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A Spectroscopic Study of a Cyclodextrin-Based Polymer and the “*Molecular Accordion*” Effect

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Abstract

The formation of host-guest complexes was studied for two hosts; β -cyclodextrin (β -CD) and a cross-linked polymer containing an equimolar ratio of β -CD and hexamethylene diisocyanate (HDI), denoted as HDI-1. The thermodynamics of host-guest binding was studied with 1-anilinonaphthalene-8-sulfonic acid (1,8-ANS) using steady-state fluorescence spectroscopy in aqueous solution at variable temperature and ambient pH. The association of 1,8-ANS with β -CD and HDI-1, showed a fluorescence enhancement of ~ 4 and 12 units, respectively. Greater fluorescence enhancement for the polymer/dye system indicates the presence of multiple binding sites (inclusion vs. interstitial). By contrast, the β -CD/dye system adopt trends that indicate the formation of well-defined inclusion complexes. HDI-1 has inclusion sites (β -CD) and interstitial domains (HDI) that afford dual binding with variable binding affinity. Simplified binding models employed herein address the role of inclusion binding without an explicit account for higher order or secondary binding equilibria. The approximate 1:1 binding constant ($K_{1:1}$) for CD/1,8-ANS is about two-fold greater over the HDI-1/1,8-ANS system. HDI-1 displays cooperative effects among the polymer subunits, according to changes in relative fluorescence intensity due to structural transitions and binding site loci. The relative fluorescence intensities of the HDI-1/1,8-ANS system relate to a reversible temperature-driven structural transition (globular $\rightleftharpoons$ extended) between 5 °C and 60 °C of the polymer, in contrast to the β -CD/1,8-ANS complex. The temperature- and guest-driven structural transition, described as the “molecular accordion” effect is supported by new insight provided by complementary fluorescence and ¹H NMR spectral results in aqueous solution.

Keywords: Fluorescence; Cyclodextrin; Host-Guest; Complex formation; Responsive polymers

1. INTRODUCTION

There is a growing interest in the design, characterization and manipulation of “smart” polymer materials for diverse applications ranging from biomedicine, food processing, and environmental remediation.¹ Several classes of stimuli-responsive polymers have been reported for native polysaccharide biopolymers.² More recently, synthetically modified polymers with stimuli-responsive properties have been widely explored that broaden the scope of application for smart functional materials.^{3,4} Stimuli-responsive materials undergo changes in their physicochemical properties (e.g., conformation, color, solubility, and conductivity) in response to external stimuli (e.g., variation in temperature, ionic strength, pH, electric and magnetic fields, or light).^{2,5,6} In the presence of external stimuli, the physicochemical properties of responsive materials can be modified due to the formation and/or breakage of non-covalent interactions (e.g. hydrogen bonding, hydrophobic effects, and electrostatic interactions) or changes in osmotic pressure.⁶ Supramolecular host materials that contain cyclodextrins (CDs) possess versatile structure and function,⁷ where the most common CDs have 6 (α -CD), 7 (β -CD) or 8 (γ -CD) α -D glucopyranose units linked by α -1,4-glycosidic bonds.⁸ CD-based polymers can be prepared with tunable composition, structure, and responsive properties. Potential applications for such CD-based materials include chemical separation processes and water treatment,^{9,10} enhanced oil recovery,^{11–13} catalysis,¹⁴ and biomedical devices.^{5,15}

A CD-based polymer with a linear morphology, denoted as HDI-1, was recently reported to display thermo- and chemo-responsive properties.¹⁶ The HDI-1 polymer was prepared by cross-linking equimolar ratios of β -CD and hexamethylene diisocyanate (HDI), as illustrated in Scheme 1.^{10,17} The molecular structure and dye adsorption properties of HDI-1 was investigated by thermoanalytical and spectroscopic techniques to reveal a responsive structural transition (*globular-to-extended*) of HDI-1 upon heating or guest binding. The responsive structural change is likened to that of a “molecular accordion”,¹⁶ where potential applications of this system include fields such as the environment, biomedical devices, and chemical sensors. However, a key challenge for the study of such materials relates to the availability of suitable techniques to characterize their structure and functional properties. In cases where sufficient levels of host and guest are present in solution, high resolution NMR spectroscopy is a versatile structural tool for the study of host-guest systems, especially those with measurable host/guest dipolar interactions.¹⁸ DSC and TGA methods can be used to follow thermal events of polymer materials with thermo-responsive properties,¹⁶ along with other complementary methods to gain molecular level insight.^{16,19} The use of fluorescent dye probes has found widespread use for the structural characterization of supramolecular and functional polymer materials due to their spectral sensitivity to changes in the microenvironment.^{20,21} Fluorescent probes, such as 1-anilinonaphthalene-8-sulfonic acid (1,8-ANS) and pyrene, have been used to study self-assembly and macromolecular binding phenomena of proteins,^{22–25}

micelles,²⁶ and host-guest inclusion complexes.^{27–30} For example, the fluorescence emission of 1,8-ANS was enhanced when bound within the apolar cavity of CDs relative to its unbound state in bulk aqueous solution.³¹ Similarly, supramolecular systems that undergo temperature-, pH- or chemical-induced polarity microenvironment changes as a result of association or aggregation phenomena are amenable to study by fluorescence spectroscopy. Stimuli-induced conformational changes of proteins in the presence of various fluorescent probes are well documented.^{19,32,33} Niskanen and coworkers³⁴ reported on the conformational responsiveness of hybrid materials such as poly-(dimethylaminoethyl methacrylate) (PDMAEMA)-grafted clay to temperature and pH effects. By contrast, related studies for CD-based polymers are sparsely reported despite their unique ability to form host-guest complexes and to undergo self-assembly. It is envisaged that fluorophores can be used to probe multiple binding sites (inclusion and non-inclusion), in conjunction with NMR spectroscopy to provide insight on the structure-function properties of supramolecular polymer-CD (poly-CD) hosts such as HDI-1.²⁹

