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7-Cyanoindole Fluorescence as a Local Hydration Reporter: Application to Probe the Microheterogeneity of Nine Water-Organic Binary Mixtures

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Abstract

Considerable efforts have been devoted to the development of spectroscopic probes that are sensitive to water and can be used to monitor, for example, biological and chemical processes involving dehydration or hydration. Continuing this line of research, herein we show that 7-cyanoindole can serve as a sensitive fluorescence probe of hydration as its fluorescence properties, including intensity, peak wavelength and lifetime, depend on the amount of water in nine water-organic solvent mixtures. Our results indicate that 7-cyanoindole is not only able to reveal the underlying microheterogeneity of these binary solvent systems, but also offers distinct advantages. These include: (1) its fluorescence intensity increases more than ten times upon going from a hydrated to a dehydrated environment; (2) its peak wavelength shifts as much as 35 nm upon dehydration; (3) its single-exponential fluorescence decay lifetime increases from 2.0 ns in water to 8-16 ns in water-organic binary mixtures, making it viable to distinguish between differently hydrated environments via fluorescence lifetime measurements; and (4) its absorption spectrum is significantly red-shifted from that of indole, making selective excitation of its fluorescence possible in the presence of naturally occurring amino-acid fluorophores. Moreover, we find that for seven binary mixtures the fluorescence lifetimes of 7-cyanoindole measured at solvent compositions where maximum microheterogeneity occurs correlate linearly with the peak wavenumbers of its fluorescence spectra obtained in the respective pure organic solvents. This suggests that the microheterogeneities of these binary mixtures bear certain similarity, a phenomenon that warrants further investigation.

Graphical abstract

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SUPPORTING INFORMATION

Electronic Supplementary Information (ESI) available.

INTRODUCTION

Recently, several cyanoindole-based unnatural amino acids^{1–4} have received much attention due to their distinct fluorescent property and hence potential utility as novel biological fluorophores. For example, Hilaire *et al.*³ demonstrated that 4-cyanotryptophan (4-CN-Trp) exhibits unique photophysical properties, including fluorescence emission in the blue region of the visible spectrum, that can be exploited for biological spectroscopy and imaging applications. Furthermore, Markiewicz *et al.*¹ showed that 5-cyanotryptophan (5-CN-Trp) can be used to probe local hydration status in proteins, whereas Talukder *et al.*² demonstrated that 6-cyanotryptophan (6-CN-Trp) and 7-cyanotryptophan (7-CN-Trp) could serve as fluorescence resonance energy transfer (FRET) donors to study interactions between proteins and nucleic acids. Continuing this line of effort, we aim to show that the fluorophore of 7-CN-Trp, 7-cyanoindole (7-CNI), can also be used as a sensitive fluorescence probe of biological processes involving protein hydration or dehydration.

In comparison to fluorophores of amino acids that have been used as fluorescence probes of protein hydration or dehydration, such as tryptophan,^{5,6} *p*-cyanophenylalanine,⁷ and 5-CN-Trp,¹ one advantage of 7-CNI is that its absorption spectrum is significantly shifted from those of aromatic sidechains found in proteins (Fig. S1, ESI). Thus, the fluorescence of 7-CNI can be selectively excited even in the presence of other aromatic amino acids by using an excitation wavelength around 308 nm. In addition, a recent study by Hilaire *et al.*⁴ showed that the fluorescence lifetimes of 7-CNI are 2.0 ns and 14.5 ns in water and dimethyl sulfoxide respectively, indicating that its fluorescence property is sensitive to hydration. However its practical utility as a fluorescence hydration probe has not been established. Therefore, to validate this idea, herein we apply it to investigate the microheterogeneity in nine water-organic solvent binary mixtures.

