Fluorescence enhancement of epicocconone in its complexes with cyclodextrins

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Abstract

The interactions of epicocconone with two supramolecular hosts, α- and β-cyclodextrin have been investigated using fluorescence spectroscopic techniques. 1:1 and 1:2 complexes formed with both the cyclodextrins are marked by the enhancement of fluorescence of epicocconone. A significantly greater fluorescence enhancement is observed with α-CD than with β-CD. This observation fortifies the contention that microviscosity has a significant influence on the excited state dynamics of epicocconone, as the space available for the fluorophore within its complex with α-CD is significantly lesser than that available in its complexes with β-CD, even though the fluorophore is shielded from water in both the cases.

1. Introduction

Excited state dynamics of fluorophores get significantly modulated upon complexation with supramolecular hosts, which offer considerably restricted microenvironments [1]. Cyclodextrins (CD) constitute the most widely studied species in this class of hosts [2,3]. Epicocconone (Scheme 1) is a natural product with a weak fluorescence at 530 nm. Its fluorescence is enhanced markedly, with a red shift in emission maximum to 610 nm, in the presence of proteins. This has been rationalized in the light of the occurrence of a covalently bonded conjugate of epicocconone, where it reacts with the amino group of the N-terminal amino acid residue of the protein [4,5]. Very recently, we have reported that upon incorporation in micelles, the position of the emission maximum remains at 530 nm and that there is a marked difference in the extent of fluorescence enhancement in Triton X-100 and sodium dodecyl sulfate micelles [6]. The fluorescence decays of epicocconone have been found to be bimodal, with components of 0.2 and 1 ns, with a likelihood of the existence of an additional ultrafast component. The contribution of the longer lifetime component increases upon incorporation in the micelles. Such enhancement in lifetime and quantum yield, upon incorporation in micelles, might be due to a decreased microviscosity, increased polarity or sequestration from water. In order to know which of these are responsible for the fluorescence enhancement, it is imperative to perform rigorous studies in homogeneous liquids, with a view to resolve the effects of the different properties of the microenvironment of the fluorophore. Unfortunately, epicocconone exhibits a rather limited range of solubility and so, a systematic study in homogeneous solutions of varied properties is not possible. However, from our experiments in H2O, D2O, acetonitrile and butanol, we have found that there is little if any effect of deuteration or polarity. On the other hand, an increase in viscosity at constant polarity decreases the efficiency of the nonradiative process markedly [6]. Quantum chemical calculations indicate the HOMO–LUMO transition to be of π–π* type. This, along with the marked dependence of the excited state process on microviscosity, leads to the contention that photoisomerization is the major nonradiative process operative in the epicocconone [6]. With this background, we present our findings on the interaction of this fluorophore
with macrocyclic hosts and the consequent modulation of
its fluorescence properties.

2. Experimental

Deep purple total protein stain from Amersham Biosciences, containing epicocconone as the active ingredient, was used as received. The analytical grade cyclodextrins were obtained from Aldrich chemicals, USA. Deionized water was distilled twice before being used as a solvent. The absorption and fluorescence spectra were recorded on JASCO V 530 spectrophotometer and Varian Cary Eclipse fluorimeter, respectively. The excitation wavelength ($\lambda_{ex}$) was 430 nm for fluorescence measurements. The absorbance at this wavelength was kept below 0.1, in order to avoid inner filter effects and ensure that the fluorophore was present in micromolar concentrations. Fluorescence quantum yields ($\phi_f$) were calculated after proper correction for changes in absorbance using Lucifer yellow CH ($\phi_f = 0.21$) as the reference [6]. Time resolved fluorescence measurements were performed at magic angle polarization using a picosecond pulsed diode laser based TCSPC fluorescence spectrometer with $\lambda_{ex} = 406$ nm from Jobin Yvon Horiba. The full width at half maximum of the instrument response function was 250 ps and the resolution was 7 ps per channel. The data were fitted to multieponential functions after deconvolution of the instrument response function by an iterative reconvolution technique, using the JY Horiba IBH DAS 6.2 data analysis software, where reduced $\chi^2$ and weighted residuals serve as parameters for goodness of fit [6].

