Complexation Behaviour of Polymers with Pendant Cyclodextrin Side Groups

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SUMMARY: The influence of the chain conformation on the formation of polymeric supramolecular complexes as well as the influence of the complexation on the conformation of the polymer chain has been studied. The complexation of pyrene into the cavity of β-cyclodextrin (β-CD) was investigated in aqueous solutions of β-CD substituted poly(allylamine) (PAA) under variation of external parameters, i.e. temperature, pH, ionic strength and addition of urea. The observed changes of the complexation constant *K* for the formation of the 2:1 β-CD/pyrene complex can be explained by a change of the chain flexibility which leads to a variation of the mean distance between neighbouring β-CD-moieties along the polymer chain. The intra-chain association of the decyl group with β-CD in PAA with co-pendant decyl and β-CD is disrupted by the addition of 1-adamantanamine HCl resulting in a more extended structure of the polymer. The β-CD moiety in PAA-CD shows one order of magnitude greater affinity to 2-(p-toluidyl)naphthalene-6-sulfonate than the native β-CD and the affinity increases further by the presence of decyl side groups.

Introduction

Covalent bonding of supramolecular functional units to a polymer backbone may lead to a large change in the physicochemical properties of the polymer as well as the functional units[1-3]. If the polymer and the functional units co-operate in a synergistic manner, new, highly selective materials are to be expected for analytical and catalytic processes. In this way it might be possible to mimic the high efficiency of biopolymers.

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Because of their good water solubility and the hydrophobic cavity, cyclodextrins (CDs) are ideal functional units for the complexation of organic compounds in aqueous solutions. They also can easily be attached to the polymer backbone of water soluble polyelectrolytes.

In this paper we want to discuss two different approaches to study the influence of the polymer on the complexation behaviour. The first part deals with the control of the complexation by controlling the chain conformation and the second part focuses on the control of the chain conformation by displacing the guest of a polymeric, intramolecular complex by a low molecular weight species.

For the concept of the first part one has to consider that the stoichiometry of a CD host-guest complex depends on both the ring size of the CD and the size of the guest molecule. In case of a 2:1 complex between two polymer-bound CD units and one low molecular weight guest molecule a strong impact of the polymer chain on the complexation is to be expected, because of the necessary co-operative process.

The fluorophor pyrene is suited as guest molecule because of its ability to form such 2:1 complex structures with β-CD and furthermore pyrene allows to detect the complexation process via its sensitivity towards micropolarity[4]. In a previous paper[5], we showed that the 2:1 complex between pyrene and β-CD attached to poly(allylamine) (PAA) in aqueous solutions has a chelate-type structure (i.e. no 1:1 intermediate) and is strongly controlled by the distance between neighbouring CD groups along the polymer chain.

The chain conformation of PAA is controlled by H-bonds and electrostatic interaction between neighbouring amino- and ammonium groups. Therefore, the desired variation of the conformation is possible by adjusting pH, ionic strength and temperature of the polymer solution. Different energetic situations of the chain should lead to a change in the entropy contributions to the driving force of the complexation.

Also, the interaction between the polymer-bound CDs and a guest can affect the properties of the polymer chain. This is the object of the second part of this paper. Therefore we prepared PAAs with co-pendant alkyl and β-CD side groups. Intra-chain association between the side groups is expected in these polymers. This should change the conformation and thus the substrate binding properties of the host polymers.