Many organic solvents, such as tetrahydrofuran, acetonitrile, and dimethyl sulfoxide, are fully miscible with water at any mole fraction, forming binary mixtures with distinct physical properties. As such, various water-organic binary mixtures have found practical applications in various scientific fields, including organic synthesis,⁸ environmental cleanup,^{9,10} chromatography,^{11–15} nanofluids,^{16,17} and enzymology.^{18–22} For example, mixtures of water and dimethyl sulfoxide have been used to denature, precipitate, crystallize, stabilize, activate, and inhibit proteins.^{23–25} It is well known that even though water and an organic solvent are miscible at the macroscopic level, the resulting binary mixture is a non-ideal solution that can contain microscopically distinguishable solvent clusters.²⁶ This behavior, commonly referred to as solvent microheterogeneity,^{27–38} has been extensively studied using

a variety of water-organic binary mixtures and techniques, including molecular dynamics simulations,^{28,32} linear and nonlinear spectroscopic methods,^{30,38} x-ray scattering,³¹ and mass spectrometry.^{33–37} Therefore, we believe that commonly encountered water-organic binary mixtures would serve as convenient model systems for us to test the applicability of 7-CNI as a fluorescence hydration probe. It is already well known that indole rings show preference for water-organic interfacial regions,³⁹ such as the headgroup regions of lipid bilayers or membranes.⁴⁰ Thus, the premise of our study is that 7-CNI will bind to solvent clusters that contain organic molecules, which, in turn, will lead to changes in its fluorescence properties and consequently provide information about the microheterogeneity of the binary solvent system in question. To prove our hypothesis, herein we studied nine water-organic binary mixtures involving the following organic solvents: tetrahydrofuran (THF), acetonitrile (ACN), dimethyl sulfoxide (DMSO), 1,4-dioxane (DIO), methanol (MeOH), ethanol (EtOH), isopropanol (IPA), tert-butanol (TBA), and ethylene glycol (EtG). Indeed, our results show that for every binary mixture studied, the change in 7-CNI fluorescence intensity is able to capture the mole fraction of the organic component (hereafter referred to as χ_O) or regions of χ_O at which maximum microheterogeneity occurs. In addition, our time-resolved measurements indicate that the solvent clusters giving rise to such microheterogeneity in the binary systems studied provide a rather special solvation environment for 7-CNI.

EXPERIMENTAL DETAILS

Materials and Sample Preparation

All materials were of the highest purity and used as received. Specifically, 5-cyanoindole (5-CNI, 99%) and 7-cyanoindole (99%) were obtained from Ark Pharm Inc., 1,4-dioxane (spectroscopic grade) and isopropanol (spectroscopic grade) were obtained from Alfa Aesar, ethanol (spectroscopic grade) was obtained from Decon Laboratories, Inc., ethylene glycol (spectroscopic grade) and tetrahydrofuran (HPLC grade) were purchased from Fisher Chemical, and all other solvents (extra dry) – dimethyl sulfoxide, acetonitrile, methanol, and tert-butanol were obtained from Acros Organics. All samples were prepared by mixing appropriate volumes of two stock solutions, both of which had a solute concentration of 10 μ M with one prepared in Millipore water and the other prepared in the desired organic solvent. For each sample, the mole fraction of the organic solvent was calculated based upon weights, using the total volume of the two corresponding solutions. Samples used in the background measurements (for both absorbance and fluorescence) were prepared in the same way, except that the stock solutions did not contain any solute.

Absorption Measurements—UV-Vis absorption spectra were collected on a Jasco V-650 UV-Vis spectrophotometer using a quartz cuvette with a path length of 1.0 cm at room temperature.

Fluorescence Measurements—Fluorescence spectra were collected on a Jobin Yvon Horiba Fluorolog 3.10 fluorometer. Specifically, the temperature of the sample in a 1.0 cm quartz cuvette was maintained at 25 °C, and the spectral resolution and scanning rate were set at 1.0 nm and 1 s/nm, respectively. The excitation wavelengths were 308 and 280 nm for

7-CNI and 5-CNI respectively. For each solvent condition, a background fluorescence spectrum measured with the corresponding binary solvent mixture was subtracted from that of the solute. For a given water-organic solvent mixture, the fluorescence intensity data (S_F) were presented in a normalized format and as a function of the organic mole fraction (χ_O), calculated using the following equation:

$$S_F(\chi_O) = \frac{I_{WO}}{I_W} \times \frac{A_W}{A_{WO}} \times \frac{(n_{WO})^2}{(n_W)^2} \quad (1)$$

Where, I_W and A_W are the integrated area of the fluorescence spectrum and the absorbance at the excitation wavelength of the fluorophore in water, respectively, whereas I_{WO} and A_{WO} are the integrated area of the fluorescence spectrum and the absorbance at the excitation wavelength of the fluorophore in the water-organic solvent binary mixture of interest at a mole fraction of (χ_O). In addition, n_W and n_{WO} represent the indices of refraction of water and the corresponding water-organic binary mixture, respectively. Except for water-DMSO solutions, the n_{WO} values for all other solution mixtures were directly measured using a Bri/RI-check refractometer (Reichert Technologies).