Herein, we report the use of fluorescence spectroscopy to study two types of host systems (β -CD and a poly-CD; hereafter referred to as HDI-1) with a fluorophore guest probe (1,8-ANS). The host-guest complexes of β -CD/1,8-ANS and HDI-1/1,8-ANS systems were characterized using 1-D/2-D ^1H NMR spectroscopy in aqueous solution. It will be shown that the spectral shifts and fluorescence enhancement of 1,8-ANS in the absence and presence of incremental amounts of host (β -CD or HDI-1) provide further details regarding the structure and binding properties of such complexes. Further studies on the structural transition of the HDI-1 polymer were probed at variable temperature (VT) using fluorescence spectroscopy. The spectral results provide support that the *globular-to-extended* structural transition of HDI-1 occurs as a function of temperature and guest concentration. The use of complementary NMR and fluorescence spectroscopic methods provide insight on the responsive properties of the “molecular accordion” effect reported herein. This study will likely catalyze further research in the field of responsive polymer materials with supramolecular functional properties as chemical sensors or “catch-and-release” carrier systems for petrochemicals, pharmaceuticals, and environmental contaminants.

2. EXPERIMENTAL SECTION

2.1. Chemicals and Materials

β -cyclodextrin (β -CD) was purchased from VWR Canada Ltd. Dimethyl acetamide (DMA), hexamethylene diisocyanate (HDI), and 1-anilinonaphthalene-8-sulfonate (1,8-ANS) were purchased from Sigma Aldrich Canada. Deuterium oxide (D_2O) was purchased from Cambridge Isotope Laboratories. The DMA was dried over 4 Å (8–12 mesh; Sigma Aldrich, Canada) molecular sieves and

its water content was estimated (ca. 0.5 %) by ^1H NMR spectroscopy. All other materials were used as received unless specified otherwise.

2.2. Methods

2.2.1. Synthesis of the β -CD (HDI-1) polymer. The synthesis of a cross-linked polymer containing β -CD was adapted from a previous report.¹⁷ In brief, the β -CD polymer (*cf.* Scheme 1) was prepared by adding 1 mmol of dried β -CD to a round-bottom flask with stirring until dissolved in 10 mL of DMA, followed by addition of 1 mmol of HDI in 30 mL of DMA to the reaction mixture. The mixture was stirred at 68 ± 2 °C for 24 h under argon gas, where the reaction was finally cooled to 23 °C upon completion. The excess DMA was removed under vacuum (~ 1 mbar), and the subsequent addition of cold methanol (~ 0 °C) to the gelled product was followed by filtration through Whatman no. 2 filter paper. The crude product was washed with methanol in a Soxhlet extractor for 24 h to remove unreacted reagents and low molecular weight oligomers. The product was dried in a pistol dryer for 24 h, ground into a powder, and passed through a size 40 mesh sieve to ensure uniform particle size. A second cycle of washing in the Soxhlet extractor with anhydrous diethyl ether for 24 h was followed by drying, grinding, and sieving, as outlined above. The product (HDI-1) was characterized using solution ^1H NMR (Figure S1 and S2-B) in the Supporting Information (SI), where the detailed structural characterization is described elsewhere.¹⁷ Some physicochemical properties of HDI-1 (solubility, surface area, etc.) are summarized in Table S1 in the SI. The HDI-1 acronym and number index designation denotes the equimolar feed ratio of reactants (β -CD: HDI cross-linker) used in the synthesis (*cf.* Scheme 1).

2.2.2. Characterization of the complexes of β -CD and HDI-1 with 1,8-ANS. The fluorescence spectra of 1,8-ANS (guest) in the absence and presence of hosts (β -CD or HDI-1) were obtained on a PTI fluorimeter with excitation and emission monochromator bypass set at 3 nm with an excitation wavelength of 340 nm in 1 cm² quartz fluorescence cells. Host-guest (β -CD/1,8-ANS and HDI-1/1,8-ANS) systems were prepared in aqueous solution at ambient pH and temperature at (i) fixed guest (0.1 mM) and variable host (0.1 – 2 mM) concentrations, and (ii) fixed host (0.1 mM) and variable guest (0.1 – 0.6 mM) concentrations. Spectra were obtained at variable temperature (VT) for the 1:1 host/guest complexes from 5 °C – 60 °C. The results were plotted as intensity or relative intensity on the ordinate against wavelength or temperature on the abscissa. Fluorescence emission enhancement (F/F_0) was determined as the ratio of integrated intensity values of the corrected fluorescence spectrum of 1,8-ANS in the presence (F) and absence (F_0) of the host.

The ^1H NMR spectra were acquired on a 3-channel Bruker Avance spectrometer operating at a ^1H resonance frequency of 500 MHz at ambient conditions. NMR samples were prepared at the 1:1, 1:3 and 1:5 host:guest mole ratios, where the mole ratio of HDI-1 denotes the β -CD content of HDI-1 relative to the guest. All ^1H NMR spectra were referenced externally to tetramethylsilane (TMS, δ 0.0 ppm) with a 2 s recycle delay and a $10\ \mu\text{s}$ 90° pulse length. For all 2-D rotating-frame Overhauser effect spectroscopy (ROESY), the spin-lock times and levels were set at 350 ms and 21.33 dB, respectively. All the spectra were acquired using a spectral width of 12 ppm in 2k data points with 8 scans (2-D gROESY) or 32k data points with 16 scans (1-D NMR).