Experimental

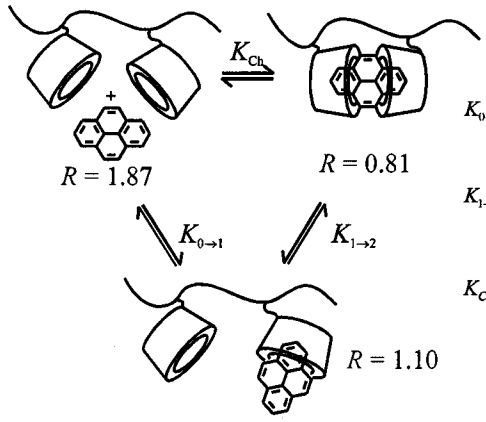
Poly(allylamine) with pendant β-CD side groups (degree of substitution DS = 2%, 3.6%, 9% and 11.3%) was synthesized by a procedure published in ref. 5. Decyl groups were linked to PAA and some of the PAA-CD polymers by reacting the appropriate amounts of 1-bromodecane with the polymers in methanol / water (4:1 V/V) media. After evaporating the solvent, the solid residue was washed with n-hexane to remove the unreacted 1-bromodecane and then dissolved in water and recovered by freeze-drying. The DS with CD and decyl groups were calculated from NMR integration data. Corrected fluorescence spectra of the pyrene- or 2-(*p*-toluidyl)-napthalene-6-sulfonate (TNS)- containing aqueous solutions were taken with a SPEX FLUOROLOG 2 or a HITACHI F3010 spectrofluorimeter. For the experiments with aqueous pyrene solutions, the sample preparation and the automatic titration experiments with the electronic dispensing system EDOS 5221 (EPPENDORF) were carried out according to ref. 5. The pyrene concentration was always set to $2 \cdot 10^{-7}$ mol/L. The pH of the solutions were adjusted with HCl and measured with an electronic pH-meter. β-CD was obtained from WACKER CHEMIE GmbH, all other chemicals were obtained from ALDRICH and were used without further purification. Viscosity measurements were made with an *Ubbelohde* capillary viscometer in a tempered water basin. The equilibrium binding constants $K_{0\rightarrow1}$, $K_{1\rightarrow2}$ and K_{Ch} (see Results and Discussion) were calculated from the first (I_1, λ = 373 nm) and third vibronic transition (I_3, λ = 384 nm) of the pyrene emission spectrum (λ_{Ex} = 335 nm) according to *Xu et al.*[1]. Changes in the micropolarity in the vicinity of the pyrene probe molecules are expressed via the intensity ratio $R = I_1/I_3$. A high value of this quantity corresponds to a high polarity and a low value to a nonpolar local environment[4].

Equilibrium dialysis was carried out with a membrane of MW cut-off 500 for at least 7 days. Concentrations of TNS were determined from fluorescence intensity in β-CD solutions. The results of inside→out and outside→in diffusion were averaged. The equilibrium constants $K_{0\rightarrow1}$ and $K_{1\rightarrow2}$ for the subsequent complexation of TNS by one and two β-CDs were calculated by fitting the TNS intensity profile according to *Martel et al.*[2].

Results and Discussion

Pyrene Complexation Behaviour of Poly(allylamine) with β-CD Side Groups.

As shown previously[5] the 2:1-complexation mechanism of pyrene in PAA-CD depends strongly on the degree of substitution (DS) of the PAA with β-CD: at low DS (< 5%) the complexation is a 2-step process, equivalent to native β-CD, i.e. with an 1:1 intermediate and the associated equilibrium constants $K_{0\rightarrow1}$ and $K_{1\rightarrow2}$ (Fig. 1). However, at higher DS the binding of pyrene can only be explained by a chelate-type complexation, i.e. a one-step process with K_{Ch} as the overall complexation constant. This is due to the smaller distance between the CDs along the polymer chain.



$$K_{0\rightarrow1} = \frac{[\text{Py}-\text{CD}]}{[\text{Py}]\cdot[\text{CD}]}$$

$$K_{1\rightarrow2} = \frac{[\text{CD}-\text{Py}-\text{CD}]}{[\text{Py}-\text{CD}]\cdot[\text{CD}]}$$

$$K_{Ch} = \frac{[\text{CD}-\text{Py}-\text{CD}]}{[\text{Py}]\cdot[\text{CD}-\text{CD}]} = \frac{[\text{CD}-\text{Py}-\text{CD}]}{[\text{Py}]\cdot 0.5\cdot[\text{CD}]}$$

Fig. 1: Schematic representation of the pyrene complexation by PAA-CD. The given R values of the individual species are calculated from the fluorescence intensities I_1 and I_3 according to ref. 1.

As mentioned above, a change in the mean distance between the CD units and thus the equilibrium constant is also expected by varying the temperature. Fig. 2 shows the ln K versus 1/T plot of the binding constant for the polymer with DS = 9% and a degree of polymerization P_n of 150-200.