Fluorescence lifetimes (τ_F) were measured on a home-built time-correlated single photon counting (TCSPC) apparatus at room temperature using a 1.0 cm quartz cuvette and an excitation wavelength of 270 nm, which was generated by frequency tripling of the fundamental output of a femtosecond Ti:Sapphire oscillator. An electro-optical pulse picking system (Conoptics, Inc.) was used to decrease the repetition rate of the pulse to 21 MHz. Fluorescence emission was isolated through a combination of a 355/45 nm short bandpass filter and a 300 nm long pass filter (Semrock) and collected under magic angle polarization condition with a MCP-PMT detector (Hamamatsu R2809U) and a TCSPC board (Becker and Hickl SPC-730). Fluorescence decay curves were fit to exponential functions using the FLUOFIT program (PicoQuant GmbH) and an experimentally determined instrument response function (IRF). All samples used in the time-resolved experiments had an absorbance around 0.2 at the excitation wavelength.

RESULTS AND DISCUSSION

Binary Mixtures Involving Aprotic Organic Solvents

We chose DMSO, THF, DIO and ACN because they are among the most commonly encountered aprotic solvents used in water-organic binary mixtures and there are ample studies on the corresponding mixtures.^{32,35,36,41–51} As shown (Fig. S2, ESI), the absorption spectrum and fluorescence excitation spectrum of 7-CNI are insensitive to the identity and mole fraction of the organic component in the binary mixture. However, the fluorescence spectra of 7-CNI in these binary mixtures clearly reveal a red-shift upon going from the pure organic solvent to water in each case (Fig. S3, ESI). This is expected as the underlying electronic transition of the indole ring produces a larger dipole moment at the electronically excited (or fluorescent) state, in comparison to that of the ground electronic state, hence rendering the excited state more stabilized in a more polar environment (e.g., in pure water).

^{52,53} In addition, these spectra show that the fluorescence intensity of 7-CNI varies with χ_O in each case. Therefore, taken together these data indicate that the fluorescence property of 7-CNI depends on the amount of water present in these binary mixtures and hence could be used as a local hydration probe. However, to determine whether the fluorescence of 7-CNI is sensitive enough to reveal the microheterogeneity of these water-organic binary mixtures, we need to take a closer look at the dependences of the peak emission wavelength ($\lambda_{\max}$) and the fluorescence intensity (S_F , calculated via Eq. 1) of 7-CNI on χ_O for each binary mixture and to compare our results with previous findings.

As shown (Fig. 1), for all four binary mixtures, the fluorescence intensity of 7-CNI shows a similar dependence on χ_O – it first increases, reaching a maximum, and then decreases (to a lesser extent than the increase). Furthermore, for each case, the fluorescence intensity at $\chi_O = 1$ is much larger than that at $\chi_O = 0$, manifesting the fact that in comparison to the organic solvents used in this study, water is a more efficient quencher of 7-CNI fluorescence.⁴ On the other hand, as indicated (Fig. 2), the dependence of $\lambda_{\max}$ on χ_O differs among these binary mixtures. Below, we discuss these results individually.