2.2.3. Stoichiometry and Binding Studies of 1,8-ANS Complexes. The stoichiometry of the 1,8-ANS complexes with β -CD and the HDI-1 polymer was evaluated by using the continuous variation (Job's plot) method.³⁵ The host-guest stoichiometry was established by measuring the fluorescence of various host/guest mole ratios where the total concentration of each solution mixture was maintained to a constant value. The solutions for the continuous variation experiment were prepared by adding known volumes (between 1 – 9 mL) of 1 mM solutions each of 1,8-ANS and the respective host (β -CD or HDI-1) in a vial to make a total fixed volume and concentration of 10 mL and 1.0×10^{-5} M, respectively. The stoichiometry was estimated by plotting the relative fluorescence intensities (F/F_0) against the mole fraction of 1,8-ANS (X_{ANS}).³⁶

The binding constants were estimated from an analysis of the fluorescence intensity results, where the fluorescence emission enhancement (F/F_0) results were plotted as a function of incremental host concentration, as given in eq. 1.^{28,30,37}

$$\frac{F}{F_0} = 1 + \left(\frac{F_\infty}{F_0} - 1\right) \frac{[\text{Host}]_0}{1 + [\text{Host}]_0 K_{1:1}} \quad (1)$$

In eq. 1, F and F_0 are the integrated fluorescence intensity values in the presence and absence of host. F_∞ is the integrated fluorescence intensity when 100% of ANS is complexed by the host and the fluorescence data was fit using eq.1 with F_∞/F_0 and K as the fit parameters.

3. Results and Discussion

3.1. NMR characterization of β -CD/Poly-CD Association with 1,8-ANS

While the characterization of the host-guest (β -CD/1,8-ANS) complexes have been reported by use of ^1H NMR spectroscopy in solution,²⁹ a comparison of spectral results for native and modified forms of β -CD are warranted to provide insight on the formation of complexes for poly-CDs such as HDI-1. NMR

spectral techniques are known to provide valuable structural information about host-guest systems.²⁹ The ¹H NMR spectral numbering assignment of 1,8-ANS and its various complexes with β-CD and HDI-1 are shown in Figure S2A-B (*cf.* SI). The complexation-induced chemical shift (CIS) values for the β-CD intra-cavity protons (H3 and H5) are summarized in Table 1, where the assigned spectral signatures for the host and guest nuclei were in agreement with previous assignments.^{18,38,39} The comparison of the CIS values in Table 1 for the H3 and H5 nuclei of β-CD/1,8-ANS and HDI-1/1,8-ANS (*cf.* Figure S2) systems reveal minor changes in the chemical shifts at these conditions. These minor changes in CIS values may provide insight on variable binding modes of the guest with β-CD and HDI-1, respectively. It is noteworthy that the relative accessibility of the β-CD inclusion sites do not vary appreciably between native β-CD and HDI-1 since steric effects at the macrocycle periphery for the polymer is negligible (*cf.* Table S1). The inclusion site accessibility is known to decrease for polymers with elevated levels of cross-linking, however; the accessibility of the β-CD inclusion sites of HDI-1 are similar when compared with native β-CD (ca. 100% accessible; Table S1) according to an indicator-based displacement assay.⁴⁰ The CIS values for HDI-1 herein are expected to vary appreciably at temperatures well below and above ambient conditions due to temperature-induced structural transition, in line with thermo-responsive properties of this polymer.¹⁶ The relatively constant CIS values (Table 1) at host/guest mole ratios above 1:3 indicates that 1,8-ANS forms 1:1 and/or 2:1 complexes with β-CD and HDI-1, respectively.

2-D ROESY NMR spectroscopy affords the measurement of homonuclear NOE effects under spin-lock conditions, where the observed cross-peaks provide evidence of *through-space* interactions between two or more nuclei via dipolar interactions within 5 Å separation.⁴¹ Figure 1 shows the 2-D ROESY results for the inclusion complexes of 1,8-ANS with β-CD (a) and HDI-1 polymer (b) at the 1:1 host/guest mole ratio for each system. Figure 1a reveals well-defined dipolar interactions between the H5 (~3.75 ppm) cavity nuclei of β-CD at the narrow rim with the phenyl moiety (H_{3',5'} and H_{2',6'}, ~7.1 – 7.2 ppm) of the fluorophore (*cf.* Figure 1a). Similar dipolar interactions are observed with the external H6 proton species at the primary annulus of β-CD. Weaker dipolar interactions are evident between the naphthyl protons (H_{3,6}, H₇ ~7.5 ppm) and the cavity nuclei of β-CD (H3 ~3.85 ppm). By contrast, there are no interactions (cross-peaks) observed between these protons and the H5 cavity nuclei of β-CD. The foregoing suggests a binding mode where the phenyl ring of 1,8-ANS is partially included within the β-CD cavity site via the wider annular face that extends into the narrow annular region of the macrocycle. The naphthyl moiety interacts peripherally at the extracavity region near the wider annulus of β-CD. The binding mode of 1,8-ANS with β-CD is depicted in Scheme 2a. The type of host-guest binding mode for β-CD in Scheme 2a is consistent with results reported by Nishijo et al.⁴² where they proposed that the phenyl ring sits within the cavity interior, while much of the naphthyl moiety resides peripheral to the β-CD inclusion site. In the case of the HDI-1/ANS complex (Figure 1b), a different binding geometry is

