. The magnetic parameters of the copper(II) ion and **1** in a 1 : 2 ratio (2×10^{-3} mol dm^{-3}) ($g_{\parallel}$ = 2.254, $A_{\parallel}$ = 0.0191 cm^{-1}) are equal to those found in the analogous complexes of copper(II) with cyhis[10] ($g_{\parallel}$ = 2.253, $A_{\parallel}$ = 0.0190 cm^{-1}). We obtained the spectrum of copper(II) and **1** in a 1 : 1 ratio, but the magnetic parameters, different from those of free metal ion, cannot be compared with those concerning the [Cu(cyhis)]$^{2+}$ complex,[10] since in this latter case only semi-quantitative information could be obtained, owing to experimental problems.

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