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A ^{1}H NMR study of inclusion complex formation between β-cyclodextrin and monohydroxypyridines

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Abstract

The inclusion complexes of 2-, 3-, and 4-hydroxypyridines with β-cyclodextrin in aqueous solution have been studied by high field ^{1}H NMR. All complexes showed a 1 : 1 stoichiometry and the apparent association constants reflected the polarity of the guest compounds in a qualitative fashion. The inclusion process has been shown to markedly affect the tautomeric equilibrium between the lactim and lactam forms of 3-hydroxypyridine with a preferential inclusion of the former, less polar tautomer.

1. Introduction

Cyclodextrins are cyclic oligosaccharides composed of (1 → 4)-linked α-D-glucopyranosyl residues, which have a remarkable ability of forming inclusion complexes with a wide variety of guest molecules [1,2]. β-Cyclodextrin (β-CD) is comprised of seven glucose units, and exhibits a toroidal shape with a hydrophobic cavity of 6.0–6.4 Å i.d. (as measured by CPK models). β-CD can act as a molecular receptor for aromatic rings.

Following a recent study [3] on the changes in the UV absorption spectra of some pyridine derivatives upon complex formation with different cyclodextrins, we now report a ^{1}H NMR investigation of the inclusion processes which take place between β-cyclodextrin and monohydroxypyridines in aqueous solution.

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2-, 3-, and 4-Hydroxypyridines have been chosen as model compounds as they are known to play a relevant role in many biological processes and their nucleus is found in a wide range of drugs with different pharmacological action.

2. Experimental

Materials.—β-Cyclodextrin (Sigma) was used without further purification. Its water content was determined by thermogravimetric analysis with a Perkin–Elmer TGA 7 instrument and the appropriate corrections were made to solution concentrations.

2-Hydroxypyridine (Aldrich), 3-hydroxypyridine (B.H. Shilling), and 4-hydroxypyridine (Shuchard) were purified by sublimation before use. D_2O (99.5% isotopic purity) was obtained from Fluka.

^1H NMR measurements.—^1H NMR spectroscopy was performed on a Bruker AMX 400 spectrometer operating at 400.13 MHz. The probe temperature was set at 298 ± 1 K using a Haake control system. Chemical shifts were measured relative to external sodium 4,4-dimethyl-4-silapentane-1-sulphonate (DSS) at 0 ppm. Spectra were collected by co-addition of 128 or 256 scans using a 45° observe pulse. Processing and plotting of the data were performed on a Bruker X32 data station.

3. Results and discussion

Fig. 1 shows ^1H NMR spectra of β-CD in the absence and presence of the guest compounds. Upfield shifts of the H-3 and H-5 signals are observed, whereas very small or no shifts were experienced by the other protons of the host compound. This finding demonstrates true inclusion complex formation, as H-3 and H-5 point toward the interior of the β-CD cavity and the shifts of their resonances are due to ring current and magnetic anisotropic effects exerted by the guest. Moreover no extra peaks appeared which could be assigned to the pure complexes, thus implying fast exchange (on the NMR time scale) between bound and free species [4]. Only very small downfield shifts were observed for the signals of the guest protons and consequently no attempt was made to analyze these data.

In analysing the experimental data, proper account was taken of the tautomeric equilibria of monohydroxypyridines in aqueous solution (Chart 1). While for 2- and 4-hydroxypyridine there is a strong prevalence of the lactam (NH) form ($K_T = 910$ and 1910, respectively [6]), both the lactim (OH) and the lactam tautomer of 3-hydroxypyridine are present in comparable amounts in aqueous solution ($K_T = 1.1$) [7] and can be included in β-CD.

Since no splitting of the β-CD signals is observed, the two inclusion complexes are assumed to be interconverting in a fast exchange regime. Therefore the observed chemical shift differences are due to the combined effects of complex formation between β-CD and both the lactim and lactam forms of 3-hydroxypyridine.

