Caging and solvent effects on the tautomeric equilibrium of 3-pyridone/3-hydroxypyridine in the ground state: A study in cyclodextrins and binary solvents

ARTICLE *in* PHYSICAL CHEMISTRY CHEMICAL PHYSICS · AUGUST 2009

Impact Factor: 4.49 · DOI: 10.1039/b823405a · Source: PubMed

2 AUTHORS, INCLUDING:

Osama Kamal Abou-Zied
Sultan Qaboos University
50 PUBLICATIONS **950** CITATIONS

SEE PROFILE

Caging and solvent effects on the tautomeric equilibrium of 3-pyridone/3-hydroxypyridine in the ground state: a study in cyclodextrins and binary solvents

Osama K. Abou-Zied* and Othman I. K. Al-Shihi

Received 7th January 2009, Accepted 25th March 2009
First published as an Advance Article on the web 20th April 2009
DOI: 10.1039/b823405a

The tautomeric equilibrium between 3-pyridone (3Py) and 3-hydroxypyridine (3HP) shows characteristic absorption peaks for the zwitterion form of 3Py in water that may be used as a probe of the hydrophobic nature inside macromolecules such as proteins and other biologically related systems. We studied this equilibrium in the ground state in aqueous cyclodextrins (CDs) and in binary solvent mixtures of 1,4-dioxane and water by absorption spectroscopy, and by *ab initio* calculations. Upon the addition of α-CD or β-CD to an aqueous solution of the 3Py/3HP system, the absorbance intensity of the zwitterion tautomer decreases with a concomitant increase in the intensity of the enol tautomer of 3HP. The results reflect the nature of the tautomeric equilibrium and point to the hydrophobic environment inside the CD cavities. The effect of inclusion is noticeably less in the case of α-CD. This is attributed to the small cavity size of α-CD which sustains only partial inclusion. Upon the addition of γ-CD, the intensity of the zwitterion tautomer slightly increased over that in water which is attributed to the direct interaction between the charged sides of the tautomer with the outer primary or secondary hydroxyls of the glycopyranose units of γ-CD. This interaction is a result of the large cavity size of γ-CD which does not support a stable complex. The largest caging effect was observed in 2,6-di-*O*-methyl-β-CD (DMβ-CD) which is an indication of a more hydrophobic environment around the guest. The large hydrophobicity of DMβ-CD is due to the presence of the two methyl groups in the β-CD derivative which reduce the amount of water inside the cavity upon encapsulation. In the binary mixtures of 1,4-dioxane and water, the change in the absorbance intensity of the enol and the zwitterion tautomers was analyzed quantitatively and three water molecules were found to solvate the polar centers of each tautomer. *Ab initio* calculations of the solvation of both tautomers by two and three water molecules were performed at the MP2/6-31++G(d,p) level. The calculations show that three water molecules are necessary to solvate the polar centers of each tautomer in a water network pattern. The results presented here suggest that the 3Py/3HP system represents a potentially useful new photophysical probe for supramolecular structures, particularly those involving inclusion.

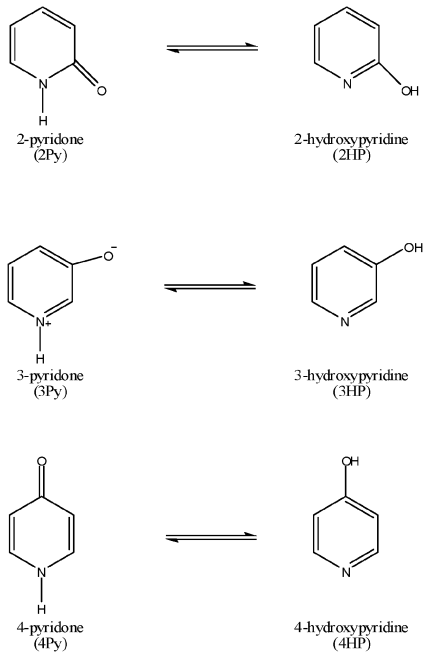
1. Introduction

The interior properties of a biological macromolecule are crucial to its structure and functionality. A large number of low molecular weight compounds bind reversibly to proteins and are widely used as extrinsic probes for the investigation of physicochemical, biochemical and biological systems. The spectral changes observed on the binding of probes with proteins are an important tool for the investigation of binding sites, conformational changes and characterization of substrate to ligand binding.[1]

Recently, we used three isomers of small aromatic molecules as probes to characterize the drug-binding site "subdomain IIA" in human serum albumin (HSA).[2] 2-Pyridone (2Py), 3-pyridone (3Py) and 4-pyridone (4Py) (shown in Scheme 1) have received much interest due to the similarity of their molecular structures with those found in a wide range of drugs of different pharmacological functions.[3] The three probes have the same functional groups and similar chemical properties, which include the possibility of keto-enolic tautomerism (solvent polarity dependent).[4–12] We found that the probes specifically bind in subdomain IIA and cause a reduction in the fluorescence intensity and lifetime of the Trp-214 residue in native HSA which is located in the same subdomain. The efficiency of energy transfer from the Trp-214 fluorescence to the probes was analyzed using Förster theory and was found to depend on the degree of the spectral overlap between the donor's fluorescence and the acceptor's absorption.