observed due to differences in the cross-peaks at the $H_{2,6'}$ sites of 1,8-ANS. The loss of interactions for these signals at the H5 nuclei of the β -CD cavity sites suggests a binding mode where the phenyl ring is less deeply included and resides closer to the wider annular hydroxyl region of β -CD. This geometry is understood in terms of the steric hindrance that may occur as a result of the presence of the HDI amide linker sites of the polymer that are located at the primary hydroxyl groups of β -CD annulus (*cf.* Scheme 2b). It was shown in a previous report that the hexamethylene units of the HDI linker were partially self-included within the β -CD cavity sites.¹⁶ In the same report, additional interactions between the guest and the interstitial domains of the HDI-1 polymer were reported for *p*-nitrophenol (PNP), where differences were noted relative to native β -CD due to the cross-linker domains of HDI-1. The differences in the 1:1 binding mode of 1,8-ANS at the narrow annular rim (H5) of β -CD with the two host systems (β -CD vs. HDI-1) is revealed by the variable CIS values for H5 nuclei listed in Table 1, albeit rather small changes. It is worthwhile noting that the chemical shift changes relate to contributions from bound and free host or guest species. In a previous report,¹⁶ the binding geometry of PNP with native β -CD resembled that observed for the HDI-1/PNP system. However, 1,8-ANS and PNP differ in their relative molecular sizes, Lewis base character and polarizability properties, as evidenced by the differing trends in the CIS values. The bulky naphthyl moiety of the 1,8-ANS guest is positioned distal to the wider annular region of the β -CD sites of the HDI-1 polymer host due to conformational and steric effects shown in Scheme 2b. The structural characterization of the host-guest complexes for 1,8-ANS with the host (β -CD or HDI-1) systems were further studied by fluorescence spectroscopy due to its high sensitivity to changes in the microenvironment, as revealed in previous studies of macromolecular systems.^{20,21,43}

3.2. Fluorescence studies of the 1,8-ANS complexes with β -CD and HDI-1

3.2.1. Binding Studies

The host/guest binding studies of β -CD/HDI-1 with 1,8-ANS afford further understanding of the role of dual binding sites of the HDI-1 polymer. In particular, an evaluation of the host/guest stoichiometry is important for estimation of the apparent host-guest binding constants for 1,8-ANS with β -CD and HDI-1, respectively. The stoichiometry was determined by the continuous variation³⁵ method described in the experimental section. The results in Figure 2 reveal a 1:1 stoichiometry for the β -CD/1,8-ANS system as shown by the maxima (dashed line) at $X = 0.5$ (Figure 2a). β -CD is commonly known to form 1:1 complexes with a wide range of naphthalene-based fluorophores, including 1,8- and 2,6-ANS.^{28,29,44,45} In the case of the HDI-1/1,8-ANS system, the continuous variation plot (Figure 2b) shows the absence of a defined maximum at $X = 0.5$. It follows that other contributions due to 2:1 complexes or factors assigned to the dual binding sites of HDI-1 contribute to secondary host-guest equilibria (*cf.* Scheme 2). It is noteworthy that binding contributions related to the cross-linker sites of the polymer may account for the

non-ideal behaviour (dashed lines) shown in Figure 2b. Harada et al.³¹ have shown that β -CD forms a 1:1 complex with 1,8-ANS; whereas, the modified poly(acryloyl- β -CD) host solely forms 2:1 complexes. The formation of other complexes at the interstitial sites of HDI-1 with 1,8-ANS provide an account for the deviations of maximum emission intensity away from $X = 0.5$ (dashed lines) observed in the Job's plot (Fig. 2b). As well, the role of multiple binding sites on the fluorescence emission intensity for HDI-1 is understood based on the occurrence of variable polymer self-assembly (globular and extended forms), in line with anticipated hydrophobic effects on the local microenvironment of 1,8-ANS due to the "molecular accordion" effect.^{16,46}

Estimates of the 1:1 binding constant ($K_{1:1}$) values were obtained by analyzing the host concentration dependence of the fluorescence emission intensity of 1,8-ANS in Figure 3. The value for β -CD/1,8-ANS (583 M^{-1}) exceeds that for HDI-1/1,8-ANS (302 M^{-1}), where the former value is greater compared to estimates reported in the literature for native and modified β -CD hosts.²⁸ The greater values of binding constants in this study are mainly related to the use of simplified models that assume a 1:1 host-guest stoichiometry. As well, the experimental conditions herein may not fully satisfy the assumption of excess host ($[\text{Host}]_0 \gg [\text{Fluorophore}]$) using the applied fitting model (eq. 1) and the Benesi-Hildebrand double-reciprocal method.^{47,48} It follows that the data obtained by the double-reciprocal method is considered as approximate given the assumptions involved. However, a relative comparison of the apparent values of $K_{1:1}$ for complexes formed between 1,8-ANS with each host (native β -CD and HDI-1) provides insight on the role of higher order equilibria (2:1) or contributions due to multiple binding sites (inclusion and interstitial). The two-fold attenuation of $K_{1:1}$ for the HDI-1/1,8-ANS relates to the key role of secondary binding sites (HDI interstitial domains). The equimolar abundance of dual sites of HDI-1 (interstitial and inclusion) binding sites along with anticipated differences in binding affinity at each site (interstitial vs. inclusion) for HDI-1 provide account for the difference in the "apparent" $K_{1:1}$ relative to the β -CD/1,8-ANS system due to competitive binding effects. A parallel argument applies for the overall lowering of the "apparent" value of $K_{1:1}$ of the β -CD/1,8-ANS system due to the formation of 2:1 host-guest complexes.^{49,50} It is noteworthy that a greater enhancement of the fluorescence intensity does not necessarily translate to greater $K_{1:1}$ values as reported for γ -CD with smaller intensity but greater $K_{1:1}$ compared to a modified CD.²⁸ Furthermore, the role of solvent effects on the fluorescence emission properties of 1,8-ANS are anticipated for HDI-1 due to the "molecular accordion" effect described herein. Thus, the formation of other complexes aside from the 1:1 β -CD/guest inclusion complexes are not explicitly accounted for in the overall mass-balance equation that result in an "apparent" lowering of the $K_{1:1}$ value.⁵¹ Moreover, the binding mode of the guest is expected to vary within the internal cavity sites of native β -CD over that of poly-CD that contains dual binding sites, as described in section 3.1 that outlines the ^1H NMR spectral results for these host-guest systems.

