Femtosecond to nanosecond dynamics of 2,2′-bipyridine-3,3′-diol inside the nano-cavities of molecular containers

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Femtosecond fluorescence upconversion measurements are employed to elucidate the mechanism of ultrafast double proton transfer dynamics of BP(OH)2 inside molecular containers (cucurbit[7]uril (CB7) and β-cyclodextrin (β-CD)). Femtosecond up-converted signals of BP(OH)2 in water consist of growth followed by a long decay component (∼650 ps). The appearance of the growth component (∼35 ps) in the up-converted signal indicates the presence of a two-step sequential proton transfer process of BP(OH)2 in water. Surprisingly, the up-converted signal of BP(OH)2 inside the CB7 nano-cavity does not exhibit any growth component characteristic of a two-step sequential process. Interestingly, the growth component exists inside the nano-cavity of β-CD (having similar cavity size as that of CB7), inferring the presence of a two-step sequential process of PT inside the β-CD nano-cavity. The different features of PT dynamics of BP(OH)2 in the above mentioned two macrocyclic hosts may be attributed to the presence and absence of water solvation network surrounding the BP(OH)2 inside the nano-cavities of β-CD and CB7, respectively. Finally, docking and DFT calculations have been employed in deciphering the molecular pictures of the interactions between BP(OH)2 and the macrocyclic host.

Introduction

Molecules exhibiting excited state intramolecular proton transfer (ESIPT) have received lot of interest owing to their current cutting-edge applications in science, such as molecular probes, logic gates and light-emitting diodes.1–5 Among the compounds exhibiting proton transfer processes, [2,2′-bipyridyl]-3,3′-diol (BP(OH)2) is of special interest, since it contains two protons, which can be involved in double proton transfer processes, and thereby, BP(OH)2 serves as a model for natural base pairs to study the tautomerization process in duplex DNA.6 BP(OH)2 is known to undergo an ultrafast excited state intramolecular double proton transfer (ESIDPT) from its dienol (DE) form (Scheme 1), and this ESIDPT process results in displacement of two protons from hydroxyl groups towards the pyridyl-ring nitrogen atoms leading to the formation of the diketo (DK) form (Scheme 1). The proton transfer dynamics of BP(OH)2 have been well explored using both experimental and theoretical studies.7–15 Femtosecond fluorescence upconversion and transient absorption experiments report that intramolecular double proton transfer in BP(OH)2 takes place via two channels, i.e., the process can be concerted (one-step) and/or sequential (two-step).10–14 The concerted proton transfer process occurs on an ultrafast time scale (<50 fs), whereas the sequential process takes place in an ultrafast first step (<50 fs) followed by a slow second step of ≥10 ps.15 Considering its unique feature of proton transfer dynamics, the photophysical properties of BP(OH)2 have been investigated in various constrained environments like cyclodextrins, micelles, proteins, protein–SDS aggregates, bile salt aggregates, zeolites, sol-gel glasses, nafion...
membranes, binary solvent mixtures and ionic liquids. However, in these confined environments, the proton transfer dynamics of BP(OH)$_2$, which takes place in an ultrafast time regime (<20 ps), are rarely monitored. Therefore, it would be very intriguing to probe proton transfer dynamics in confined environments with the help of fluorescence up-conversion techniques. In continuation of this effort, we have chosen cucurbit[7]uril (CB7), which is another attractive class of macrocyclic hosts. Cucurbit[n]urils (Cbn) are a class of caged compounds, whose skeleton consists of $n$ glycoluril units linked by a pair of methylene groups, providing different outer diameters ranging from 4.5 to 12.5 Å for CB5 to CB10, respectively, thereby allows guest molecules of various sizes within its cavity.\(^{26-28}\) The internal hydrophobic cavity is then responsible for the complexation with smaller guest molecules by hydrophobic interactions, whereas the two portals of the pumpkin-shaped CBn, lined by ureido carbonyl groups are responsible for the complexion with positively charged molecules by ion-dipole and/or hydrogen bonding interactions.\(^{26-30}\) In most of the cases, the complexion of drugs by CBn has pronounced effect on their $pK_a$ values,\(^{31-33}\) which might be useful in the recognition and delivery of medicinally and biologically important guests. Very recently, we have reported the effect of CB7 nano-cavities on the excited state proton transfer (ESPT) of an anti cancer drug topotecan (TPT).\(^{34}\) It was observed that ESPT was inhibited in the cavity of CB7 and it could be seen by naked eye through a fluorescence switch from green to blue.