Studies of the above pyridone systems in different solvents proved that the 2- and the 4-isomers are distinctly different from the 3-isomer.[5–8] The former isomers exist predominantly in the pyridone form in neutral solution, whereas evidence for a zwitterion has been reported for the 3-isomer.[11,12] While in

Department of Chemistry, Faculty of Science, Sultan Qaboos University, P.O. Box 36, Muscat, Postal Code 123, Sultanate of Oman. E-mail: abouzied@squ.edu.om; Fax: +968 2414 1469; Tel: +968 2414 1468



Scheme 1 Tautomerization between pyridones and hydroxypyridines.

the gas phase or in nonpolar solvents the hydroxypyridine forms (HP) dominate (Scheme 1), in aqueous solution or in polar solvents the pyridone forms predominate. The change in equilibrium constants may be attributed to the greater stability of pyridine–solvent complexes.[10–12] The *meta* position of the hydroxyl group in 3HP opens new ways with regard to its tautomeric reactions, with two or more water molecules involved in forming a hydrogen-bond network.

The change in the absorption spectrum of 3Py/3HP in water may suggest the use of this equilibrium system as a water sensor in macromolecules. Two additional peaks at 247 and 315 nm were only measured when 3Py/3HP is dissolved in water or buffer containing water.[2,4] A very important criterion in the choice of a probe is its sensitivity to a particular property of the nanoenvironment in which it is located (*e.g.* polarity, acidity, *etc.*). For probing binding sites in macromolecules, the spectroscopic changes associated with the chosen probe are usually similar to the changes observed when the probe molecule is transferred from a polar to a nonpolar environment.[13,14] For this purpose, 3Py/3HP is expected to provide a good measure of the nature of its surrounding environment when it is incorporated inside a macromolecule.

In this paper, we investigate the caging effect on the absorption spectra of 3Py/3HP aqueous solution. This caging effect can be induced in heterogeneous environments that are capable of providing nanopockets of desired polarity and viscosity. These environments can be attained by aqueous cyclodextrins (CDs) which quite often provide nanoenvironments very similar to biological environments. CDs are linked glucopyranose rings forming a doughnut-shaped structure.[15] The number of glucose units and, consequently, the cavity diameter increases on going from α-CD to β-CD to γ-CD.[16] CDs with different cavity diameters have been used advantageously to sequester guests on the basis of size. This property has been exploited to use CDs in drug delivery techniques. They are, thus, interesting nanovessels for appropriately sized molecules and the resulting supramolecules serve as excellent miniature models of enzyme-substrate complexes. The reduced polarity and restricted space provided by the CD cavity markedly influence a number of photophysical and photochemical processes.[17,18] For example, Shizuka *et al.* have demonstrated that the proton transfer reaction in 2-naphthol is affected by inclusion in different CD cavities.[19,20]

The present study is expected to contribute to the current understanding of the chemistry of CD caged drugs, in which photophysics and photochemistry of the drug upon encapsulation by CD nanocavities show significant changes due to the local environment.[21] We also investigate the mechanism of water solvation of the polar centers in 3Py/3HP, experimentally in mixtures of binary solvents, and theoretically by *ab initio* calculations.

2. Experimental and theoretical methods

3Py (98%) was obtained from Aldrich and used without further purification. α-CD (≥ 98%), β-CD (≥ 99%), 2,6-di-*O*-methyl-β-CD (DMβ-CD) (≥ 98%) and γ-CD (≥ 98%) were purchased from Fluka and used as received. Spectroscopic grade cyclohexane was purchased from BDH Chemicals. Anhydrous 1,4-dioxane was obtained from Sigma-Aldrich Chemical Co. The concentration of 3Py in all solvents and in CDs was 50 μM. For the studies in binary aqueous mixtures, a stock solution of 3Py in 1,4-dioxane (5 mM) was prepared. Equivolume amounts (50.0 μL) of 3Py in 1,4-dioxane were pipetted into separate test tubes and diluted with the appropriate amounts of 1,4-dioxane–water to make volume : volume (v/v) mixtures. Deionized water (Millipore) was used in the preparations.

Absorption spectra were obtained with an HP 845× Diode Array spectrophotometer. In all the experiments, samples were contained in a 1 cm path length quartz cell and the measurements were performed at 23 ± 1 °C. All prepared solutions were kept to equilibrate for 2 h before taking the measurements. Measurements were then repeated after 12 h, and no significant differences were detected.

Geometry optimization of the 3Py and 3HP tautomers and their water complexes was carried out using the GAMESS program[22] at the second-order Møller–Plesset (MP2)/6-31++G(d,p) level of calculation.