3.2.2. Guest-Dependence Conformations of HDI-1

The use of 1,8-ANS as a fluorescent probe provides further insight on the host/guest binding and dynamic processes that occur upon formation of non-covalent complexes via fluorescence spectroscopy.⁵² The sensitivity of 1,8-ANS to changes in its microenvironment are reflected in the fluorescence spectral titrations with incremental amounts of host (β -CD or HDI-1) as shown in Figure 4a-b. The utility of 1,8-ANS as a sensitive probe to microenvironment effects has been reported in studies of host-guest interactions and self-assembly of CD-based host systems.⁴⁴ In particular, modified CDs may possess more apolar microenvironments with greater fluorescence enhancement as noted for grafted²⁷ and cross-linked CD systems.³¹ In Figure 4, the fluorescence intensity of 1,8-ANS in its bound state increases monotonically with increasing host concentration (β -CD or HDI-1). The greater enhancement of the fluorescence intensity in the presence of HDI-1 (~12 times; Figure 4b) exceeds that for β -CD (~4 times; Figure 4a). The enhancement effect noted for HDI-1 is ascribed to its polymer structure that affords additional apolar sites (HDI linker domains) that may vary according to the conformational preference (globular vs. extended) of HDI-1. Similarly, the greater enhancement of fluorescence emission occurs at the highest mole ratio of the host molecule because the abundance of such apolar binding sites at these conditions is greater due to the “molecular accordion” effect and adsorption processes in line with 2-D lattice adsorption models.⁵³ It is noteworthy that the emission spectrum of 1,8-ANS at λ_{max} 559 nm is accompanied by a significant shift to shorter wavelengths ~497 nm (blue shift) in the presence of HDI-1 (*cf.* Figure 4b). By contrast, the β -CD/1,8-ANS host-guest system shows a reduced blue spectral shift (559 to 524 nm). Wagner and Fritzpatrick⁴⁴ reported that usage of hydroxypropyl (HP)-substituted β -CD led to larger fluorescence enhancements upon complex formation with 1,8-ANS due to the enhanced apolar nature of HP- β -CD over unmodified β -CD. A greater fluorescence enhancement and significant blue shift effects were noted for a poly-(acryloyl- β -CD)/potassium-2-*p*-toluidinylnaphthalene-6-sulfonate (TNS) system reported by Harada et al.³¹ The observed fluorescence enhancement for TNS was related to the cooperative binding among adjacent β -CD moieties in the pendant polymer chain. In the case of HDI-1 reported herein, the large enhancement and blue shifts in Figure 4b are inferred due to an increased interaction of the fluorophore as a result of cooperative interactions and self-assembly between adjacent β -CD moieties and/or secondary interactions with the HDI cross-linker units (*cf.* Schemes 1 and 2). This trend coincides with the shift of the emission spectrum of 1,8-ANS to shorter wavelengths upon its complexation.⁵² Previous results indicate that HDI-1 undergoes a *globular-to-extended* conformational change as the guest concentration and temperature varies,¹⁶ where such conformational changes result in substantial solvation reorganization of the microenvironment of the fluorophore, in line with the “molecular accordion” effect.

The guest interactions with respective host systems (β -CD and HDI-1) can be explored with incremental amounts of 1,8-ANS to probe the active binding sites and structure of each macromolecular host system. Figure 5 reveals the interaction of incremental amounts of 1,8-ANS at fixed concentrations of β -CD (a) and HDI-1 (b). Similar to the results in Figure 4, greater fluorescence emission of 1,8-ANS occur when there is maximal contact with the host, especially at the 1:1 host/guest mole ratios in this study. Generally, the HDI-1/1,8-ANS system is characterized by a large fluorescence enhancement upon going from the 1:6 (**1**) to the 1:1 (**7**) host-guest mole ratios (*cf.* Figure 5a,b). However, a significant jump is observed from the 1:2 (**6**) to the 1:1 (**7**) host/guest mole ratios for the 1,8-ANS/HDI-1 system in Figure 5b, unlike the β -CD/ANS complex in Figure 5a. The foregoing observations are consistent with the presence of guest-induced conformational effects and possible formation of higher order complexes, as described for the NMR spectral results and the continuous variation studies. Single-molecule fluorescence studies^{23,24,54} were used to provide valuable insights on the structural transitions of proteins between the extended and helical structures using fluorescent probes. In the foregoing results, the sudden decrease in intensity at the 1:2 versus the 1:1 host-guest systems is inferred due to a chemical (guest)-induced conformational change of HDI-1. At the 1:2 host:guest ratio, a conformation where the polymer exists in an extended (uncoiled) form is anticipated with reduced fluorescence intensity relative to a globular (coiled) conformational state. In the latter case, maximum contact of HDI-1 with 1,8-ANS is expected because of an optimal *size-fit* association between the host and guest. It is understood that the uncoiled form of HDI-1 may result in a microenvironment that is more polar due to exposure of the polymer to the bulk aqueous environment versus a globular or aggregated form.

3.2.3. Temperature-Dependence Conformations of HDI-1

To this end, fluorescence studies at variable temperature (VT) were necessary in order to further investigate the *globular-to-extended* conformation of HDI-1 to provide additional support for the structural transition of such responsive materials.³⁴ The 1:1 HDI-1/1,8-ANS complex provides a unique system to investigate the role of folding and unfolding of HDI-1 at VT due to its thermo-responsive nature.¹⁶ The folded conformation of HDI-1 at the 1:1 host-guest (HDI-1/1,8-ANS) mole ratio is anticipated to result in maximal intermolecular contacts, as shown by the greater fluorescence enhancement of 1,8-ANS. Figure 6 shows the trend in fluorescence emission at VT conditions, where the integrated emission intensity for the 1:1 host-guest (HDI-1/1,8-ANS) system are plotted versus temperature (5 – 60 °C), where the results for the corresponding β -CD/1,8-ANS system are shown for comparison. In Figure 6, 1,8-ANS shows no observable change in fluorescence emission versus temperature in the presence of β -CD, indicative of the complex stability over this VT range. However, in the presence of HDI-1, observable intensity enhancements are noted on going from higher (60 °C) to