For the water-ACN binary mixture, the S_F versus χ_{ACN} plot (Fig. 1) shows that the fluorescence intensity of 7-CNI reaches a maximum at $\chi_{ACN} \approx 0.5$ (Table S1, ESI) where its value is about 8.1 times of that obtained in pure water (hereafter referred to as $S_{FW} = 1$), whereas in pure ACN, the value of S_F is only about 6 times that of S_{FW} . Taken together, these results argue against the idea that the change in the fluorescence intensity of 7-CNI arises from preferential interactions between the fluorophore and the organic solvent molecules. If that were the case, one would expect to observe a curve where S_F either continuously increases with increasing χ_{ACN} or reaches a plateau at a certain χ_{ACN} value. Instead, these results manifest the underlying microheterogeneity of the binary solvent system. This notion is further supported, perhaps even more evidently, by the $\lambda_{\max}$ versus χ_{ACN} plot (Fig 2), which clearly shows that the dependence of $\lambda_{\max}$ on χ_{ACN} can be divided into three χ_{ACN} regions. In the first region where $\chi_{ACN} < 0.25$, $\lambda_{\max}$ decreases with increasing χ_{ACN} ; in the second region where $0.25 < \chi_{ACN} < 0.75$, $\lambda_{\max}$ does not show any appreciable change; whereas in the third region where $\chi_{ACN} > 0.75$, $\lambda_{\max}$ once again decreases with increasing χ_{ACN} . This dependence is remarkably consistent with the study of Douhéret *et al.*,⁵⁴ which, based on measurements of various physical properties of the water-ACN binary mixture, including viscosity, density, partial molar volume of mixing, and dielectric constant, concluded that this binary solvent system can be classified into three regions, i.e., $\chi_{ACN} < 0.2$, $0.2 < \chi_{ACN} < 0.6$, and $\chi_{ACN} > 0.6$. Similar conclusions have also been reached by many other studies where different techniques, including infrared (IR) spectroscopy,^{26,55} terahertz spectroscopy,⁵⁶ mass spectrometry,³⁵ X-ray diffraction,²⁶ and MD simulations,⁴⁸ were used.

As shown (Fig. 1), the S_F versus χ_O plots obtained with water-DIO and water-THF binary mixtures are comparable. For both systems, the maximum S_F is approximately 10.2 times of S_{FW} and is reached at a similar χ_O value (i.e., $\chi_{DIO} \approx 0.3$ and $\chi_{THF} \approx 0.2$). The major difference between these two systems is that for the water-THF binary mixture, the S_F value undergoes a more significant decrease beyond the maximum, which presumably reflects the structural difference between THF and DIO. Interestingly, several previous studies^{57,58} that

employed various scattering methods to characterize the mixing properties of these binary mixtures have concluded that the microheterogeneity of these binary solvent systems reaches a maximum at $\chi_O \approx 0.3$, where organic clusters and water-organic clusters coexist, and beyond this point, organic clusters become dominant.⁵⁹ Hence, our results indicate that the fluorescence intensity of 7-CNI is sensitive enough to report on the formation of such solvent clusters.

Similar to that observed in the water-ACN binary solvent, the $\lambda_{\max}$ of 7-CNI in these binary mixtures also exhibits a rather complex dependence on the corresponding χ_O (Fig. 2). This result is qualitatively consistent with many previous studies⁵⁷⁻⁵⁹ showing that these cyclic ethers can form various clusters in water and hence render the binary mixture heterogeneous at the microscopic level (see below for further discussion).

For the DMSO-water binary mixture, our results show that the S_F ($\lambda_{\max}$) of 7-CNI increases (decreases) with increasing χ_{DMSO} in the region where χ_{DMSO} is less than ~ 0.3 (Figs. 1 and 2). This is in good agreement with previous observations that various solvent properties of this binary mixture, such as viscosity and excess mixing volume, exhibit a maximum in the χ_{DMSO} region of 0.25-0.35.⁶⁰ Additionally, our result is consistent with a previous dielectric relaxation study,⁴² which demonstrated that at $\chi_{\text{DMSO}} = 0.33$, maximum hydrogen bonding interactions occur between water and DMSO molecules.

Taken together, the results discussed above provide compelling evidence that the fluorescence of 7-CNI can serve as a sensitive fluorescence probe of local hydration environment and is able to reveal the intrinsic microheterogeneity of a binary mixture consisting of water and an organic solvent. To further verify this point, we also carried out similar measurements using 5-CNI, the fluorescence of which has been shown to be sensitive to hydration.¹ As shown (Fig. S4), the results obtained with 5-CNI are in agreement with those obtained with 7-CNI (Fig. 1). Therefore, these results validate the notion that both 7-CNI and 5-CNI are capable of probing the microheterogeneity of water-organic binary mixtures. However, in comparison to 7-CNI, the main disadvantage of 5-CNI is that in water its fluorescence lifetime is extremely short (< 200 ps), making it inconvenient to use in applications where the fluorescence lifetime needs to be determined.