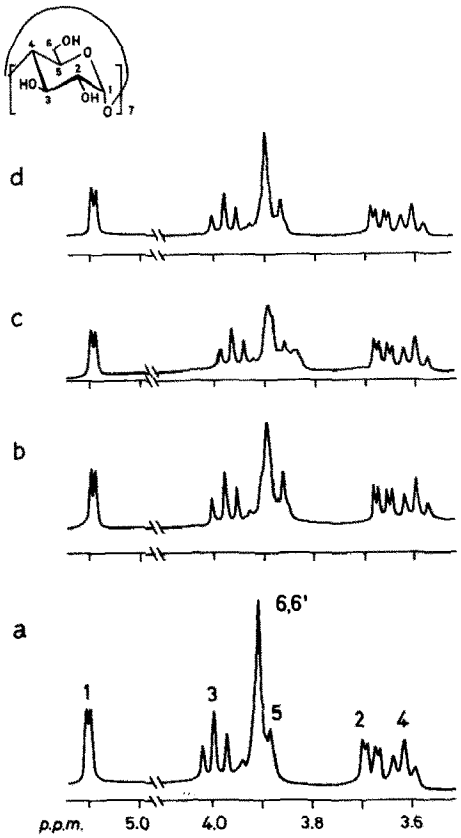

Fig. 1. 400-MHz ^{1}H NMR spectra in D_2O of: (a) 5×10^{-3} M β-CD; (b) 5×10^{-3} M β-CD+2.5×10^{-2} M 2-hydroxypyridine; (c) 5×10^{-3} M β-CD+2.5×10^{-2} M 3-hydroxypyridine; (d) 5×10^{-3} M β-CD+2.5×10^{-2} M 4-hydroxypyridine. The assignments of the spectral lines are based on literature data [4].

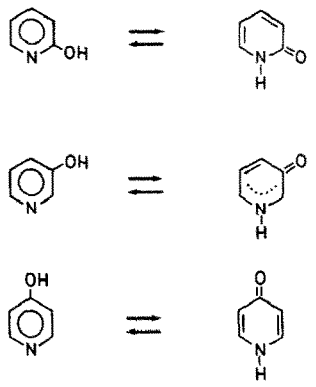

Chart 1. Tautomeric equilibria of monohydroxypyridines.

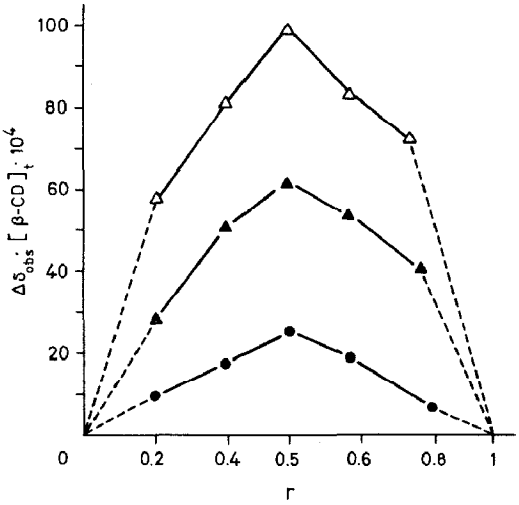

Fig. 2. Continuous variation plot of $\Delta\delta_{obs} \cdot [\beta\text{-CD}]_t$ as a function of $r = [\beta\text{-CD}]_t / ([\text{OHpy}]_t + [\beta\text{-CD}]_t)$ for H-3 of β-CD in the presence of: (●) 2-hydroxypyridine; (△) 3-hydroxypyridine; (▲) 4-hydroxypyridine.

The stoichoimetry of the complexes was obtained by the continuous variation method [5]. The total concentration of the interacting species was kept constant at 10^{-2} M, while the ratio $r = [\beta\text{-CD}]/([\text{OHpy}] + [\beta\text{-CD}])$ (where [OHpy] is the concentration of the guest compound) was varied between 0.2 and 1.