Here in, we report the effect of nano-confinement by cucurbit[7]uril (CB7) on the photophysics of BP(OH)$_2$ with the help of steady state and time-resolved fluorescence spectroscopic techniques and compared with that of β-cyclodextrin (β-CD), which has an almost similar cavity size to CB7. To the best of our knowledge, we are reporting for the first time the dynamics of ESIDPT of BP(OH)$_2$ using a femtosecond fluorescence up-conversion technique in the two above mentioned macrocyclic hosts. Moreover, docking and DFT quantum chemical calculations have been employed in deciphering the molecular pictures of the interactions between BP(OH)$_2$ and macrocyclic hosts.

**Experimental section**

Cucurbit[7]uril (CB7), β-cyclodextrin (β-CD) and [2,2′-bipyridyl]-3,3′-diiol (BP(OH)$_2$) were purchased from Sigma-Aldrich and used as received. All the samples are prepared in Millipore water. CB7/β-CD was gradually added to the solution containing BP(OH)$_2$, and the solution was gently shaken after each addition of CB7/β-CD until complete solubilization of CB7/β-CD took place. Moreover, we have given 20 minutes equilibration time for each addition of host.

Absorbance measurements were performed on a spectrophotometer (Shimadzu, UV-2600), and steady-state fluorescence spectra were recorded in a FluoroMax-4 spectrofluorimeter (Horiba Scientific, USA). All time-resolved fluorescence measurements (both lifetime as well as anisotropy) were taken on a time correlated single photon counting (TCSPC) spectrometer (Horiba Jobin Yvon IBH, UK). The detailed descriptions of the instruments are described elsewhere.\(^{35-37}\) Notably, we have used a 375 nm diode laser for excitation of BP(OH)$_2$ molecules. The analysis of the lifetime is done by IBH DA6 analysis software. We have fitted both lifetime data with a minimum number of exponentials. Quality of each fit was judged by $\chi^2$ values and the visual inspection of the residuals. The value of $\chi^2 \approx 1$ was considered as best fit for the plots.

In our femtosecond up-conversion setup (FOG 100, CDP) the sample was excited at 380 nm using the second harmonic of a mode-locked Ti-sapphire laser (Mai-Tai, Spectra Physics). The fundamental beam was frequency doubled in a nonlinear crystal. The fluorescence emitted from the sample was upconverted in another nonlinear crystal using a gate pulse of the fundamental beam. The sum frequency of the fluorescence and gate pulse was detected as a function of the time delay between excitation and gate pulses. The angle between the polarization of the pump and gate pulses was kept at the magic angle to eliminate effects from rotational diffusion. The up-converted signal was dispersed in a monochromator and detected using photon counting electronics. A cross-correlation function obtained using the Raman scattering from ethanol displayed a full-width at half-maximum (fwhm) of ~350 fs.