Binary Mixtures Involving Alcohols

To show that 7-CNI can also be used to probe the microheterogeneity of binary mixtures consisting of water and a protic solvent, we extended our study by including five water miscible alcohols, MeOH, EtOH, IPA, TBA and EtG.

As indicated (Figs. 3 and 4, Table S1, ESI), the experimental data obtained with 7-CNI in these water-alcohol binary mixtures provides a clear indication that the fluorescence properties of this fluorophore (i.e., S_F and $\lambda_{\max}$) vary not only with the mole fraction of the alcohol but also with its identity. More specifically, they indicate that (1) the mole fraction of alcohol at which S_F reaches its maximum decreases upon increasing the size of the hydrophobic group in these mono-alcohols, i.e., in the order of MeOH $>$ EtOH $>$ IPA $\approx$ TBA, (2) the maximum value of S_F follows the same order, (3) the dependence of S_F on χ_{EtG} is dissimilar to those obtained with other water-alcohol binary systems and is much

less deviated from a straight line, suggesting that due to the additional –OH group in the alcohol, EtG and water can mix more thoroughly at the microscopic level, resulting in almost an ideal mixture, and (4) similar to that observed with binary mixtures composed of water and an aprotic solvent, the λ_{max} versus χ_{O} plots of these water-alcohol binary mixtures (Fig. 4), especially those containing EtOH, IPA, or TBA, indicate that the underlying microheterogeneity is more complex than that inferred from the corresponding fluorescence intensity plots. This finding also indicates that the energy of the fluorescent state of 7-CNI is more sensitive to subtle change in the solvation environment than its decay kinetics. This is expected as the electron-withdrawing cyano (CN) group in this case enhances the charge transfer from the pyrrole to benzene ring of indole upon photoexcitation, leading to a large increase in the dipole moment of the singlet excited state(s) of 7-CNI.

In addition, our results are consistent with previous studies on these water-alcohol binary systems.^{37,61–69} For example, for the water-MeOH binary system, our observation that S_{F} reaches a maximum at $\chi_{\text{MeOH}} \approx 0.7$ (Fig. 3, Table S1, ESI) is consistent with studies^{71,72} showing that MeOH-MeOH association is less common at lower χ_{MeOH} values. For the water-EtOH system, our results, especially the dependence of λ_{max} on χ_{O} (Fig. 4), are in agreement with previous studies^{62,64,73} demonstrating that the microheterogeneity of this binary mixture can be discussed in terms of four χ_{EtOH} regions and that beyond 0.2, EtOH clusters and EtOH-water clusters can coexist and interactions between EtOH molecules reach a maximum at $\chi_{\text{EtOH}} \approx 0.5$ (Table S1, ESI). Similarly, for the water-IPA and water-TBA mixtures, our results (Fig. 3 and 4, Table S1, ESI) are in line with various studies^{61,70,74} indicating that organic clusters can form at a rather low alcohol mole fraction (~ 0.2) and that these clusters begin to break up when the alcohol mole fraction is greater than ~ 0.7 .

Thus, taken together our results support the well-accepted notion that mixing water with certain alcohols can be a non-ideal process and alcohol clusters of different sizes and compositions can form in the corresponding binary mixtures. Moreover, it is worth noting that for all water-organic binary mixtures studied herein many solvent properties, such as polarity, dipolarity, acidity, and polarizability, are expected to change with χ_{O} . Therefore, it would be interesting to investigate in future studies the dependence of the fluorescence property of 7CNI on these parameters, at both the macroscopic and microscopic (i.e., cluster) levels.

Homogeneity within Heterogeneity

A qualitative picture emerging from our measurements and earlier studies is that for a water-organic solvent mixture, microheterogeneity can occur. Although the mole fraction at which the system becomes most heterogeneous (χ_{OM}) depends on the organic component, the entire concentration range can be divided into three to four regions, when discussing the physical behavior of any binary system. In the first or water-rich region, the organic molecules are mostly incorporated in the structural network of water. In the second region, organic-organic clusters and water-organic clusters begin to form and their populations reach a maximum at a certain χ_{O} value, where the system becomes most heterogeneous

microscopically. In the third region, water solvated organic clusters become predominant. In the fourth or organic-rich region, organic-clusters gradually disappear and small water aggregates can form. In an attempt to further characterize the distribution of these clusters, we carried out fluorescence lifetime measurements.