3. Results and discussions

3.1 Absorption spectra of 3Py/3HP in solution and in aqueous CDs

The absorption spectra of 3Py/3HP in cyclohexane, water, and aqueous CDs are displayed in Fig. 1 for the spectral region from 235 to 355 nm. The absorption peak in cyclohexane at 278 nm represents the first π–π* transition and is due to the neutral enol form (3HP in Scheme 1).[8] Two additional peaks at 247 and 315 nm are obtained in water. These two peaks have been attributed to the zwitterion form (3Py).[8,9] Comparing the absorption spectra in cyclohexane and water,

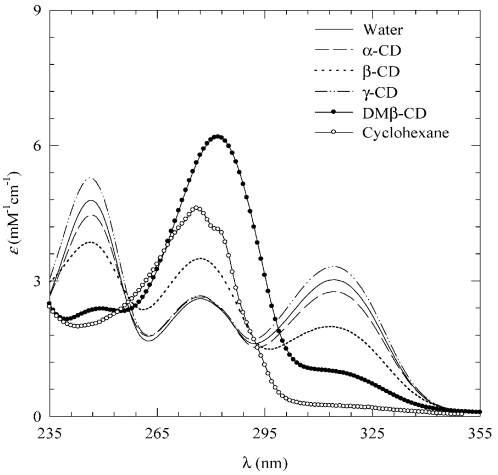


Fig. 1 Absorption spectra of 3Py/3HP in water, cyclohexane, and in aqueous solutions of four CDs. The concentration of all CDs was 10 mM. The concentration of 3Py/3HP was 0.05 mM.

the absorption band in cyclohexane shows more structured transitions which vanish in water. This observation indicates the less solute–solvent interaction in nonpolar solvents.

As shown in Fig. 1, the two additional peaks in water gain their intensity at the expense of that at 278 nm. This observation reflects the equilibrium between the enol and the zwitterion tautomers of the 3-isomer in aqueous solution, unlike the 2- and 4-isomers which show a strong prevalence for one tautomeric form in water.[8] The equilibrium constant for the 3Py/3HP equilibrium was reported to be 1.1 in aqueous buffer of pH 7.0.[23] This equilibrium is expected to be affected by inclusion in the hydrophobic cavity of CD.

The absorption spectra of 3Py/3HP in different CDs point to the degree of encapsulation. This is manifested in the change in the absorbance intensity of the enol and zwitterion tautomers. With the exception of γ-CD, the absorbance in all other CDs shows a decrease in intensity at the zwitterion peaks and an increase in intensity at the enol peak compared to the absorbance of 3Py/3HP in water. The results reflect the hydrophobic nature of the different CD cavities through a preferential complexation of the enol tautomer.

The encapsulation of 3Py/3HP by β-CD is shown to be stronger than that by α-CD. The cavity size of the former (an inner diameter of 0.78 nm)[16] is large enough to include the guest molecule, whereas the cavity size of α-CD (with an inner diameter of 0.57 nm)[16] is too small to enhance the inclusion of the whole guest molecule and only partial inclusion may be possible. On the other hand, the cavity size of γ-CD (0.95 nm)[16] is too large to sustain any complex stability. The slight increase in the absorbance intensity of the peaks due to the zwitterion tautomer upon mixing with γ-CD can be explained in terms of a direct interaction between the zwitterionic moiety with the primary or secondary hydroxyls of the glycopyranose units of γ-CD. This interaction is expected to increase the stability of the zwitterion form due to the increased polarity around the outer rims of CD. Previous work on the complex formation between 3Py/3HP and α-, *β*-, and γ-CDs showed that the estimated binding constants for the 3Py/3HP–β-CD complexes are larger than those estimated for the 3Py/3HP–α-CD complexes with a stoichiometry of 1 : 1 and a preferential inclusion of the neutral enol tautomer in both cases.[24,25] The authors mentioned that the effect of γ-CD on the absorbance of 3Py/3HP was significantly small to allow any data analysis.[24] Our results are in substantial agreement with this work.

The reported binding constants for the 3Py/3HP–α-CD complexes seem to be reasonable (less than 50 M^{-1}) given the weak nature of the complex formation.[24] On the other hand, discrepancies have been reported for the binding constants of the 3Py/3HP–β-CD complexes using different techniques.[24,25] It is worth noting here that the solubility of the CDs in aqueous media vary in an irregular manner, with a relatively low solubility of β-CD.[26] The reported data for the 3Py/3HP–β-CD complexes show a variation of the β-CD concentration with a maximum concentration of less than 10 mM.[24,25] This range of host concentrations seems to be too small to accurately estimate the binding constant, particularly for a weak complex as in the present case. Attempts to raise the concentration of β-CD would be very difficult due to its low solubility. This fact was a reason for the modification of the β-CD structure in order to improve its solubility as its annulus is of a size particularly suitable for the complexation of drug complexes which are more soluble than the drugs in their free states.[16,26] We show next our results for the complex formation between 3Py/3HP and one of the β-CD derivatives (DMβ-CD).