In comparison with the data for native β-CD[5] (also shown in Fig. 2), K of the polymer decreases much stronger with increasing temperature. The data analysis also revealed a change in the complexation mechanism from chelate-type complexation below 35 °C to subsequent 1:1 and 2:1 complexation above this temperature.

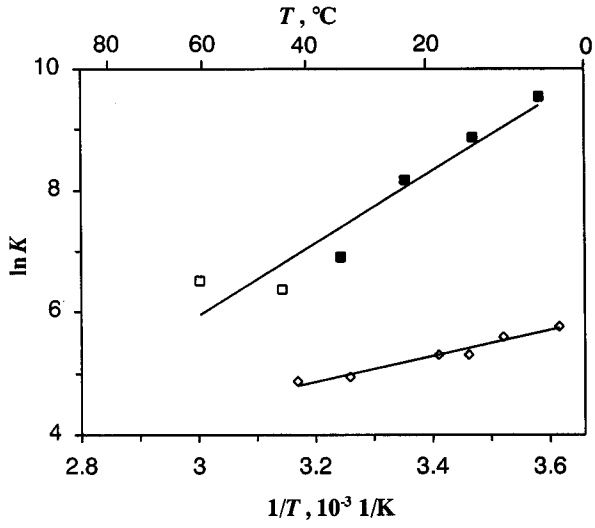


Fig 2: Temperature dependence of the equilibrium constant K for PAA-CD (DS = 9%) (□, ■) and β-cyclodextrin (◊). Closed symbols: $K = K_{Ch}$, open symbols: $K = (K_{0\to1} \cdot K_{1\to2})^{1/2}$; —: regression lines.

The thermodynamic data calculated from Fig. 2 are ΔH = -34.4 kJ/mol, and ΔS = -29.2 J/(mol·K) for β-CD and ΔH = -49 kJ/mol, ΔS = -95 J/(mol·K) for PAA-CD. This shows that both systems are enthalpy driven with a negative reaction entropy. In case of the CD polymer a smaller entropy loss compared to native β-CD was expected due to a restricted mobility of the CD units. The observed higher entropy loss is attributed to the temperature dependence of the polymer chain flexibility. Viscosity measurements of aqueous solutions of the PAA-CD exhibited a significant coil expansion with increasing temperature. A decreasing fraction of lipophile interacting methylene- and methine groups is the reason for this coil expansion, which leads to increasing distances between neighbouring CD units. As a consequence, the complexation constant decreases much stronger with increasing temperature compared to the native β-CD.

The lower ΔH value of the CD polymer is not a result of a direct interaction of the pyrene guest with the polymer backbone in the investigated concentration range, as proved by titration experiments with pure PAA[5]. Hence, stronger interactions between the polymer backbone and CD moieties in the complexed state have to be assumed.

The pH shows a significant influence on the reduced viscosity of aqueous solutions of PAA-CD (DS = 9%) (Fig. 3). At pH = 7 the typical viscosity behaviour of polyelectrolytes can be observed. On first glance the decrease of the viscosity in acidic solutions contradicts to this behaviour, but can be explained with the strong control of the conformation by H-bonds along the polymer chain[2]. At pH = 7 the polymer chain has the most extended conformation because of a high number of stiffening H-bonds between amino- and ammonium groups. Increasing the degree of ionization leads to a decrease of the number of this H-bonds and consequently to smaller coil dimensions in spite of the electrostatic repulsion between the ammonium groups. On the other hand, alkaline solutions of CD polymers lead to a coil collapse because of a lack of the stabilizing electrostatic interactions. The observed strong viscosity decrease in salt solutions can be explained by partial screening of electrostatic interactions and breaking of H-bonds because of counter ion condensation.