lower (5 °C) temperature. This phenomenon is reversible as evidenced by a decrease in spectral intensity upon reversing the temperature cycle. Parallel types of fluorescence studies using 1,8-ANS were carried out by Kudou et al.⁵⁵ and Massey et al.⁵⁶ to investigate the thermoresponsive structure of protein residues. In these reports, the reversible VT changes in the fluorescence intensity of the fluorophore were attributed to conformational changes. In another study, Niskanen et al.³⁴ observed a reduction in fluorescence emission intensity for solutions of 1,8-ANS and PDMAEMA as a function of increasing temperature. In turn, Niskanen et al.³⁴ suggest that the probe may be squeezed out of the polymer binding sites to account for the decreased fluorescence intensity upon exposure to a more polar microenvironment. Herein, the dissociation of the HDI-1/1,8-ANS complex is unlikely over the VT range and ambient pH conditions, as supported by the nearly constant fluorescence emission for the β -CD/1,8-ANS system. Thus, the enhanced fluorescence of 1,8-ANS at lower temperature relates to the increased contact of 1,8-ANS with HDI-1 due to its globular polymer morphology as depicted in Scheme 3. At higher temperatures, the unfolded form of the polymer reduces its apolar contact/association with 1,8-ANS upon exposure to the polar solvent, accounting for the reduced fluorescence emission. The foregoing results provide support for the “molecular accordion” effect, where the *globular-to-extended* structure of HDI-1 can be sensitively monitored via trends in fluorescence emission, in agreement with complementary insight from an independent study.¹⁶ By contrast, native β -CD retains its well-defined macromolecular structure, where it does not display responsive properties that are noted for the HDI-1 polymer.

4. Conclusions

In this study, the complex formation of two types of host-guest systems were investigated by NMR and fluorescence spectroscopy under steady-state conditions where the effects of temperature and host-guest mixing ratios were studied. β -CD and its polymeric form (HDI-1) are shown to form inclusion complexes with evidence of secondary complexes in the case of the HDI-1/1,8-ANS system due to the presence of dual binding sites (inclusion and interstitial) in the polymer. By contrast, β -CD forms well-defined 1:1 inclusion complexes with 1,8-ANS up to equimolar concentrations. By contrast, the formation of 2:1 host-guest complexes with 1,8-ANS occurs at excess β -CD concentration. In the case of HDI-1, the occurrence of 1:1 and 2:1 complexes cannot be ruled out, however; it is more likely that complexes involve binding at the inclusion and interstitial binding sites for the HDI-1/1,8-ANS system. The thermo- and chemo-responsive properties of HDI-1 were monitored via fluorescence spectroscopy where this structural transition (globular $\rightleftharpoons$ extended morphology) is likened to that of a “molecular accordion”. The results are supported by the variable temperature and concentration effects, where this study contributes further insight on the role of hydration effects⁵⁷ of a uniquely responsive CD-based urethane polymer via complementary spectral methods. We anticipate that supramolecular systems of this type will have

manifold applications as responsive carrier systems in biomedicine for controlled-release, along with the removal of waterborne contaminants in aquatic environments.

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DEDICATION

This work is dedicated to Emeritus Professor, Ronald P. Steer, to honour his meritorious research contributions in the field of spectroscopy and advanced training of generations of students during his career at the University of Saskatchewan.

References

- (1) Gandhi, M. V.; Thomson, B. S. *Smart Materials and Structures*, 1st ed.; Chapman & Hall: Melbourne, 1992.
- (2) Meléndez-Ortiz, H. I.; Varca, G. H. C.; Zavala-Lagunes, E.; Bucio, E. State of the Art of Smart Materials: From Fundamentals to Final Applications. In *Polymer Science: Research advance, Practical Applications and Educational Aspects*; Mendez-Vilas, A.; Solano, A. Eds, Formatex Research Center, **2016**; Ed. 1, p 476.
- (3) Jochum, F. D.; Theato, P. Temperature- and Light-Responsive Smart Polymer Materials. *Chem. Soc. Rev.* **2013**, *42*, 7468. doi: 10.1039/C2CS35191A.
- (4) Pasparakis, G.; Vamvakaki, M. *Polym. Chem.* **2011**, *2*, 1234. doi:10.1039/C0PY00424C
- (5) Crucho, C. I. C. *Chem. Med. Chem.* **2015**, *10*, 24. doi: 10.1002/cmdc.201402290.
- (6) Koetting, M. C.; Peters, J. T.; Steichen, S. D.; Peppas, N. A. *Mater. Sci. Eng. R Rep.* **2015**, *93*, 1. doi:10.1016/j.mser.2015.04.001.
- (7) Yan, X.; Wang, F.; Zheng, B.; Huang, F. *Chem. Soc. Rev.* **2012**, *41*, 6042. doi:10.1039/C2CS35091B.
- (8) Szejtli, J.; Osa, T. *Comprehensive Supramolecular Chemistry*, Vol. 3; Cyclodextrins; Pergamon: Oxford, 1996.
- (9) Wilson, L. D.; Mohamed, M. H.; Headley, J. V. *Rev. Environ. Health* **2014**, *29*, 5.