**Results and discussion**

**Steady-state results**

Absorption spectra of BP(OH)$_2$ in water with varying concentrations of cucurbit[7]uril (CB7) are depicted in Fig. 1a. BP(OH)$_2$ in water exhibits a peak maximum at 345 nm and the double peak absorption band in the region of 400–450 nm. The 345 nm band originated from the π to π* transition of the dienol (DE) form and the 400–450 nm band appears due to the water solvated diketo (DK) form.\(^{16-18}\) The increase in absorbance at 345 nm along with a bathochromic shift with the gradual addition of CB7 indicates the occurrence of an interaction between the DE form and CB7. In addition to the changes at 345 nm, the absorbance of the water solvated DK band (400–450 nm) decreases in the presence of CB7. The suppression of the low energy band in the 400–450 nm region clearly demonstrates that the water solvation network around BP(OH)$_2$, which is believed to be responsible for the formation of the DK form, is perturbed in presence of CB7. As a result, the DK form of BP(OH)$_2$ is converted to the DE form in the presence of CB7. The presence of an isosbestic point in the absorption spectra further confirms the existence of a simple equilibrium between the two states.

To get clear insight about the complexation processes, we have monitored the emission profiles of BP(OH)$_2$ in absence and presence of CB7 (Fig. 1b). BP(OH)$_2$ in water exhibits an emission peak at 465 nm, when it is excited at 345 nm. The large Stokes-shifted emission of BP(OH)$_2$ is attributed to the DK form generated after the ESIDPT process in the excited state,
i.e., excitation of the DE form results in the emission from the DK form of BP(OH)$_2$. With gradual addition of CB7, the intensity at the peak maximum (465 nm) increases significantly with a ~35 nm red shift. Here it is relevant to mention that the caging effect generally reduces the water assisted non-radiative channels of BP(OH)$_2$. Moreover, ~35 nm red shift is observed in going from water to non-polar solvent. Therefore, the hike in intensity as well as the red shift in fluorescence spectra of BP(OH)$_2$ is a manifestation of the caging effect by the CB7 nano-cavity. We have also monitored the emission profiles by exciting the BP(OH)$_2$ molecules at 425 nm, where the DK form of BP(OH)$_2$ molecules are selectively getting excited, and the corresponding emission spectra are depicted in Fig. 1c. Increasing CB7 concentration leads to a decrease in the emission intensity of BP(OH)$_2$ at 465 nm and the observation is well corroborated with absorption studies. Similar observation was also reported previously when BP(OH)$_2$ is encapsulated in other hydrophobic constrained environments, like cyclodextrins, micelles and proteins. Therefore, the decrease in emission intensity upon excitation at 425 nm further confirms the effect of the hydrophobic environment of the CB7 cavity on BP(OH)$_2$. In a nutshell, steady-state results clearly indicate that CB7 forms an inclusion complex with BP(OH)$_2$. The interaction scenario between BP(OH)$_2$ and CB7 will be further verified through time-resolved fluorescence and computational studies.

In order to determine the stoichiometry as well as the binding constant of the inclusion complex, the changes in fluorescence intensity were plotted against the concentration (Inset of Fig. 1b) of host using the Benesi–Hildebrand (BH) equation
given below;

$$\frac{1}{F - F_0} = \frac{1}{K[F_1 - F_0][\text{host}]} + \frac{1}{F_1 - F_0}$$

where $F_0$, $F$ and $F_1$ are the fluorescence intensities of BP(OH)$_2$ in the absence and presence of host, and in the inclusion complex, respectively. The double reciprocal plot monitored at 465 nm is observed clearly to be linear ($R = 0.997$), indicating formation of a 1:1 inclusion complex between BP(OH)$_2$ and CB7, and the association constant ($K_1$) is estimated to be $\sim 3.1 \times 10^3 \text{ M}^{-1}$.