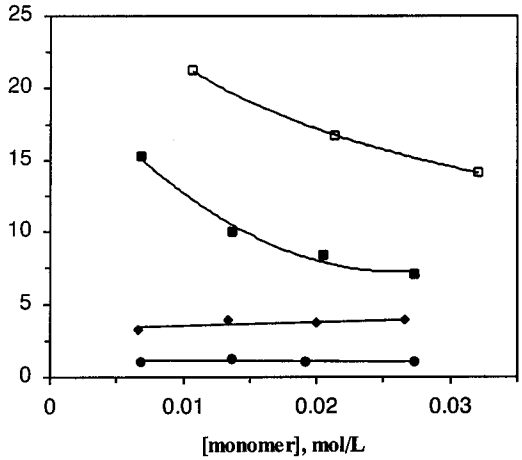


Fig. 3: Reduced viscosity of aqueous solutions of poly(allylamine), P_n = 150-200, at pH = 7 (□) and of PAA-CD (DS = 9%) at pH = 7 (■), pH = 0.5 (◆) and pH = 7, with [NaCl] = 3.5 mol/L (●); T = 295.1K. Polymer concentration is given in terms of allyl monomeric units.

The intensity ratios $R = I_1/I_3$ resulting from titration experiments in neutral, acidic or salt-containing solutions of PAA-CD (DS = 9%) are shown in Fig. 4. In acidic and neutral solutions pyrene is complexed in the same concentration range but the final R values are significantly higher in acidic solutions. This cannot be attributed only to a higher solvent polarity but rather indicates a change in complexation behaviour in favour of the 1:1 complex.

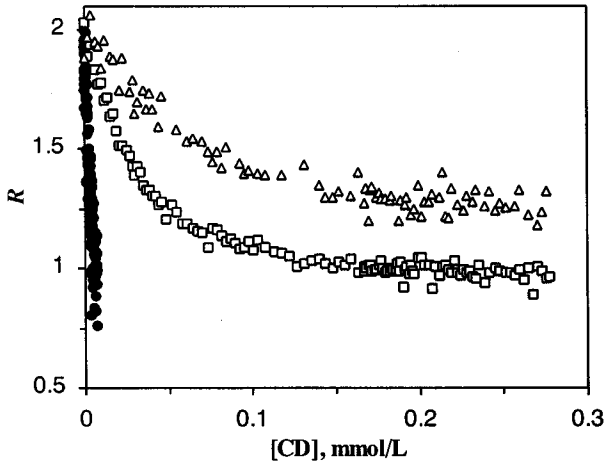


Fig. 4: Micropolarity $R = I_1/I_3$ versus CD concentration in aqueous solutions of PAA-CD (DS = 9%) at pH = 7 (□), pH = 1 (Δ) and pH = 7, [NaCl] = 3.5 mol/L (●); T = 295.1K.

The hindered formation of chelate-type structures in acid solutions might be explained with a folding of the polymer backbone around the CDs as already postulated by *Martel et al.*[2] for poly(vinylamine) in the presence of native β-CD. The chain folding is the result of numerous H-bonds between OH- groups of the CDs and ammonium groups of the polymer backbone. Furthermore, enthalpic interactions between complexed pyrene and the polymer backbone have to be assumed.

In the presence of salt the complexation of pyrene is shifted about two orders of magnitude to smaller CD concentrations (Fig. 4). This is a hint for the higher dominance of the chelate complex and a high equilibrium constant in comparison to neutral salt-free polymer solutions. Reason for this behaviour is an additional entropic contribution of the chain as a consequence of the reduced distance between neighbouring CDs. K_{Ch} could not be calculated for these solutions, because the pyrene concentration ([Py] = $2 \cdot 10^{-7}$ mol/L) is no longer negligibly small in comparison to that of CD (see ref. 1).

Similar to biopolymers the conformation may even be affected by denaturing agents[6] like urea. If H-bonds control the polymer structure in PAA-CD, the distance between adjacent CD moieties should therefore be controllable by the addition of urea. However, the interactions of urea with organic molecules in the presence of CDs are not fully enlightened up to now[7]. Interactions of urea with both the polymer and pyrene are possible in PAA-CD.

The hindered formation of chelate structures in the presence of urea is indicated by a significant shift of the decrease of R on the concentration scale (Fig. 5). Reason for this is an energetic stabilization of free pyrene in combination with a coil expansion attributed to decreasing hydrophobic interactions and a break up of H-bonds. K_{Ch} could not be determined for these solutions.