- doi:10.1515/reveh-2014-0005.
- (10) Karoyo, A. H.; Wilson, L. D. *J Colloid Interface Sci.* **2013**, *402*, 196.
doi:10.1016/j.jcis.2013.03.065.
- (11) Raffa, P.; Broekhuis, A. A.; Picchioni, F. *J. Pet. Sci. Eng.* **2016**, *145*, 723.
doi:10.1016/j.petrol.2016.07.007.
- (12) Leslie, T.; Xiao, H.; Dong, M. *J. Pet. Sci. Eng.* **2005**, *46*, 225. doi:10.1016/j.petrol.2005.01.003
- (13) Zou, C.; Zhao, P.; Ge, J.; Lei, Y.; Luo, P. *Carbohydr. Polym.* **2012**, *87*, 607.
doi:10.1016/j.carbpol.2011.08.031.
- (14) Hapiot, F.; Menuel, S.; Monflier, E. *ACS Catal.* **2013**, *3*, 1006. doi:10.1021/cs400118c.
- (15) Mano, J. F. *Adv. Eng. Mater.* **2008**, *10*, 515. doi:10.1002/adem.200700355.
- (16) Karoyo, A. H.; Wilson, L. D. *Langmuir* **2016**, *32*, 3066. doi:10.1021/acs.langmuir.6b00099.
- (17) Mohamed, M. H.; Wilson, L. D.; Headley, J. V. *Carbohydr. Res.* **2011**, *346*, 219.
doi:10.1016/j.carres.2010.11.022.
- (18) Karoyo, A. H. Structural Studies of Supramolecular Host-Guest Systems. University of Saskatchewan, 2014.
- (19) Qadeer, A.; Rabbani, G.; Zaidi, N.; Ahmad, E.; Khan, J. M.; Khan, R. H. *PLoS One* **2012**, *7*, 1.
doi:10.1371/journal.pone.0050633.
- (20) Dsouza, R. N.; Pischel, U.; Nau, W. M. *Chem. Rev.* **2011**, *111*, 7941. doi:10.1021/cr200213s.
- (21) Yao, C.; Kraatz, H. B.; Steer, R. P. *Photochem. Photobiol. Sci.* **2005**, *4*, 191.
doi:10.1039/B414577C.
- (22) Chattopadhyay, K.; Mazumdar, S. *Biochemistry* **2000**, *39*, 263. doi:10.1021/bi990729o.
- (23) Ferreon, A. C. M.; Gambin, Y.; Lemke, E. A.; Deniz, A. A. *Proc. Natl. Acad. Sci.* **2009**, *106*, 5645. doi:10.1073/pnas.0809232106.
- (24) Lee, J. C.; Langen, R.; Hummel, P. A.; Gray, H. B.; Winkler, J. R. *Proc. Natl. Acad. Sci.* **2004**, *101*, 16466. doi:10.1073/pnas.0407307101.
- (25) Lee, S. H.; Suh, J. K.; Li, M. *Bull. Korean Chem. Soc.* **2003**, *24*, 45.
doi:10.5012/bkcs.2003.24.1.045.
- (26) Wettig, S. D. Studies of the Interaction of Gemini Surfactants with Polymers and Triblock Copolymers, University of Saskatchewan, 2000.
- (27) Wagner, B. D.; Stojanovic, N.; Day, A. I.; Blanch, R. J. *J. Phys. Chem. B* **2003**, *107*, 10741.
doi:10.1021/jp034891j.
- (28) Wagner, B. D.; MacDonald, P. J. *J. Photochem. Photobiol. A Chem.* **1998**, *114*, 151.
doi:10.1016/S1010-6030(98)00210-X.
- (29) Schneider, H. J.; Blatter, T.; Simova, S. *J. Am. Chem. Soc.* **1991**, *113*, 1996.

- doi:10.1021/ja00006a020.
- (30) Baglole, K. N.; Boland, P. G.; Wagner, B. D. *J. Photochem. Photobiol. A Chem.* **2005**, *173*, 230. doi:10.1016/j.jphotochem.2005.04.002.
- (31) Harada, A.; Furue, M.; Nozakura, S. I. *Macromolecules* **1977**, *10*, 676. doi:10.1021/ma60057a036.
- (32) Albrecht, T.; Elben, H.; Jaeger, R.; Kimmig, M.; Steiner, R.; Strobl, G.; Stahn, B.; Schwickert, H.; Ritter, C. *J. Chem. Phys.* **1991**, *95*, 2807. doi:10.1063/1.460932.
- (33) Abbate, V.; Bassindale, A. R.; Brandstadt, K. F.; Taylor, P. G. *J. Incl. Phenom. Macrocycl. Chem.* **2012**, *74*, 223. doi:10.1007/s10847-011-0103-7.
- (34) Niskanen, J.; Karesoja, M.; Rossi, T.; Tenhu, H. *Polym. Chem.* **2011**, *2*, 2027. doi:10.1039/C1PY00143D.
- (35) Renny, J. S.; Tomasevich, L. I.; Tallmadge, E. H.; Collum, D. B. *Angew Chem. Int. Ed. Engl.* **2013**, *52*, 11998. doi:10.1002/anie.201304157.
- (36) Sueishi, Y.; Fujita, T.; Nakatani, S.; Inazumi, N.; Osawa, Y. *Acta - Part A Mol. Biomol. Spectrosc.* **2013**, *114*, 344. doi:10.1016/j.saa.2013.05.052.
- (37) Munoz de la Pena, A.; Ndou, T.; Zung, J. B.; Warner, I. M. *J. Phys. Chem.* **1991**, *95*, 3330.
- (38) Schneider, H.; Hacket, F.; Rüdiger, V.; Ikeda, H. *Chem. Rev.* **1998**, *98*, 175585. doi:10.1021/cr970019t.
- (39) Penzer, G. R. *Euro J Chem.* **1972**, *25*, 218. doi:10.1111/j.1432-1033.1972.tb01687.x.
- (40) Mohamed, M. H.; Wilson, L. D.; Headley, J. V. *Carbohydr. Polym.* **2010**, *80*, 186. doi:10.1016/j.carbpol.2009.11.014.
- (41) Bax, A.; Davis, D. G. J. *Magn. Reson.* **1985**, *63*, 207. doi:10.1016/0022-2364(85)90171-4
- (42) Nishijo, J.; Nagai, M.; Yasuda, M. *Carbohydr. Res.* **1993**, *245*, 43. doi:10.1016/0008-6215(93)80058-M.
- (43) Maciejewski, A.; Szymanski, M.; Steer, R. P. *J. Phys. Chem* **1986**, *90*, 6314. doi:10.1021/j100281a051.
- (44) Wagner, B. D.; Fitzpatrick, S. J. *J. Incl. Phenom. Macrocycl. Chem.* **2000**, *6*, 467. doi:10.1023/A:1008198825835.
- (45) Sideris, E. E.; Valsami, G. N.; Koupparis, M. A.; Macheras, P. E. *Pharm. Res.* **1992**, *9*, 1568. doi:10.1023/A:1015808307322.
- (46) Blokzijl, B. W.; Engberts, J. B. F. N. *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 1545. doi:10.1002/anie.199315451.
- (47) Martin, R. B. *J. Chem. Educ.* **1997**, *74*, 1238. doi:10.1021/ed074p1238.
- (48) Qureshi, P. M.; Varshney, R. K.; Singh, S. B. *Spectrochim. Acta Part A Mol. Spectrosc.* **1994**, *50*, 1789. doi:10.1016/0584-8539(94)80184-3.

