^{1}H NMR AND UV SPECTROSCOPIC STUDY OF INCLUSION COMPLEX FORMATION BETWEEN PYRIDOXINE AND β- AND γ-CYCLODEXTRINS

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ABSTRACT

Inclusion complex formation between pyridoxine and β- and γ-cyclodextrins in aqueous solution has been investigated by ^{1}H NMR and UV spectroscopy. Both complexes exhibited a 1:1 stoichiometry and the inclusion process has been shown to perturb the equilibrium between the lactim and the lactam tautomer of pyridoxine, with a preferential inclusion of the former, less polar tautomer.

1. INTRODUCTION

Pyridoxine (2-methyl-3-hydroxy-4,5-bis(hydroxymethyl)pyridine) and structurally related compounds play a relevant role in a large number of biological systems. Many reactions catalyzed by the coenzymes of the pyridoxal group are known to take place in a rather hydrophobic environment inside the enzyme active site [1]. In the study of these systems it is therefore of fundamental importance to devise a biomimetic model which can simulate such hydrophobic interactions. α-, β- and γ-Cyclodextrins (CD_S), which exhibit a toroidal shape with a hydrophobic cavity, may afford suitable biomimetic models in the study of hydrophobic interactions of biological relevance.

2. MATERIALS AND METHODS

2.1. Materials

β-Cyclodextrin (β-CD) and γ-cyclodextrin (γ-CD), both from Sigma, were used without further purification; pyridoxine·HCl (> 98.5% purity) was purchased from Carlo Erba. D_2O (> 99.5% isotopic purity) was obtained from Fluka. All solutions for UV absorption measurements were made in phosphate buffer, pH 6.8±0.1. The pD of the solutions for ^{1}H NMR experiments was set at 7.4 ± 0.1 (pH-meter reading plus 0.4 to correct for isotopic effects).

2.2. Methods

UV absorption spectra were recorded at 298 ± 0.5 K on a Perkin Elmer Lambda 16 spectrometer. Due to the known instability of pyridoxine in neutral aqueous medium [2], all solutions were freshly prepared immediately before recording the spectra and protected from light. ^{1}H NMR spectroscopy was performed on a Bruker AMX spectrometer operating at 400.13 MHz. The probe temperature was set at 298 ± 1 K using a Haake

Journal of Inclusion Phenomena and Molecular Recognition in Chemistry **25**: 113–116, 1996.

control system. Chemical shifts were measured relative to external sodium 4,4-dimethyl-4-silapentane-1-sulphonate (DSS) at 0 ppm.

3. RESULTS AND DISCUSSION

3.1. UV spectroscopic study

The UV absorption spectrum of pyridoxine has been shown to be markedly solvent dependent. Such dependence is also related to the tautomeric equilibrium between the lactam and the lactim forms of pyridoxine. The apparent tautomerization constant K_T in aqueous medium (buffered solution, pH 7) has been reported to be 3.92 [3], with a preferential stabilization of the more polar lactam form.
A spectral change is therefore to be expected following inclusion of pyridoxine in the hydrophobic cavity of the cyclodextrins. This is indeed the case as depicted in Fig. 1 which shows the UV spectra of pyridoxine both in the absence and in the presence of β-CD.

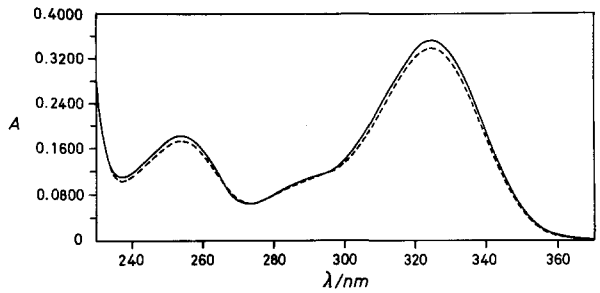

Fig. 1. UV absorption spectra of pyridoxine ($5.0 \cdot 10^{-5}$ M) in phosphate buffer solution (pH = 6.8) at 298 K in the absence (——) and in the presence of $1.0 \cdot 10^{-2}$ M β–CD (----).