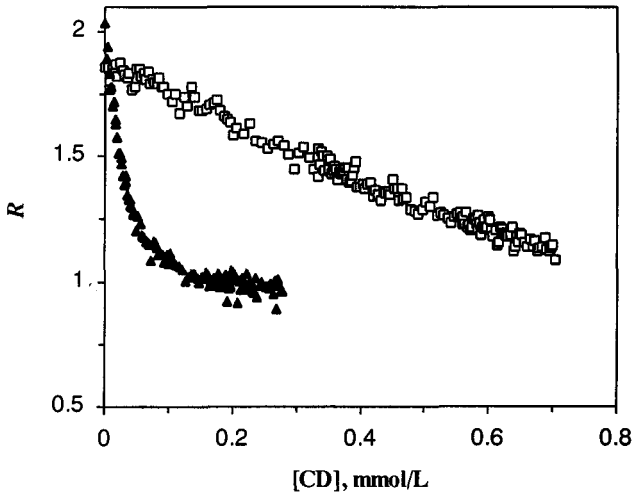


Fig. 5: Pyrene fluorescence intensity ratio R as a function of CD concentration for PAA-CD (DS = 9%) in the absence (▲) and presence (□) of urea (c = 5 mol/L); [Py] = $2 \cdot 10^{-7}$ mol/L, T = 293.1 K.

Viscosity and TNS Binding Properties of Poly(allylamine) with Co-pendant Decyl and β-Cyclodextrin Side Groups.

Alkyl chains show high binding affinity towards the β-CD cavity[8]. Thus the intra-chain association of alkyl with β-CD is expected in PAA with co-pendant alkyl and β-CD groups (PAA-alkyl-CD), resulting in a compact structure of the polymer. The addition of 1-adamantanamine HCl, of which the binding constant with native β-CD was reported as 18,000 L/mol[9], has little effect on the viscosity of PAA solution, but increases the viscosity of PAA-decyl-CD solution (Fig. 6).

Also, the intrinsic viscosity of PAA-decyl-CD (DS of decyl 6%; DS of CD 3.6%) increased from 1.05 dL/g in the absence of 1-adamantanamine HCl to 1.08 dL/g in the presence of 6 mM 1-adamantanamine HCl at pH 3.1, ionic strength 0.04 mol/L and 25°C (data not shown). This clearly indicates that PAA-decyl-CD molecules indeed adopt a compact conformation by intra-chain association of decyl group with β-CD, but the association is disrupted by the inclusion of 1-adamantanamine in the β-CD moiety (Fig. 7).

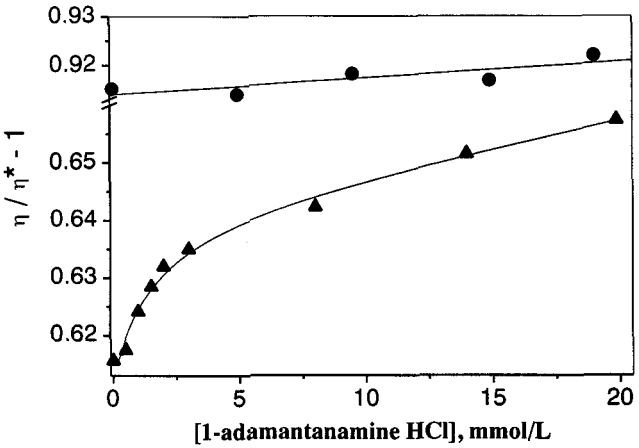


Fig. 6: Dependence of the specific viscosity of 5.0 g/L PAA (●) and PAA-decyl-CD (▲) on the concentration of 1-adamantanamine HCl at 25 °C, pH 3.1 and ionic strength I = 0.04 mol/L. The number of monomer units of PAA is 530-640, DS of decyl is 6%, and that of β-CD is 3.6%.

Another evidence for intra-chain association is the shape of the viscosity titration curve. The concentration of β-CD moiety in the solution used in Fig. 6 is 1.26 mmol/L. If the β-CD is not associated with the decyl group and the binding affinity for 1-adamantanamine is the same as that of free β-CD, the titration curve should show a saturation behaviour above about 1.5 mmol/L of 1-adamantanamine. In fact, the curve exhibits a rather heterogeneous binding and the average binding constant is less than 1000 L/mol. This is in good agreement with our expectation that the β-CD cavity is occupied with a decyl group and inclusion of 1-adamantanamine requires the release of the decyl unit.