The presence of DMβ-CD in the aqueous solution of 3Py/3HP shows a much stronger effect on the absorption spectrum (Fig. 1). The dramatic decrease in the absorbance of the zwitterion tautomer and the subsequent increase in the absorbance of the enol tautomer is an indication of a strong guest : host complex formation. The result also indicates a more hydrophobic cavity than in the parent β-CD.

Both β-CD and DMβ-CD have the same cavity diameter, but the hydrogen-bonding ability of the alcoholic hydrogens in β-CD is low compared to that of DMβ-CD because, in the former, the secondary alcoholic –OH groups at the 2- and 3-positions of the adjacent glucopyranose rings are engaged in intramolecular hydrogen-bonding with each other. As a result, β-CD is less soluble in water than DMβ-CD. In the case of DMβ-CD, however, this intramolecular hydrogen-bonding is destroyed due to substitution of the alcoholic proton at the 2-position with a methyl group. This structure enhances the intermolecular hydrogen-bonding ability of the alcoholic –OH group at the 3-position with water which leads to more solubility. Replacing OH by OCH_3 at the 2- and 6-positions in DMβ-CD also increases the hydrophobicity of the cavity during the process of encapsulation.[26,27] This increases penetration of the guest inside the cavity of DMβ-CD compared with the case of β-CD. The result is more interaction between the CD interior and the hydrophobic moiety of the guest molecule. This is shown in the dramatic decrease in the absorbance intensity in the region of the zwitterion tautomer and the concomitant increase in the absorbance due to the enol tautomer (Fig. 1).

The stoichiometric ratio and the binding constant of the 3Py/3HP–DMβ-CD complex was estimated from the Benesi–Hildebrand (BH) double reciprocal plots.[28,29] The

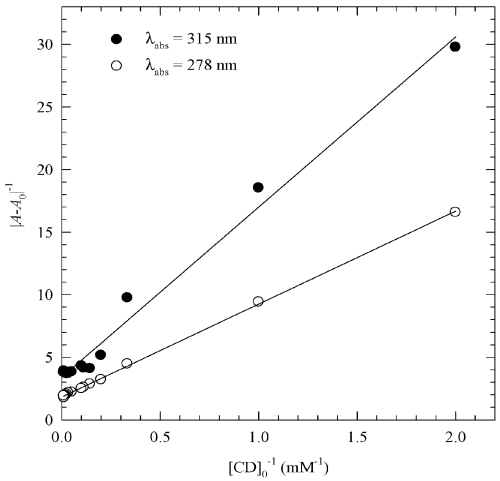


Fig. 2 BH double-reciprocal plots (eqn (1)) for the change in the absorbance intensity for the 3Py/3HP–DMβ-CD complex as a function of the DMβ-CD initial concentration. Zwitterion tautomer: solid circles, enol tautomer: open circles.

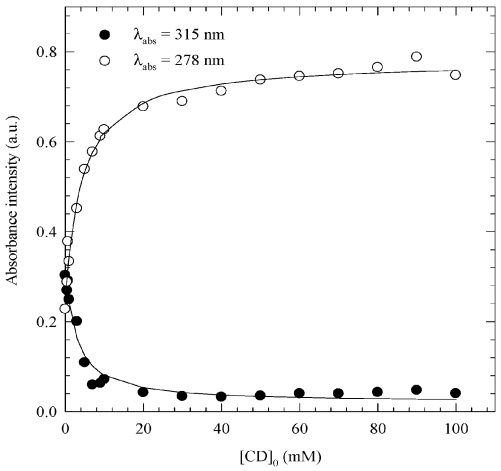


Fig. 3 Absorbance change as a function of DMβ-CD initial concentration for the zwitterion tautomer (solid circles) and the enol tautomer (open circles). The solid lines represent the best NLR fits using eqn (2) for $n = 1$.

change in the absorption intensity is correlated to the initial CD concentration according to

$$\frac{1}{A - A_0} = \frac{1}{(\varepsilon_c - \varepsilon_0)[N]_0} + \frac{1}{(\varepsilon_0 - \varepsilon_0)[N]_0 K[CD]_0^n} \qquad (1)$$

where A and A_0 are the absorbance per cm of 3Py/3HP aqueous solution in the presence and absence, respectively, of CD; ε_c and ε_0 are the molar absorption coefficients of the 3Py/3HP–DMβ-CD complex and uncomplexed 3Py/3HP, respectively; $[N]_0$ is the initial concentration of 3Py/3HP; $[CD]_0$ is the initial concentration of DMβ-CD; n represents the stoichiometry of the equilibrium reaction in relation to CD; and K is the binding constant for the formation of 3Py/3HP–DMβ-CD. Since the concentration of DMβ-CD is much higher than that of the guest, $[CD]_0$ is assumed constant before and after complexation.