It can be seen that the absorption centered at 324 nm, which is attributed to the long wavelength $\pi \rightarrow \pi^*$ transition of the lactam tautomer, decreases . Similar effects were observed with γ-CD while α-CD did not perturb the pyridoxine spectrum to any noticeable extent.
A 1:1 inclusion mechanism has been assumed throughout the present study because pyridoxine presents only one interacting moiety (the aromatic ring) and its molecular dimensions seem to preclude inclusion in more than one CD molecule. Moreover the guest is not known to give rise to autoassociation processes from which a 2:1 (guest:host) inclusion complex may arise.
On the assumption that the molar absorptivity and λ_{max} of the complexed pyridoxine do not differ from those of the free guest, only the relative concentration of the different tautomers being affected by the inclusion process [4] the following equation was derived ([CD] » [B_6]):

$$\frac{A \cdot 1/K_T}{[B_6] \cdot \varepsilon - A \cdot (1 + 1/K_T)} = \frac{1}{[CD]} \cdot \frac{1}{K_1 - K_2} + \frac{K_1}{K_1 - K_2} \qquad (1)$$

where A and ε are the absorbance value and the molar absorptivity, respectively, at λ = 324 nm; [B_6] is the total concentration of the guest which was kept constant at $5 \cdot 10^{-5}$ M while the CD concentrations were set at 0.002, 0.004, 0.006, 0.008 and 0.01 M (β-CD) and at 0.01, 0.02, 0.03, 0.04 and 0.05 M (γ-CD); K_1 and K_2 are the apparent equilibrium constants for inclusion complex formation between the cyclodextrins and the lactim and lactam tautomer of pyridoxine, respectively. The apparent association constants are reported in Table 1.

Table 1. UV determined apparent association constants for the inclusion complex formation between pyridoxine and β- and γ-cyclodextrins at 298 K in aqueous solution.

Host	K_1 / (M^{-1})	K_2 / (M^{-1})	K_{Tc}
β-CD	31.5 ± 10.5	7.6 ± 3.5	0.94 ± 0.4
γ-CD	12.9 ± 4.2	2.6 ± 1.3	0.80 ± 0.4

The K values found confirm a preferential inclusion of the less polar lactim tautomer of pyridoxine. The tautomerization constant (K_{Tc}) values of pyridoxine in β-CD (0.94) and γ-CD (0.8) are similar to those found in water-dioxane mixtures where the volume fraction of the less polar solvent is ~ 0.3.

3.2. ^{1}H NMR study

Fig. 2 shows the ^{1}H NMR spectra of β-CD in the absence and in the presence of pyridoxine.
More pronounced upfield shifts of the H-3 and H-5 signals are observed while smaller effects are experienced by the other hydrogens of the host compound, thus supporting the hypotesis of true inclusion complex formation. Similar conclusions could be drawn for inclusion complex formation between pyridoxine and γ-CD. The 1:1 stoichiometry of the last complex was obtained by the continuous variation (Job) method. For the β-CD·pyridoxine complex we were unable to obtain a Job plot due to the very small chemical shift differences observed when both the guest and the host concentrations were of the order of 0.01 M which approaches the solubility limit of β-CD. The stoichiometry of the complex was thus inferred from the UV measurements.

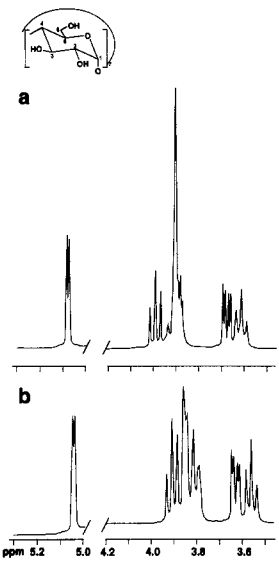

Fig.2. 400-MHz ^{1}H NMR spectra in D_2O: (a) $5 \cdot 10^{-3}$ M β-CD; (b) $5 \cdot 10^{-3}$ M β-CD + $2.0 \cdot 10^{-1}$ M pyridoxine.