**Pico-second time-resolved fluorescence study**

Modulation in radiative properties and excited state dynamics of BP(OH)$_2$ upon interaction with cucurbit[7]uril (CB7) can also be clarified by fluorescence lifetime measurements. Fluorescence decays of BP(OH)$_2$ in absence and presence of CB7 collected at the respective emission maxima are shown in Fig. 2 and the corresponding fitting parameters are tabulated in Table S1 (ESI†). BP(OH)$_2$ exhibits single exponential decay in water with a lifetime component of ~650 ps, which is in good accordance with the previously reported value. In the presence of CB7, a longer lifetime component (~8 ns) appears in the decay profile along with a ~650 ps component (Table S1, ESI†). With the gradual addition of CB7, the contribution of a longer lifetime component enhances progressively (Fig. 2b), and becomes the major component (~80%) at higher concentration of host (500 μM). This newly appeared component is likely to be an outcome of the interaction between BP(OH)$_2$ and CB7. The increased lifetime of BP(OH)$_2$ on complexation with CB7 may be attributed to the decrease in non-radiative decay pathways of the dye inside the molecular container. In addition
to the non-radiative decay channels, polarity of the surrounding environment may affect the lifetime of BP(OH)$_2$. It is already evident that emission of BP(OH)$_2$ is originating from the DK form as result of the ESIDPT process, and the lifetime of this DK form is known to be sensitive to the polarity of the medium.$^{16-19}$ Recently, it was found that BP(OH)$_2$ shows a longer fluorescence lifetime in the hydrophobic pocket of cyclodextrin, proteins and bile salt aggregates compared to water.$^{16-19}$ Therefore, the longer lifetime component of BP(OH)$_2$ originated from the encapsulation inside the hydrophobic nano-cavity of CB7. Apparently, this lifetime component of BP(OH)$_2$ in CB7 is much longer than any other observed nano-cavities having hydrophobic environments, and it may be attributed to the inaccessibility of water molecules inside the CB7 cavity.

It is well known that time resolved fluorescence anisotropy measurements can provide valuable information regarding the rotational motion of a fluorophore, and hence, it can be used to probe the encapsulation process of BP(OH)$_2$ inside CB7. The rotational relaxation of BP(OH)$_2$ in water takes place on $\approx 70$ ps time scale (Fig. 3). The anisotropy decay displays noteworthy changes in the rotational relaxation time of BP(OH)$_2$ in the presence of CB7 (Fig. 3). The rotational correlation time monitored at 465 nm ($\lambda_{\text{ex}}$ = 375 nm) increased to 350 ps (Fig. 3) in the presence of CB7 due to the increased rigidity for BP(OH)$_2$ inside the cavity of CB7. The $\tau_T$ value is used to determine the hydrodynamic volume of the above mentioned stiochiometry from the Stokes–Einstein relationship$^{39}

\begin{equation}
\tau_T = \frac{1}{6D_r} = \frac{\eta V}{kT} \tag{2}
\end{equation}

Where $D_r$ and $\eta$ are the rotational diffusion coefficient and viscosity of the medium, respectively, $V$ is the hydrodynamic molecular volume of the complex, and $T$ is the absolute temperature. By using the above relation and assuming the viscosity of the medium is same as that of water, the calculated effective hydrodynamic diameter of BP(OH)$_2$ and the inclusion complex (CB7 : BP(OH)$_2$) are 8.24 Å and 14.09 Å, respectively. Therefore, the increased hydrodynamic diameter of BP(OH)$_2$ is a proof for the formation of an inclusion complex with CB7, which supports our previous interpretations in steady state and time resolved studies.

**Fluorescence up-conversion study of BP(OH)$_2$ inside the molecular containers: a comparative study in the nano-cavities of β-cyclodextrin (β-CD) and cucurbit[7]uril (CB7)**