A plot of $(A - A_0)^{-1}$ *vs.* $[CD]_0^{-n}$ should yield a straight line for the correct stoichiometry (n). Typical double reciprocal plots are shown in Fig. 2 for the change in the absorbance intensity of the zwitterion peak (315 nm) and the enol peak (278 nm). As shown in the Figure, a straight line is obtained for each case when $n = 1$. No linear plot was observed for other values of n. These results suggest that the stoichiometry of the 3Py/3HP–DMβ-CD complex is dominated by 1 : 1.

The binding constant for the complex formation was estimated from the slope and intercept of the BH plot and used as an initial guess in an iterative nonlinear regression (NLR) fit using the following equation[29–32]

$$A = \frac{A_0 + A_c K[CD]_0^n}{1 + K[CD]_0^n} \qquad (2)$$

where A_c is the absorbance of the 3Py/3HP–DMβ-CD complex. More accurate K values are usually obtained from NLR fits than those estimated from double reciprocal plots.[30–35] Eqn (2) was found to correctly fit the experimental data when $n = 1$, confirming the stoichiometry of the complex. The NLR fits are displayed in Fig. 3 and the estimated K values are $255 \pm 25\ M^{-1}$ for the zwitterion peak and $250 \pm 5\ M^{-1}$ for the enol peak. The similar K values in both cases is an indication of the presence of one equilibrium only which shifts dramatically towards forming more of the enol species inside the CD cavity.

We compare here the results of the inclusion of 3Py/3HP in different CDs with our recent results on the inclusion of 2,2′-bipyridine-3,3′-diol ($BP(OH)_2$) in the same CDs.[30,31] $BP(OH)_2$ is composed of two molecules of 3HP joined together by a chemical bond at the 2-position. It shows two unique absorption peaks in water due to a zwitterion tautomer, similar to the case of 3Py. The results showed that decreasing the cavity size and increasing its hydrophobicity cause a large reduction in the absorbance intensity of the zwitterion peaks accompanied by an increase in the absorbance intensity of the enol form. We interpreted the results to be due to the decrease in water accessibility inside the CD cavities. Unlike the case of 3Py/3HP in γ-CD, $BP(OH)_2$ is slightly buried inside the γ-CD cavity which is attributed to the large molecular size of $BP(OH)_2$ and its low solubility in water, all together favor inclusion inside the γ-CD cavity. The maximum caging effect was observed in α-CD where the stoichiometric ratio in the $BP(OH)_2$–(α-CD) complex is dominated by 1 : 2. The caging effects of β-CD and DMβ-CD on $BP(OH)_2$ showed a trend similar to that observed for 3Py/3HP with a similar 1 : 1 stoichiometry. The latter result is an indication of the suitable cavity size of β-CD which tends to stabilize intramolecular complexes with a wide range of molecular guests.[36]

3.2 Absorption spectra of 3Py/3HP in binary mixtures

We measured the absorption spectra of 3Py/3HP in binary mixtures of 1,4-dioxane and water. 1,4-dioxane and water are miscible in all proportions and thus provide an opportunity to study the effect of a broad range of solvent polarity. Their mixtures are proposed as media to study probes in microenvironments similar to those encountered in vesicles and at interfaces.[37–39]

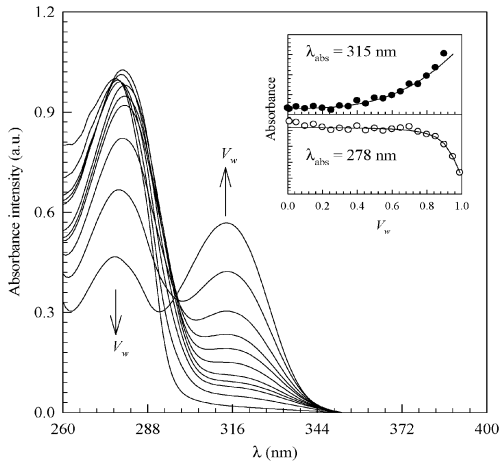


Fig. 4 Selected absorption spectra of 3Py/3HP in 1,4-dioxane–water binary mixtures. The inset shows the intensity change as a function of water volume fraction (V_w). Solid circles: zwitterion peak at 315 nm, open circles: enol peak at 278 nm. The solid lines are the best NLR fits to eqn (5).

The absorption spectra of 3Py/3HP in the 1,4-dioxane/water binary mixtures are shown in Fig. 4 for different water content. In the pure 1,4-dioxane solvent, the absorption shows one band which peaks at 278 nm and is due to the enol tautomer. Upon increasing the water content (volume fraction, V_w), the intensity of the enol peak decreases and the peak due to the zwitterion tautomer at 315 nm starts to emerge and its intensity increases as a function of the water content.