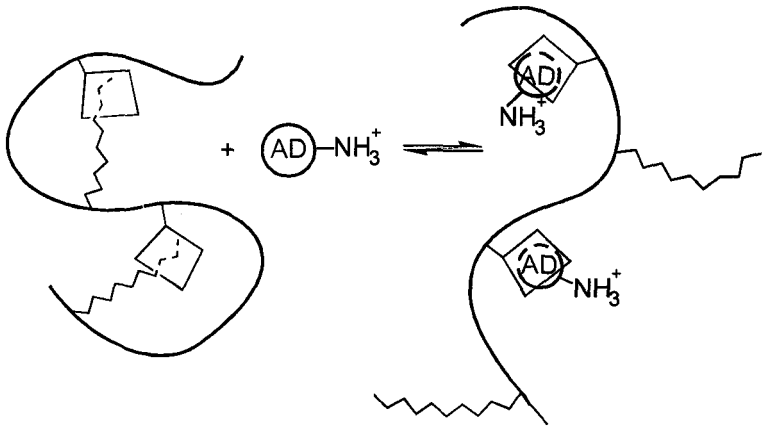


Fig. 7: Schematic representation of association of 1-adamantanamine with PAA-decyl-CD and structural change of the polymer.

TNS is virtually non-fluorescent in aqueous media. However, the addition of PAA or its derivatives and β-CD to aqueous TNS solutions enhances the fluorescence, reflecting transfer of the dye to apolar polymer domain. Fig. 8 shows the dependence of TNS fluorescence intensity on the concentration of polymers and β-CD. Analysis of the TNS fluorescence intensity profile in terms of 1:1 binding[2] for β-CD and CD polymers did not give reasonable fitting. However, the fitting of TNS binding to β-CD to a scheme of 1:1 and 2:1 successive binding (Eq. 1) gave the $K_{0\to1}$ and $K_{1\to2}$ values of 3600 and 14 L/mol, respectively, and the fluorescence intensity ratio of the complex to the free TNS were found to be 21 for 1:1 complex and 173 for 2:1 (β-CD:TNS) complex. The binding constants are in reasonable agreement with the report of Kondo et al.[10] and Jobe and coworkers.[9]

$$I = \frac{I_{TNS} + I_{CD-TNS} \cdot K_{0\to1} \cdot [CD] + I_{CD-TNS-CD} \cdot K_{0\to1} \cdot K_{1\to2} \cdot [CD]^2}{1 + K_{0\to1} \cdot [CD] + K_{0\to1} \cdot K_{1\to2} \cdot [CD]^2} \quad (1)$$

I_{TNS}, $I_{CD\text{-}TNS}$, and $I_{CD\text{-}TNS\text{-}CD}$: fluorescence intensities of the free TNS, the 1:1 and 2:1 complex respectively.

Though the results of fluorescence titration (Fig. 8) do not give quantitative information on the TNS binding with the polymers, it is shown that the binding affinity of TNS to the polymers and the fluorescent quantum yield of the polymer-bound TNS is highly dependent on the nature of side groups and degree of substitution.

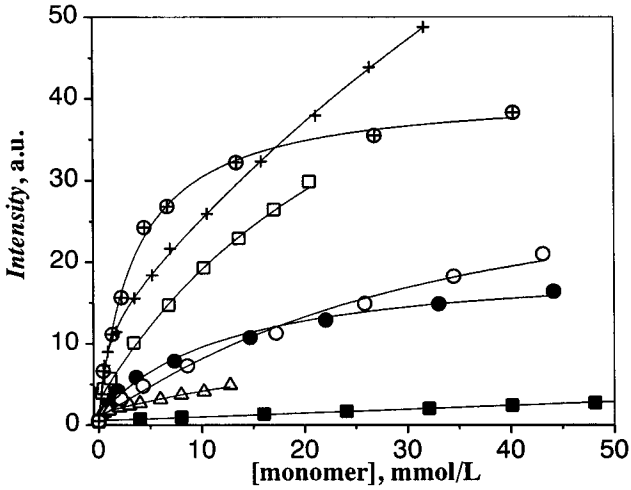


Fig. 8: Dependence of fluorescence intensity of $1.6 \cdot 10^{-6}$ mol/L TNS solutions at 438 nm on the concentration of PAA (■), β-CD(Δ), PAA-decyl (DS 6%) (●), PAA-CD (DS 2%) (○), PAA-CD (DS 11.3%) (□), PAA-decyl (DS 13%) (⊕), and PAA-decyl(DS 6%)-CD(DS 3.6%) (+) at 25 °C. The concentration of polymer is given in terms of allyl monomeric units.