Recently, Hilaire *et al.* showed that 7-CNI affords single-exponential fluorescence decay kinetics in various solvents with a lifetime (τ_F) that is sensitively dependent on the solvent, for example, $\tau_F = 2.0$ ns in water, $\tau_F = 8.2$ ns in MeOH, and $\tau_F = 14.5$ ns in DMSO.⁴ Because of this sensitivity, we believe that measurement of the fluorescence lifetime of 7-CNI in a water-organic binary mixture would help elucidate the nature of its microheterogeneity. Our working hypothesis is that in the presence of an ensemble of solvent clusters of different sizes and compositions, which should provide a distribution of solvation environments for the fluorophore, the fluorescence decay kinetics of 7-CNI will be characterized by a distribution of lifetimes. However, contrary to our expectation, in all of the binary mixtures studied and at any χ_O value, even under condition where maximum microheterogeneity is supposed to occur, the fluorescence decay kinetics of 7-CNI could be well described by a single-exponential function (Figs. S5 and S6, Table S2, ESI). Furthermore, we found that for a given water-organic binary mixture the τ_F of 7-CNI shows a similar dependence on χ_O as its S_F (Fig. 5, Table S2, ESI), which suggests that τ_F is also sensitive to the underlying microscopic phase separation. Despite this similarity, an interesting question to ask is why the fluorescence lifetime measurements did not reveal the existence of different solvent clusters. There are three possible interpretations. The first one is that the changes in τ_F are caused by preferential interactions between 7-CNI and the organic solvent molecules and, as a result, this quantity does not capture the intrinsic heterogeneity of the system. As discussed above, this possibility can be largely ruled out as none of the measured fluorescence properties (i.e., S_F , λ_{max} , and τ_F) of 7-CNI exhibits a dependence on χ_O that supports this argument. The second one is that at any given solvent condition, there is only one dominant cluster formed in the binary mixture. This scenario, however, disagrees with results obtained from previous mass spectrometric studies.^{35,36,62} The last and most probable interpretation is that the solvent clusters in the system undergo dynamic exchanges or re-configurations on an ultrafast timescale (i.e., faster than 2 ns – the fluorescence lifetime of 7-CNI in water), making the fluorophore ‘see’ only an average environment at any given solvent composition. In other words, from a solute’s perspective, these water-organic binary mixtures appear homogenous even though they are intrinsically heterogeneous. Finally, given the prevalent use of fluorescence decay measurement in assessing local solvation or environmental heterogeneity, it is worth noting, based on our current finding, that the observation of a single-exponential fluorescence decay kinetics is insufficient to conclude that the system in question is homogenous.

Interestingly, in every case, the fluorescence decay kinetics of 7-CNI is the slowest at or near the χ_{OM} value (Table S2, ESI). For example, in the binary mixture composed of water and THF the τ_F of 7-CNI is 13.3 ns at $\chi_{THF} = 0.25$, comparing to 6.9 ns obtained in pure THF. This is an important result as it indicates that even though microscopic phase separations can occur in these water-organic binary solvent systems, the resultant solvent clusters afford properties that are different from those of the corresponding pure organic phase. In other words, both water and organic molecules must be integral parts of these clusters and they