We can then write the following equilibrium as a result of solvation by water:

$$3\text{Py/3HP} + n\text{H}_2\text{O} \overset{K_{eq}}{\rightleftharpoons} 3\text{Py/3HP}-(\text{H}_2\text{O})_n \qquad (3)$$

Eqn (3) assumes that increasing the water content leads to local solvation of the polar centers in 3Py/3HP by n water molecules. In this case, water molecules in the first solvation shell will only participate by a certain number (n) and the rest of the water molecules will have the same effect as bulk water molecules. In this equation, $K_{eq} = [BS_n]/[B][S]^n$, where K_{eq} is the equilibrium constant, $[BS_n]$ represents the concentration of solvated 3Py/3HP by water molecules, [B] is the concentration of unsolvated 3Py/3HP, and [S] is the water concentration. An expression relating the relative concentrations to the observed absorbance (A_{obs}), and to the absorbance of solvated 3Py/3HP (A_{BS_n}) and unsolvated 3Py/3HP (A_B) can be written as:[29,40,41]

$$\frac{[BS_n]}{[B]} = \frac{(A_{obs} - A_B)}{(A_{BS_n} - A_{obs})} \qquad (4)$$

Using eqn (4) to rewrite the equilibrium constant, one obtains the following expression:

$$A_{obs} = \frac{A_B + A_{BS_n} K_{eq}[S]^n}{1 + K_{eq}[S]^n} \qquad (5)$$

Eqn (5) is similar to eqn (2) and both represent a binding isotherm between two species in equilibrium. Similar equations have been successfully used to estimate the stoichiometries and equilibrium constants for association of several compounds complexed with water[37–42] and other solvents,[38,39,43] and with metals[44] from the change in the steady state spectra.

The change in the absorbance intensity as a function of water volume fraction is displayed in the inset in Fig. 4 for the zwitterion and the enol peaks. The best NLR fits to eqn (5) are also shown. Both fits yielded similar results with $n = 2.8 \pm 0.2$ and $K_{eq} = (1.2 \pm 0.3) \times 10^{-5}$ M^{-3}. The n value indicates that three water molecules solvate the 3Py/3HP molecule. Accordingly, K_{eq} is expressed in the units of M^{-3}.

3.3 Structures of the 3Py–water and 3HP–water complexes

The structures of the zwitterion and enol tautomers with two and three water molecules were calculated in the ground state using *ab initio* methods at the MP2/6-31++G(d,p) level of theory. The calculations were also carried out for each of the two tautomers without water molecules in order to predict the most stable tautomer in the ground state. The structures have been fully optimized without any symmetry constraint. For the bare molecule (without water molecules), the calculations show that the molecule is planar in the ground state for both tautomers and the most stable tautomer was found to be the enol by 13.34 kcal mol^{-1}. Similar results where obtained at the HF/6-31G(d,p) and MP2/6-31+G(d,p) levels.[45]

By adding two water molecules to the polar sides of both tautomers and optimizing the total structures, the water molecules interact with the hydroxyamino region of the enol tautomer (structure I) and with the oxoamino region of the zwitterion tautomer (structure II) as shown in Fig. 5. For the zwitterion tautomer, the optimized structure indicates that one of the water molecules forms a strong hydrogen bond with the oxo part of the molecule ($-O^-\cdots H_2O$), whereas the second

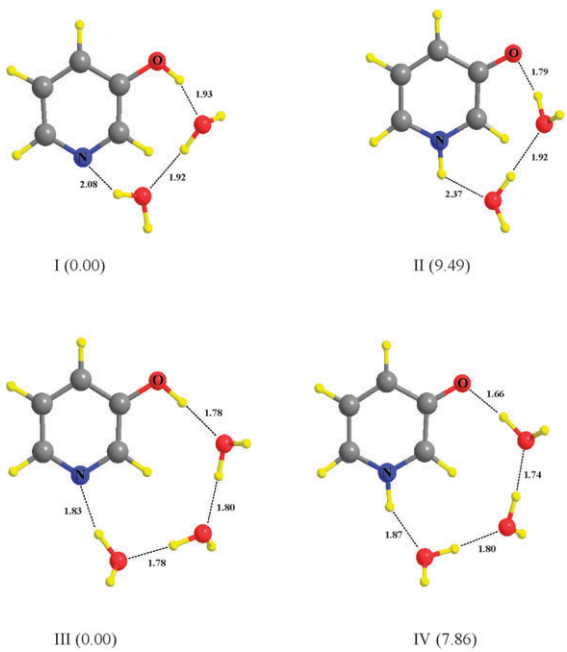


Fig. 5 Structures of the most stable minimum configurations of the 3Py and 3HP tautomers with two and three water molecules. The structures were obtained from *ab initio* calculations described in the text. The values in parentheses are relative energies in kcal mol^{-1}. The lengths of the hydrogen bonds are shown (in Å).

water molecule forms a relatively weak hydrogen bond with the amino moiety (N^+–H···OH_2). The calculated hydrogen bonds are 1.79 Å in the former and 2.37 Å in the latter. The two water molecules interact with each other by a hydrogen bond of 1.92 Å. The calculated structure for the 3Py–$2H_2O$ complex indicates that the pyridone ring is not planar and is distorted by *ca.* 12°. This non-planarity of the molecular structure can be explained as a result of the length of the solvent network, composed of only two water molecules, which is too short to solvate the two polar centers of the pyridone molecule, yet maintain their hydrogen bond link without distorting the molecular skeleton.