The plots of $\Delta\delta_{H\text{-}3} \cdot [\beta\text{-CD}]_t$ (where $\Delta\delta_{H\text{-}3}$ is the chemical shift difference for H-3 of β-CD between the free host and the host in the presence of various amounts of monohydroxypyridines) versus r always showed a maximum for $r = 0.5$, thus indicating 1:1 complex stoichiometry (Fig. 2). The signal position of β-CD H-3 was chosen to determine the stoichiometry and the apparent association constants of the complexes (as later reported), as this signal appears most sensitive to the inclusion processes and is most easily observed.

For the determination of the apparent association constants, K, β-CD concentration was kept constant at 5×10^{-3} M while the guest concentrations were in the range 5×10^{-3}–2.5×10^{-2} M.

The following equation was used:

$$K = \frac{[\text{C}]}{([\beta\text{-CD}]_t - [\text{C}]) \cdot ([\text{OHpy}]_t - [\text{C}])} \cdot \qquad (1)$$

where [C], $[\beta\text{-CD}]_t$, and $[\text{OHpy}]_t$ indicate the complex, total β-CD, and total monohydroxypyridine concentrations, respectively. The complex concentration was expressed as a function of the chemical shift differences of β-CD H-3, $[\text{C}] = \Delta\delta_{obs} \cdot [\beta\text{-CD}]_t / \Delta\delta_c$, where $\Delta\delta_c$ is the chemical shift difference for the pure complex and eq. (1) was accordingly modified.

For 3-hydroxypyridine, the presence of comparable amounts of the two tautomers had to be taken into account when considering complexation processes in

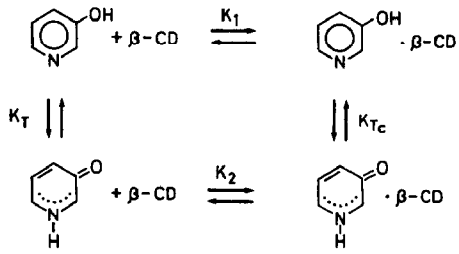

Chart 2. Complex formation and tautomeric equilibria of 3-hydroxypyridine in the presence of β-CD.

aqueous solution (Chart 2). Therefore, two such equations were constructed and solved simultaneously, one referring to the complexation of the lactam form and the other pertaining to the lactim tautomer. Thus, for 3-hydroxypyridine,

$$K_1 = \frac{[C_1] \cdot (1 + K_T)}{([OHpy]_t - [C_1] - [C_2]) \cdot ([\beta\text{-CD}]_t - [C_1] - [C_2])} \quad (2)$$

and

$$K_2 = \frac{[C_2] \cdot (1 + 1/K_T)}{([OHpy]_t - [C_1] - [C_2]) \cdot ([\beta\text{-CD}]_t - [C_1] - [C_2])} \quad (3)$$

where C_1 and C_2 are the concentrations of complexes with the lactim and the lactam tautomer, respectively and $C_2 = C_1 \cdot K_2 \cdot K_T / K_1 \cdot K_T$ is the tautomeric constant whose value in aqueous solution is 1.1. An apparent equilibrium constant can also be defined as

$$K_{ap} = \frac{[C_1] + [C_2]}{([OHpy]_t - [C_1] - [C_2]) \cdot ([\beta\text{-CD}]_t - [C_1] - [C_2])}. \quad (4)$$

$[C_1]$ and $[C_2]$ can be related to the observed chemical shift, difference of β-CD H-3, $\Delta\delta_{obs}$, through the relationship, $\Delta\delta_{obs} \cdot [\beta\text{-CD}]_t = [C_1] \cdot \Delta\delta_1 + [C_2] \cdot \Delta\delta_2$, where $\Delta\delta_1$ and $\Delta\delta_2$ are the chemical shift differences of the pure complexes with the lactim and the lactam tautomer, respectively (fast exchange regime). If we define K_3 as $\Delta\delta_2 = K_3 \cdot \Delta\delta_1$ we can write:

$$\Delta\delta_{obs} \cdot [\beta\text{-CD}]_t = ([C_1] + [C_2]) \cdot \frac{K_1 + K_T \cdot K_2 \cdot K_3}{K_1 + K_T \cdot K_2} \cdot \Delta\delta_1$$

and

$$[C_1] + [C_2] = \frac{\Delta\delta_{obs} \cdot [\beta - CD]_t \cdot (K_1 + K_T \cdot K_2)}{(K_1 + K_T \cdot K_2 \cdot K_3) \cdot \Delta\delta_1}$$