To get insight into the ultrafast proton transfer dynamics of BP(OH)$_2$, which take place within several tens of picoseconds time scale, we have performed femtosecond fluorescence up-conversion measurements in water as well as in the presence of CB7. For comparison, we have also probed the dynamics inside β-CD, having similar cavity size as that of CB7. The steady-state and TCSPC results obtained in the presence of β-CD are briefly explained in Note S1 and Fig. S1 and S2 (ESI†), and match well with those of literature reports.$^{16,40}$ Femtosecond transients of BP(OH)$_2$ in water and in the presence of CB7/β-CD are shown in Fig. 4. The fluorescence up-converted decay profile of BP(OH)$_2$ in water exhibits growth and decay components having lifetimes of $\approx 30$ ps and 650 ps, respectively. Notably, although there are reports about the fluorescence up-converted decay profiles of BP(OH)$_2$ in several aprotic and protic solvents,$^{41}$ this is the first report of fluorescence up-converted transient of BP(OH)$_2$ in water. The longer lifetime component of $\approx 650$ ps can be assigned to the lifetime of the diketo (DK) form after the ESIDPT process and is consistent with the TCSPC results. We have already mentioned that the ESIDPT process can take place via a concerted...
or two step sequential mechanism.\textsuperscript{10–14} The concerted and first step of the sequential process takes place on a \(<50\) fs time scale (Scheme 1), which cannot be detected from the present setup (IRF = 350 fs); whereas the second step of sequential process (MK to DK conversion) takes place over several tens of picoseconds (Scheme 1).\textsuperscript{15} Therefore, the ultrafast instantaneous rise component of \(<35\) ps reflects the proton transfer dynamics during conversion of the mono (MK) to diketo (DK) form of BP(OH)\(_2\). However, the proton transfer dynamics in water is slower than the reported time in hexane, which takes place in \(<10\) ps.\textsuperscript{15} The sluggish PT dynamics in water may be attributed to the presence of a hydrogen bonding network, which disturbs and competes with the proton transfer process from the MK to DK form in the excited state. In the presence of CB7, a significant change in the fluorescence decay transient of BP(OH)\(_2\) is observed (Fig. 4). Astonishingly, fluorescence up-converted transient of BP(OH)\(_2\) is devoid of any growth component in the presence of CB7, and it exhibits single exponential fluorescence decay having a lifetime of \(>3\) ns, which may be ascribed to the lifetime of the DK form inside the CB7 nano-cavity. This observation clearly indicates that the MK \(\rightarrow\) DK conversion process is inhibited inside the CB7 nano-cavity. The steady state and TCSPC results suggest that even inside the CB7 cavity the final emission is coming from the DK form of BP(OH)\(_2\). Therefore, the absence of a growth component infers that the concerted mechanism, which occurs in \(<100\) fs, is favoured over a two step sequential proton transfer process (which takes place over several tens of picoseconds) inside the CB7 nano-cavity. The reason of favoring a concerted pathway over a sequential pathway may be attributed to the absence of water molecules inside the cavity of CB7. Very recently, Biedermann \textit{et al.} reported that the largest energy gain in the course of the complexation between neutral guests and CB\(_7\) occurs due to the complete removal/displacement of water molecules from the nano-cavity of CB7.\textsuperscript{42} The absence of an intermolecular hydrogen bonding network with water molecules destabilizes the MK form inside the CB7 cavity, and thereby, it blocks the proton transfer process \textit{via} MK to DK form. As a result, BP(OH)\(_2\) encaged with CB7 exhibits ESIDPT \textit{via} a concerted mechanism in which two protons move simultaneously from the hydroxyl groups toward the pyridyl-ring nitrogen atoms. BP(OH)\(_2\) shows biexponential transient (28 ps and 1 ns) features inside the \(\beta\)-CD cavity, likewise in water (Fig. 4). Here it is relevant to mention that the features of steady state and TCSPC results of BP(OH)\(_2\) with \(\beta\)-CD are very similar to those for CB7. Hence, it is expected that the feature of PT dynamics of BP(OH)\(_2\) in both the host systems would be very similar. However, the presence of an ultrafast rise/growth component of \(<28\) ps clearly infers that proton transfer dynamics inside the \(\beta\)-CD cavity is significantly different from that of the CB7 nano-cavity. Moreover, the appearance of an ultrafast rise/growth component of ~28 ps demonstrates the sequential proton transfer pathway is feasible inside the \(\beta\)-CD host. Notably, in case of \(\beta\)-CD water molecules have access in the vicinity of the included molecule,\textsuperscript{43} so that BP(OH)\(_2\) can form an intermolecular hydrogen bonding network with water even inside the cavity, likewise in water. The presence of a water shell around BP(OH)\(_2\) inside the \(\beta\)-CD cavity is the main reason for the appearance of the ultrafast growth component (~28 ps) corresponding to a sequential two step proton transfer pathway, which is absent inside the CB7 cavity.