3. Results and discussion

The principal absorption maximum of epicocconone, which occurs at 435 nm in aqueous solution, undergoes a red shift of 10 nm in presence of $\alpha$- and $\beta$-CD (Fig. 1). This is similar to our earlier observation in sodium dodecyl sulfate and triton X-100 micelles and indicates the incorporation of the dye in the apolar interior of the cyclodextrins [6]. A concrete evidence for binding of epicocconone with all the three hosts is obtained from the remarkable increase in stability of its solutions containing the macrocycles. The fluorescence spectra undergo more prominent changes. The fluorescence maximum, which is at 533 nm in aqueous solution, undergoes a red shift to 542 nm in the cyclodextrins. Significant fluorescence enhancement occurs with both the hosts. The fluorescence quantum yield increases from 0.008 in aqueous solution to 0.099 in $\alpha$-CD and 0.027 in $\beta$-CD (Fig. 1b, Table 1). The enhancement may be due to two reasons: shielding from water and suppression of the nonradiative process, which we have earlier proposed to be photoisomerization [6], in the restricted microenvironment provided by the hosts. The stoichiometry of the complexes of the fluorophore with the supramolecular hosts is studied by using a modification of the well known Benesi–Hildebrand method (Fig. 2, Table 1) [7,8].
The Benesi–Hildebrand plots deviate from linearity at higher concentrations of the hosts, indicating the formation of one epicocconone: 2CDs complexes, as has been observed earlier with \(N,N\)-diphenylbenzidine and several other guest molecules [9]. The linear portions at lower concentrations of cyclodextrins have been fitted to a straight line, in order to calculate the binding constant for the 1:1 complexes. The value of this constant, obtained for \(\beta\)-cyclodextrin, is comparable to that of 1.22 mM/C0 of coumarin 153 with \(\beta\)-CD derivative (Table 1) [10]. With \(\alpha\)-CD, the binding constant of the 1:1 complex is found to be markedly higher than that with \(\beta\)-CD. In order to ascertain the binding constants and the fluorescence quantum yields of the 1:2 complexes with the cyclodextrins, the plot of the fluorescence quantum yield (\(\phi_f\)) against the host concentration ([CD]), has been fitted to the equation:

\[
\phi_f = \left( \phi_0 + \phi_1 K_1 [CD] + \phi_2 K_2 [CD]^2 \right) / \left( 1 + K_1 [CD] + K_2 [CD]^2 \right)
\]

where \(\phi_0\), \(\phi_1\) and \(\phi_2\) are the fluorescence quantum yields of the free fluorophore, 1:1 complex and 1:2 complex, respectively [10]. The values of \(\phi_0\) and \(K_1\) from Benesi–Hildebrand analysis are used as constants in the equation. The binding constants and the quantum yields are then calculated by iterative non-linear least squares regression (Fig. 2, Table 1). The fluorescence quantum yield (\(\phi_f\)) is found to be significantly higher in the 1:2 complexes with \(\alpha\)-CD than in any of the other complexes. It is almost five times as much as that in the 1:2 complexes with \(\beta\)-CD. In fact the \(\phi_f\) in the 1:1 complex with \(\alpha\)-CD turns out to be comparable to that in its 1:2 complex with \(\beta\)-CD. This is an important pointer towards the cause of fluorescence enhancement of epicocconone in these complexes. If the major reason for enhancement is shielding from water, then similar extents of enhancement should have been observed in the two kinds of 1:2 complexes. However if microviscosity or confinement effect is primarily responsible, then the effect of the smaller \(\alpha\)-CD is expected to be more pronounced than that of \(\beta\)-CD, which has a larger cavity. Since this is indeed the case, we propose that the major reason for the greater fluorescence enhancement in the complexes with \(\alpha\)-CD is the more restrictive microenvironment in these complexes, which is expected to hinder the flexing motion of the alkenyl chain of epicocconone.

![Fig. 2. (A) Benesi–Hildebrand plots for determination the binding constants of epicocconone with the supramolecular hosts. The binding constants are calculated from the ratios of intercepts and slopes. The two linear plots in the different concentration ranges in case of \(\alpha\)-CD and \(\beta\)-CD denote two kinds of stoichiometries: 1:1 at low concentrations and 1:2 at higher ones. (B) Plot of emission quantum yield (\(\phi_f\)) of Epicocconone against the concentration of cyclodextrins [CD] in water with varying concentrations of (a) \(\alpha\)-CD (○) and (b) \(\beta\)-CD (●). The points represent experimental values, and the solid lines represent the non-linear least-squares fit to Eq. (1).](image)
Since such a motion is essential for photoisomerization, a considerable fluorescence enhancement may be expected. This issue is explained further using the time resolved fluorescence studies.

In aqueous solutions, the fluorescence decay of epicocconone is biexponential, with components of 0.2 ns (80%) and 1 ns (20%). They become slower with the addition of the supramolecular hosts (Fig. 3). With α-CD, the lifetimes do not change considerably, but the relative amplitude of the longer lifetime increases gradually till the decay becomes single-exponential at a concentration of 9 mM α-CD (Table 2, Fig. 3). A similar variation of amplitudes, with little change in lifetimes is observed with β-CD. However in this case, the amplitudes change to a lesser extent (Fig. 4). In our previous study, the 0.2 ns component has been assigned to the fluorophore that undergo photoisomerization and the 1 ns component has been assigned to those which do not [6]. It has been shown that the excited state dynamics is governed mainly by microviscosity and not micropolarity. Besides, no deuterium isotope effect is observed. Consequently, the marked decrease in the amplitude of the shorter component upon addition of surfactants has been explained in the light of increased microviscosity in the micelles [6].