For the enol tautomer with two water molecules (3HP–$2H_2O$), the optimized structure shows a similar geometry as that for 3Py–$2H_2O$. Fig. 5 shows the complex (I) with the calculated hydrogen bonds. 3HP–$2H_2O$ is calculated to be more stable than 3Py–$2H_2O$ by 9.49 kcal mol^{-1}. The 3HP ring is planar, but the OH group makes a dihedral angle of *ca.* 30° with respect to the plane of the molecule. This dihedral angle compensates for the overall stability of the short water network which solvates the hydroxyl group and the tertiary nitrogen heteroatom in 3HP without distorting the molecular skeleton.

Adding the third water molecule to both tautomers results in planar structures with a stretched water network that reaches the two polar regions in each tautomer. The structures are shown in Fig. 5 with the corresponding hydrogen bonding lengths (III for 3HP–$3H_2O$ and IV for 3Py–$3H_2O$). As shown in the Figure, the water network (or water wire) forms strong hydrogen bonds with each polar center in each tautomer. The structures suggest that three water molecules are an optimum number in order to solvate the polar region in each tautomer. These results support those concluded from the binary mixtures study mentioned above. The 3HP–$3H_2O$ complex was calculated to be more stable than the 3Py–$3H_2O$ complex by 7.86 kcal mol^{-1}. It is noticed that by increasing the number of water molecules in the complex, the energy difference between the two solvated tautomers gets smaller. This may indicate that after complete solvation of the two tautomers by water, the relative stability is reversed in which the solvated 3Py becomes more stable than the solvated 3HP as observed experimentally.[4–7,10,11]

4. Conclusions

The equilibrium between the zwitterion and the enol tautomers of 3Py/3HP was examined in the ground state by measuring the absorption spectra in different CDs and in binary mixtures of 1,4-dioxane and water. The zwitterion form is observed only in water and the ground state equilibrium was found to depend on the degree of encapsulation in the CD cavity. In α-CD and β-CD, the increase in hydrophobicity is reflected in the reduction of the absorbance intensity of the zwitterion tautomer which is accompanied by an increase in the enol absorbance. The effect is less in the case of α-CD due to its small cavity size which may sustain partial inclusion. The slight increase in the zwitterion absorbance upon the addition of γ-CD to aqueous 3Py/3HP is attributed to the direct interaction between the polar sides of the zwitterion tautomer with the outer primary or secondary hydroxyls of the glycopyranose units of γ-CD. A large caging effect was observed in DMβ-CD which is due to the more hydrophobic cavity in the methylated derivative of β-CD.

In the binary mixtures of 1,4-dioxane and water, the change in the absorbance intensity of the enol and the zwitterion tautomers was analyzed quantitatively and three water molecules were found to solvate the polar centers of the 3Py/3HP moieties. *Ab initio* calculations of the solvation of both tautomers by two and three water molecules were performed at the MP2/6-31++G(d,p) level. The calculations show that three water molecules are optimum for solvating the polar centers of each tautomer in a water network pattern.

The results presented here suggest that the 3Py/3HP system represents a potentially useful new photophysical probe for supramolecular structures, particularly those involving inclusion.

Acknowledgements

This work was supported by the Sultan Qaboos University (grant # IG/SCI/CHEM/05/03).