work together synergistically to create a dynamic and yet distinct solvent environment (at χ_{OM}) that supports a prolonged fluorescence decay kinetics for 7-CNI. Based on this notion, we further hypothesized that for all the water-organic binary mixtures studied herein, the property of this unique environment, as manifested by the fluorescence lifetime of 7-CNI, can be correlated with a suitable solvent parameter of the organic component. While for a pure organic solvent there are many empirical parameters that could be used to characterize its effect on the fluorescence property of a solute, the one we chose for this purpose is the peak wavenumber (in cm^{-1}) of the 7-CNI fluorescence spectrum in this solvent (not the corresponding water mixture). This is because this quantity (hereafter referred to as ω_O) is an effective measure of the unique solvation environment provided by the organic solvent of interest for 7-CNI. It is worth noting that for a given water-organic binary system the ω_O value is different from that corresponding to λ_{max} (Table S1). As indicated (Fig. 6), for all the binary systems studied, except water-EtG, which exhibits more or less ideal behavior, and water-DMSO, the τ_F measured at χ_{OM} indeed exhibits a linear correlation with the corresponding ω_O . This correlation can even extend further to include the data point obtained in pure water, which, as discussed above, suggests that water molecules play an important role in defining the property of the solvent clusters formed at χ_{OM} . In addition, and perhaps more importantly, this correlation suggests that the structures and dynamics of such clusters formed in different water-organic binary mixtures share similar features. Furthermore, this correlation indicates that the microheterogeneous environment of clusters formed by organic molecules with a lower dielectric constant (e.g., THF and DIO) exhibits a more significant deviation from the environment in the pure organic solvent. Finally, as shown (Fig. S7, ESI), the corresponding fluorescence intensity obtained at χ_{OM} (i.e. $S_{F\text{max}}$ in Table S1, ESI) also exhibits a similar, linear dependence on ω_O , indicating that the radiative rate constant of 7-CNI does not significantly depend on solvent conditions.

CONCLUSIONS

Aiming to show that the fluorophore of the unnatural amino acid 7-cyanotryptophan, 7-cyanoindole (7-CNI), is a useful fluorescence hydration probe, we measured how its fluorescence properties, including intensity (S_F), peak wavelength (λ_{max}), and lifetime (τ_F), change as a function of the organic mole fraction (χ_O) in a series of water-organic binary mixtures, where the organic solvent is either dimethyl sulfoxide, tetrahydrofuran, 1,4-dioxane, acetonitrile, methanol, ethanol, isopropanol, tert-butanol or ethylene glycol. We found that in each case – (1) the dependence of S_F on χ_O provides a simple and convenient means to determine the mole fraction (χ_{OM}) at which maximum microheterogeneity occurs, (2) the dependence of λ_{max} on χ_O provides a more comprehensive description about the heterogeneous status of the system, and (3) the dependence of τ_F on χ_O shows that the fluorescence decay kinetics of 7-CNI can be much longer at χ_{OM} than in the corresponding organic solvent, indicating that the organic clusters formed in the binary mixture can provide a very different solvation environment for the solute than the pure solvent. Moreover, we found that under all solvent conditions studied, the fluorescence decay kinetics of 7-CNI can be well fit to a single-exponential function, suggesting that the solute sees a homogeneous environment on the timescale of a few nanoseconds. Finally, we found that a linear correlation exists between τ_F measured at χ_{OM} and an empirical solvation parameter of the

organic solvent. This suggests that the organic clusters formed in different water-organic binary mixtures provide a similar solvation environment for 7-CNI that can be fine-tuned by the nature of the organic solvent. Taken together, these results not only confirm the proposed utility of 7-CNI as a sensitive fluorescence hydration probe but also provide new insights into the microheterogeneity of water-organic binary mixtures. Given the fact the stretching vibration of a cyano group on the indole ring can be used as an infrared probe of local environment,⁷⁵ we expect that 7-CNI will find wide applications.⁷⁶

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlight

The fluorescence intensity, lifetime and spectrum of 7-cyanoindole sensitively depend on the amount of water in a series of binary solvent systems, indicating its utility as a hydration probe.

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Figure 1.

Normalized fluorescence intensity (S_F) of 7-CNI as a function of χ_O in different water-organic binary mixtures, as indicated. In each case, the smooth line serves to guide the eyes. Also shown in the DMSO panel is the chemical structure of 7-CNI.

Figure 2. Peak emission wavelength (λ_{max}) of 7-CNI as a function of χ_O in different water-organic binary mixtures, as indicated. In each case, the smooth line serves to guide the eyes.

Figure 3. Normalized fluorescence intensity (S_F) of 7-CNI as a function of χ_O in different water-alcohol binary mixtures, as indicated. In each case, the smooth line serves to guide the eyes.

Figure 4. Peak emission wavelength (λ_{max}) of 7-CNI as a function of χ_O in different water-alcohol binary mixtures, as indicated. In each case, the smooth line serves to guide the eyes.

Figure 5. Dependence of fluorescence lifetime (τ_F) of 7-CNI on χ_O for different water-organic binary mixtures, as indicated.

Figure 6.
Fluorescence lifetime of 7-CNI measured at χ_{OM} as a function of ω_0 .