Table 1
Apparent association constants and chemical shift differences (H-3 of β-CD) for complex formation between monohydroxypyridines and β-CD in aqueous solution at 25°C

Guest compound	K (M^{-1})	$\Delta\delta_c$ [a] (ppm)
2-Hydroxypyridine	41 ± 9 [b]	0.029 ± 0.004 [b]
3-Hydroxypyridine (lactim isomer)	95 ± 5	0.097 ± 0.004
3-Hydroxypyridine (lactam isomer)	46 ± 3	0.069 ± 0.004
4-Hydroxypyridine	79 ± 25	0.027 ± 0.004

[a] Defined as $\delta_0 - \delta_c$, where δ_0 and δ_c are the chemical shifts in the uncomplexed and fully complexed states, respectively. [b] Standard deviation.

Substituting for ($[C_1] + [C_2]$) into eq. *4* and rearranging the resulting equation, we obtain:

$$\frac{[\mathrm{OHpy}]_t}{\Delta\delta_{obs}} = \frac{K_1 + K_T \cdot K_2}{(K_1 + K_T \cdot K_2 \cdot K_3) \cdot \Delta\delta_1} + \frac{[\mathrm{OHpy}]_t \cdot (K_1 + K_T \cdot K_2)}{K_{ap} \cdot (K_1 + K_T \cdot K_2 \cdot K_3) \cdot \Delta\delta_1} + \frac{[\beta\text{-CD}]_t \cdot (K_1 + K_T \cdot K_2)}{(K_1 + K_T \cdot K_2 \cdot K_3) \cdot \Delta\delta_1} - \frac{\Delta\delta_{obs} \cdot [\beta\text{-CD}]_t \cdot (K_1 + K_T \cdot K_2)^2}{(K_1 + K_T \cdot K_2 \cdot K_3)^2 \cdot (\Delta\delta_1)^2} \quad (5)$$

This equation can be solved by non-linear least squares fitting to obtain the five parameters K_{ap}, K_1, K_2, K_3, and $\Delta\delta_1$ from the experimental values of [OHpy], $\Delta\delta_{obs}$, and their ratio $[\mathrm{OHpy}]_t/\Delta\delta_{obs}$, also taking into account the results of a recent UV spectrophotometric study [3] of the same complexes from which a value of 0.48 for the ratio K_2/K_1 has been obtained.

The values of K and $\Delta\delta_c$ for the various complexes were obtained by non-linear least squares fitting of the relative equations using BMDP software package [8] and are reported in Table 1. The values of the apparent association constants for the various complexes were similar in magnitude. The highest K value was observed with the lactim form of 3-hydroxypyridine. Taking into account the hydrophobic nature of the β-CD cavity, this finding agrees with the qualitative predictions based on the relative polarity of monohydroxypyridines as measured by their dipole moments [9]. The lactim form of 3-hydroxypyridine is the least polar of the assayed, compounds. Moreover there is the possibility of hydrogen bonding between the non-bonding electron pairs of the glycosidic oxygen bridges lining the β-CD cavity and the hydrogen of the OH-group in the lactim tautomer of 3-hydroxypyridine which may stabilize the inclusion complex.

4. Conclusions

^{1}H NMR spectroscopy showed that 2-, 3-, and 4-hydroxypyridines form inclusion complexes with β-CD in aqueous solution with a host-guest molar ratio of

1:1. The inclusion phenomena can perturb the tautomeric equilibria of the guest compounds since the hydrophobic cavity of β-CD mimics a less polar environment compared to water. This perturbation is remarkable when, as in the case of 3-hydroxypyridine, the different tautomers are present in comparable amounts. A tautomeric constant (K_{Tc}) value of 0.53 was obtained for the equilibrium between the complexed lactam and lactim forms of 3-hydroxypyridine compared to the value of 1.1 in aqueous solution.

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