References

1 C. A. Royer, *Chem. Rev.*, 2006, **106**, 1769.
2 O. K. Abou-Zied and O. I. K. Al-Shihi, *J. Am. Chem. Soc.*, 2008, **130**, 10793.
3 S. El Gezawi, N. Omar, N. E1 Rabbat, H. Ueda and J. H. Perrin, *J. Pharm. Biomed. Anal.*, 1988, **6**, 399.
4 O. K. Abou-Zied and O. I. K. Al-Shihi, *Proc. SPIE-Int. Soc. Opt. Eng.*, 2008, **6867**, 68670K.
5 A. R. Katritzky, *Physical Methods in Heterocyclic Chemistry*, vol. 1, New York, 1962.
6 A. Albert and J. N. Phillips, *J. Chem. Soc.*, 1956, **1**, 294.
7 R. H. Cox, A. A. Bothner-By and A. Aksel, *J. Phys. Chem.*, 1969, **73**, 2465.
8 A. Weisstuch, P. Neidig and A. C. Testa, *J. Lumin.*, 1975, **10**, 137.
9 D. Metzler and E. Snell, *J. Am. Chem. Soc.*, 1955, **77**, 2431.
10 P. Beak and J. B. Convington, *J. Am. Chem. Soc.*, 1978, **100**, 3961.
11 S. Suradi, N. El Saiad, G. Pilcher and H. A. Skinner, *J. Chem. Thermodyn.*, 1982, **14**, 45.
12 M. Chevrier, J. Guillerez and J. E. Dubois, *J. Chem. Soc., Perkin Trans. 2*, 1983, 979.
13 J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, Springer, New York, 2006.
14 G. Sudlow, D. J. Birkett and D. N. Wade, *Mol. Pharm.*, 1975, **11**, 824.
15 M. L. Bender and M. Komiyama, *Cyclodextrin Chemistry*, Springer, Berlin, 1978.
16 (*a*) V. T. D'Souza and M. L. Bender, *Acc. Chem. Res.*, 1987, **20**, 146; (*b*) J. Szejtli, *Cyclodextrins and their Inclusion Complexes*, Akademiai Kiado, Budapest, 1982.
17 V. Ramamurthy, *Photochemistry in Organized and Constrained Media*, VCH, New York, 1991.
18 (*a*) A. Douhal, *Chem. Rev.*, 2004, **104**, 1955; (*b*) S. Li and W. C. Purdy, *Chem. Rev.*, 1992, **92**, 1457.
19 T. Yorozu, M. Hoshino, M. Imamura and H. Shizuka, *J. Phys. Chem.*, 1982, **86**, 4422.
20 H. Shizuka, M. Fukushima, T. Fuju, T. Kobayashi, H. Ohtani and M. Hoshino, *Bull. Chem. Soc. Jpn.*, 1985, **58**, 2107.
21 M. El-Kemary and A. Douhal, in *Cyclodextrin Materials, Photochemistry, Photophysics and Photobiology*, ed. A. Douhal, Elsevier, Amsterdam, 2006.
22 M. W. Schmidt, K. K. Baldridge, J. A. Boatz, S. T. Elbert, M. S. Gordon, J. H. Jensen, S. Koseki, N. Matsunaga, K. A. Nguyen, S. J. Su, T. L. Windus, M. Dupuis and J. A. Montgomery, *J. Comput. Chem.*, 1993, **14**, 1347.

23 J. M. Sanchez Ruiz, J. Llor and M. Cortijo, *J. Chem. Soc., Perkin Trans. 2*, 1984, 2047.
24 M. Cotta Ramusino, L. Rufini and C. Mustazza, *J. Inclusion Phenom.*, 1993, **15**, 368.
25 M. Cotta Ramusino and S. Pichini, *Carbohydr. Res.*, 1994, **259**, 13.
26 C. J. Easton and S. F. Lincoln, *Modified Cyclodextrins*, Imperial College Press, London, 1999.
27 T. Steiner and W. Saenger, *Carbohydr. Res.*, 1995, **275**, 73.
28 A. H. Benesi and J. H. Hildebrand, *J. Am. Chem. Soc.*, 1949, **71**, 2703.
29 K. A. Connors, *Binding Constants. The Measurements of Molecular Complex Stability*, Wiley, New York, 1987.
30 O. K. Abou-Zied, *J. Phys. Chem. B*, 2007, **111**, 9879.
31 O. K. Abou-Zied and A. T. Al-Hinai, *J. Phys. Chem. A*, 2006, **110**, 7835.
32 O. K. Abou-Zied, *Spectrochim. Acta, Part A*, 2005, **62**, 245.
33 A. G. Mwalupindi, A. Rideau, R. A. Agbaria and I. M. Warner, *Talanta*, 1994, **41**, 599.
34 S. Mitra, R. Das and S. Mukherjee, *J. Phys. Chem. B*, 1998, **102**, 3730.
35 E. L. Roberts, J. Dey and I. M. Warner, *J. Phys. Chem.*, 1996, **100**, 19681.
36 K. A. Connors, *Chem. Rev.*, 1997, **97**, 1325.
37 E. C. C. Melo, S. M. Costa, A. L. Maçanita and H. Santos, *J. Colloid Interface Sci.*, 1991, **141**, 439.
38 M. Belletête, M. Lachapelle and G. Durocher, *J. Phys. Chem.*, 1990, **94**, 5337.
39 M. Belletête, G. Lessard and G. Durocher, *J. Lumin.*, 1989, **42**, 337.
40 O. K. Abou-Zied, *J. Photochem. Photobiol., A*, 2006, **182**, 192.
41 O. K. Abou-Zied, *Chem. Phys.*, 2007, **337**, 1.
42 S. M. Andrade and M. B. Costa, *Phys. Chem. Chem. Phys.*, 1999, **1**, 4213.
43 J. C. Penedo, M. Mosquera and F. Rodríguez-Prieto, *J. Phys. Chem. A*, 2000, **104**, 7429.
44 M. P. Bernstein, F. E. Romesberg, D. J. Fuller, A. T. Harrison, D. B. Collum, Q.-Y. Liu and P. G. Williard, *J. Am. Chem. Soc.*, 1992, **114**, 5100.
45 J. Wang and R. J. Boyd, *J. Phys. Chem.*, 1996, **100**, 1641.