For the determination of the apparent association constants, K_1 and K_2, β-CD and γ-CD concentrations were kept at $5 \cdot 10^{-3}$ M while the guest concentration was set at 0.05, 0.1, 0.15, 0.20 and 0.25 M (with β-CD) and at 0.1, 0.2, 0.3, 0.4 and 0.5 M (with γ-CD). In devising an equation for estimating the apparent association constant, the presence of comparable amounts of the two pyridoxine tautomers had to be taken into account. Therefore, two equations were constructed and solved simultaneously:

$$K_1 = \frac{[C_1] \cdot (1 + K_T)}{([B_6]_t - [C_1] - [C_2]) \cdot ([CD]_t - [C_1] - [C_2])} \quad (2)$$

$$K_2 = \frac{[C_2] \cdot (1 + 1/K_T)}{([B_6]_t - [C_1] - [C_2]) \cdot ([CD]_t - [C_1] - [C_2])} \quad (3)$$

where $[C_1]$ and $[C_2]$ are the concentrations of the complexes with the lactim and the lactam tautomer, respectively . Moreover $[C_2] = [C_1] \cdot K_2 \cdot K_T/K_1$. $[C_1]$ and $[C_2]$ can be related to the observed chemical shift differences of CD_s H-3, $\Delta\delta_{obs}$, through the relationship $\Delta\delta_{obs} \cdot [CD]_t = [C_1] \cdot \Delta\delta_1 + [C_2] \cdot \Delta\delta_2 = [C_1] \cdot (\Delta\delta_1 + \Delta\delta_2 \cdot K_2 \cdot K_T/K_1)$, 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. Substituting for $[C_1]$ into eq. 2 and assuming that $[B_6]_t - [C_1] - [C_2] \cong [B_6]_t$ and defining $\Delta\delta_{ap}$ as $(K_1 \cdot \Delta\delta_1 + \Delta\delta_2 \cdot K_2 \cdot K_T)/(K_1 + K_2 \cdot K_T)$, we obtain :

$$\frac{1}{\Delta\delta_{obs}} = \frac{1}{\Delta\delta_{ap}} + \frac{1 + K_T}{\Delta\delta_{ap} \cdot (K_1 + K_2 \cdot K_T)} \cdot \frac{1}{[B_6]_t} \quad (4)$$

The apparent association constants are reported in Table 2; they support the hypotesis of a preferential inclusion of the less polar lactim tautomer of pyridoxine both in β-CD and in γ-CD.

Table 2. NMR determined apparent association constants and $\Delta\delta_{ap}$ for inclusion complex formation between pyridoxine in β- and γ-cyclodextrins at 298 K in aqueous solution.

Host	K_1 / (M^{-1})	K_2 / (M^{-1})	$\Delta\delta_{ap}$ / p.p.m.
β-CD	20.2 ± 7.5	4.9 ± 2.0	0.100 ± 0.003
γ-CD	5.5 ± 2.0	1.2 ± 0.5	0.166 ± 0.003

4. CONCLUSION

The formation of 1:1 inclusion complexes between β- and γ-CD and pyridoxine has been demonstrated both by ^1H NMR and UV spectroscopic techniques. The apparent equilibrium constants for these processes are characteristic of rather weak intermolecular interactions,which are nevertheless able to perturb the tautomeric equilibrium of pyridoxine with a preferential stabilization of the lactim form .

REFERENCES

[1] Cortijo , M., Shaltiel, S. , On the microenvironment of the pyridoxamine 5-phosphate residue in $NaBH_4$-reduced glycogen phosphorylase b, *Eur. J. Biochem.*, **29** 134-142 (1972)

[2] Hochberg, M., Melnick, D., Siegel , L., Oser, B. L., Destruction of vitamin B_6 (pyrdoxine) by light, *J. Biol. Chem.*, **148** 253-254 (1943)

[3] Sanchez-Rui, J. M., Llor , J., Cortijo, M., Thermodinamic constants for tautomerism and ionization of pyridoxine and 3-hydroxypyridine in water-dioxane, *J. Chem. Soc. Perkin Trans. II* , 2047-2051 (1984)

[4] Cotta Ramusino, M., Rufini , L., Mustazza, C., UV spectroscopic study of the interaction between α-, β-, and γ- cyclodextrins and pyridine derivatives, *J. Incl. Phen. and Mol. Rec. in Chem.* , **15** 359-368 (1993)