- (49) Palepu, R.; Richardson, J. E.; Reinsborough, V. C. *Langmuir* **1989**, *5*, 218.
doi:10.1021/la00085a041.
- (50) Wilson, L. D.; Siddall, S. R.; Verrall, R. E. *Can. J. Chem.* **1997**, *75*, 927. doi:10.1139/v97-111.
- (51) Wilson, L. D.; Verrall, R. E. *Langmuir* **1998**, *14*, 4710. doi:10.1021/la9802365.
- (52) Edelman, G. M.; McClure, W. O. *Acc. Chem. Res.* **1968**, *1*, 65. doi:10.1021/ar50003a001.
- (53) Mohamed, M.H.; Wilson, L.D.; Headley, J.V.; Peru, K. M. *Energy and Fuels* **2015**, *29*, 3591.
doi:10.1021/acs.energyfuels.5b00289.
- (54) Michalet, X.; Weiss, S.; Jäger, M. *Chem. Rev.* **2006**, *106*, 1785. doi:10.1021/cr0404343.
- (55) Kudou, M.; Shiraki, K.; Takagi, M. *Sci. Technol. Adv. Mater.* **2004**, *5*, 339.
doi:10.1016/j.stam.2003.12.012.
- (56) Massey, V.; Curti, B.; Ganther, H. *J. Biol. Chem.* **1966**, *241*, 2347.
- (57) Dehabadi, L.; Karoyo, A. H.; Wilson, L. D. *ACS Omega* **2018**, *3*, 15370.
doi:10.1021/acsomega.8b01663.

Draft

Scheme 1. Formation of HDI-1 polymer from equimolar mixtures of β -cyclodextrin (β -CD) and hexamethylene diisocyanate (HDI), where n denotes the polymer repeat unit.

Scheme 2. Guest binding mode for 1:1 host/guest complexes of β -CD/1,8-ANS and HDI-1/1,8-ANS systems where the structural fragment in parentheses represents the repeat unit of the polymer. Note that the linker units of HDI-1 may be self-included within the CD cavity, where the structures are not drawn to scale and n denotes the polymer repeat unit.

Scheme 3. The *globular-to-extended* “molecular accordion” structural transition of HDI-1 (host) as a function of temperature and incremental amounts of 1,8-ANS (guest).

Figure 1. 2D NMR ROESY spectra of the complexes of 1,8-ANS with (a) β -CD and (b) HDI-1 polymer. Selected resonance lines are labelled using structures of β -CD and 1,8-ANS in the insets, where n denotes the polymer repeat unit.

Figure 2. The continuous variation for the complexes of 1,8-ANS with (a) β -CD and (b) HDI-1 polymer at ambient pH and temperature. The dashed lines are a visual guide for the reader.

Figure 3. The binding isotherm for β -CD/1,8-ANS and HDI-1/1,8-ANS host/guest systems.

Figure 4. Fluorescence spectra for ANS (fixed) with incremental levels of (a) β -CD and (b) HDI-1 at ambient temperature and pH conditions.

Figure 5. Fluorescence spectra titration of ANS (varied) with a fixed level of (a) CD and (b) HDI-1 at ambient temperature and pH conditions.

Figure 6. Integrated emission intensity of 1,8-ANS in the presence of β -Cyclodextrin and HDI-1 polymer as a function of temperature. The fluorescence intensities of β -CD and the complexes converge at higher temperature near 50 - 60°C, as denoted by arrows.

TABLE

Table 1. Complexation-Induced Chemical Shift (CIS) Data for the intra-cavity protons (H3 and H5) of β -CD in various inclusion complexes β -CD and HDI with 1,8-ANS. The CIS values are shown in brackets as $\delta_{\text{CIS}} = \delta_{\text{host}} - \delta_{\text{complex}}$.

¹ H Resonances	β -CD	β -CD:ANS 1:1	β -CD:ANS 1:3	β -CD:ANS 1:5
H3	3.86	3.83 (0.03)	3.82 (0.04)	3.82 (0.04)
H5	3.75	3.70 (0.05)	3.67 (0.08)	3.67 (0.08)
	HDI-1	HDI-1:ANS 1:1	HDI-1:ANS 1:3	HDI-1:ANS 1:5
H3	3.86	3.83 (0.03)	3.82 (0.04)	3.82 (0.04)
H5	3.75	3.69 (0.06)	3.68 (0.07)	3.67 (0.08)

Figures

Scheme 1.

Scheme 2.

Scheme 3.

Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.

Figure 6.