\textbf{Docking and quantum chemical calculations}

To obtain the molecular picture of orientation of BP(OH)\(_2\) in the inclusion complexes as well as to gain insight into the stabilization achieved due to encapsulation, we have docked the DE form of BP(OH)\(_2\) into cucurbit[7]uril (CB7), followed by DFT quantum chemical optimization. The docking has been performed using AutoDock (4.2) software,\textsuperscript{44} and the detailed docking protocol was described in our earlier reports.\textsuperscript{33,37} Initially, all the chemical structures (host and guest) are geometry optimized using density functional theory (DFT) using the B3LYP functional with 3-21G basis set in Gaussian09.\textsuperscript{45} During docking, the receptor was kept rigid and the ligand was flexible. Finally, the inclusion complex obtained from docking studies is further optimized using DFT with the B3LYP functional and 3-21G basis set. Since the docking and geometry optimization were done without consideration of any solvent and other parameters, these geometries provide only qualitative pictures of the structures in the ground state. Optimized structure of the inclusion complex is shown in Fig. 5. Further, we have evaluated the interaction energy for the inclusion complex by subtracting the energy of individual molecules from the complex.\textsuperscript{46} The interaction energy obtained from quantum chemical optimization method is negative (~31.90 kcal mol\(^{-1}\)), suggesting that 1:1 inclusion complex formation between BP(OH)\(_2\) and CB7 is energetically feasible. It is clear from the geometry optimized structure of the inclusion complex that the BP(OH)\(_2\) molecule is completely sequestered in the centre of the CB7 cavity (Fig. 5). Therefore, theoretical studies support the experimental evidence that the hydrophobic nano-cavity of
CB7 is responsible for the complexation process which affects the photophysical properties of BP(OH)$_2$.

**Conclusion**

In this work, host–guest interactions between the molecular containers cucurbit[7]uril (CB7) and β-cyclodextrin (β-CD) and BP(OH)$_2$ are investigated with the help of steady state and time-resolved fluorescence measurements. Ground state absorption study indicates that the conversion of the diketo (DK) to dienol (DE) form in BP(OH)$_2$ takes place in the presence of CB7. Steady state and time-resolved fluorescence studies confirm that the main emissive species is the DK form of BP(OH)$_2$ inside the CB7 nano-cavity. Femtosecond fluorescence upconversion measurements are employed to elucidate the mechanism of ultrafast proton transfer dynamics of BP(OH)$_2$ inside nano-containers (CB7 and β-CD). Femtosecond up-conversion study of BP(OH)$_2$ in water reveals that the two step sequential proton transfer process via MK to DK form appears as arowth component (∼35 ps) in the up-converted signal. Astonishingly, the fluorescence up-converted signal of BP(OH)$_2$ is devoid of any growth component in the presence of CB7, and it supports that a concerted mechanism of proton transfer (taking place on a <100 fs time scale) is present inside the nano-cavity of CB7 instead of the two step-sequential process of PT dynamics. Interestingly, a two step sequential process is feasible in the case of β-CD, as inside the nano-cavity of β-CD a growth component of ∼28 ps is detected. The reason for this observation is the presence of a surrounding water solvation network of BP(OH)$_2$ inside the cavity of β-CD, which is absent in case of the CB7 nano-cavity. Finally, docking and DFT quantum chemical calculations have been employed in deciphering the molecular orientation of BP(OH)$_2$ in the inclusion complex with the macrocyclic host. Theoretical calculations confirm that the BP(OH)$_2$ molecule resides at the centre of the hydrophobic nano-cavity of CB7, and thereby it is believed to affect the double proton transfer dynamics significantly.

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**References**