With this background, we proceed to explain the observations in the supramolecular hosts. The increase in the abundance of the species with longer lifetime in α-CD is explained by confinement effect experienced by the fluorophore within the cavities of these macrocycles. The decrease in the amplitude of the shorter component signifies a hindrance to photoisomerization, which is in line with earlier studies of similar molecules and processes in this microenvironment [11]. The greater degree of such hindrance in α-CD than in β-CD is rationalized by the smaller size of α-CD, which provides a greater restriction to any kind of motion of the fluorophore. The fact that the fluorescence decays become single-exponential at 9 mM α-CD, which is a concentration at which 1:2 complexes predominate (Fig. 3), indicates a total suppression of photoisomerization in the very rigid microenvironment provided by these complexes. It may be noted that such differences in the effect of α- and β-cyclodextrins on photoisomerization processed have been reported for other compounds [11–15]. Various explanations have been offered for such behavior. For example, a marked effect of α-CD is observed, on the photoisomerization of phenylethlenethioamide. However, no such effect

![Fig. 3. The fluorescence decays of epicocconone in (a) water (b) 35 mM α-CD (c) 15 mM β-CD. λ<sub>em</sub> = 530 nm, λ<sub>ex</sub> = 406 nm. The instrument response function is shown in dashed line. The weighted residuals are shown below the decays. The range of the y-axes in the plots of weighted residuals is −4 to +4.](image)

![Fig. 4. Variation of the amplitudes of the components of biexponential global fits to the fluorescence decays of epicocconone with concentrations of the macromolecular hosts: (a) α-CD, (b) β-CD. a<sub>1</sub>, a<sub>2</sub> are the amplitudes of the shorter and the longer components, respectively, of the biexponential fits to the decays. λ<sub>em</sub> = 530 nm, λ<sub>ex</sub> = 406 nm in all cases.](image)

Table 2
The fluorescence decay parameters of epicocconone in its inclusion complexes with the supramolecular hosts

<table>
<thead>
<tr>
<th>Medium</th>
<th>t&lt;sub&gt;1&lt;/sub&gt; (ns)</th>
<th>t&lt;sub&gt;2&lt;/sub&gt; (ns)</th>
<th>a&lt;sub&gt;1&lt;/sub&gt;</th>
<th>a&lt;sub&gt;2&lt;/sub&gt;</th>
<th>χ&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>0.30</td>
<td>1.16</td>
<td>0.81</td>
<td>0.19</td>
<td>1.13</td>
</tr>
<tr>
<td>35 mM α-CD</td>
<td>–</td>
<td>1.14</td>
<td>–</td>
<td>–</td>
<td>1.05</td>
</tr>
<tr>
<td>15 mM β-CD</td>
<td>0.22</td>
<td>1.48</td>
<td>0.58</td>
<td>0.42</td>
<td>1.07</td>
</tr>
</tbody>
</table>
is observed with β-CD [13]. This has been explained in the light of the difference in the orientation of the molecules in the complexes with the two cyclodextrins, which results from a difference in the size of their cavities. Another example of the effect of the cavity size is that of the photoisomerization of trans-stilbene, which forms 1:1 complex with α-cyclodextrin, resulting in a relatively single-exponential decay. 1:1 as well as 1:2 inclusion complexes in the presence of β-cyclodextrin, resulting in a double-exponential fluorescence decay of trans-stilbene. The longer lifetime is ascribed to a tightly bound form (inside the cavity), and short life time is due to a loosely associated form. The difference in the extents of enhancement of fluorescence quantum yield of epicocconone induced by the two cyclodextrins may be explained similarly. A tighter complex with the smaller α-CD may be expected to undergo isomerization to a lesser extent, compared to the loose complex with β-CD. This may be expected to cause a greater enhancement of fluorescence in the former than in the latter.

4. Conclusion

Epicocconone is found to exhibit substantial fluorescence enhancement in its inclusion complexes with α- and β-cyclodextrins. The rigidity of these complexes is the major cause of the enhancement of fluorescence. In both the supramolecular hosts, photoisomerization is hindered and fluorescence enhancement occurs without a change in the position of the fluorescence peak, as the aliphatic chain of the fluorophore, containing conjugated double bonds, is inserted partially into the cavities of the hosts. The maximum enhancement occurs in α-cyclodextrin, where tight 1:2 complexes are formed and the chain is incorporated completely in these complexes. The fluorescence enhancement is found to be due to a change in the relative populations of the longer of the two components of the lifetime of epicocconone.

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