From the initial slope of the variation of the fluorescence intensity on the concentration of polymers, it is estimated that the order of binding affinity of TNS to the polymers is

PAA < β-CD < PAA-CD(DS 2%) < PAA-decyl (DS 6%) < PAA-CD (DS 11.3%)
< PAA-decyl(DS 6%)-CD(DS 3.6%) < PAA-decyl (DS 13%).

Fig. 8 also indicates that the fluorescence quantum yield of bound TNS is much higher for the polymer with CD and / or decyl side groups than that for the native β-CD or unsubstituted PAA.

To obtain a quantitative information on the binding affinity of TNS with the polymer, we carried out equilibrium dialysis experiments. The results are summarized in Tab. 1: K refers to the apparent binding constant with the allyl unit of the polymer and K' to the binding constant per amine, decyl or CD functionality.

Table 1. Results of equilibrium dialysis of TNS in PAA and its derivative solutions (pH = 3.0, I = 0.04 mol/L)

polymer	$[P]_{tot}$	$[TNS]_{bound}$	$[TNS]_{free}$	K^a	K'
	10^{-2} mol/L	10^{-5} mol/L	10^{-7} mol/L	L/mol	L/mol
PAA	5.62	0.15	5.8	44	44
	2.81	1.30	60.6	80	80
PAA-decyl (DS 6%)	4.74	5.05	5.8	1800	30000[b]
	2.50	44.3	60.6	3000	50000[b]
PAA-decyl (DS 13%)	2.29	119	60.6	9000	70000[b]
PAA-CD (DS 2%)	4.93	2.15	5.8	750	40000[c]
PAA-CD (DS 11.3%)	1.22	29.2	60.6	4000	40000[c]
PAA-decyl-CD (DS 6%, 3.6%)	3.56	6.0	5.8	2900	
β-CD					3600[d], <16[e]

[a] Apparent 1:1 complexation constant of TNS with monomeric unit of the polymer
[b] Assumed 1:1 binding of TNS with decyl group of PAA-decyl.
[c] Assumed 1:1 binding of TNS with CD moiety of PAA-CD.
[d] $K_{0\to1}$ of β-CD / TNS binding.
[e] $K_{1\to2}$ of β-CD / TNS binding.

The binding constant of TNS with PAA-CD is one order of magnitude greater than that with the native β-CD. *Martel et al.*[2] reported the binding constant of TNS with poly(vinylamine) at pH 9.5 as $0.3\text{-}2\cdot10^4$ L/mol, which is significantly less than the value obtained here, presumably due to the absence of electrostatic effect in their systems.

The high binding affinity of TNS to CD moiety of PAA-CD seems to be the consequence of combined effects of electrostatic interaction with an ammonium group and hydrophobic interaction with the polymer backbone as well as the CD cavity. The agreement of the binding constant of the PAA-CD complex with that of the product of the binding constants of unsubstituted PAA and native β-CD complexes supports this.

The fraction of TNS bound to a polymer (denoted as P) becomes $K[P] / (1+K[P])$ for 1:1 binding. This relationship predicts that the concentration of polymers required for more than 90% binding is 200 mmol/L for PAA, 5 mmol/L for PAA-decyl (DS 6%), 12 mmol/L for PAA-CD (DS 2%), and 3.2 mmol/L for PAA-decyl(DS 6%)-CD(DS 3.6%). However, the fluorescence intensity *vs* concentration profile (Fig. 8) exhibits a large enhancement of fluorescence intensity by further increase of the polymer concentration, especially for the polymers with CD side groups. This implies a change in the microenvironment of the dye bound to the polymers. Two groups concluded that TNS forms 2:1 CD complexes with PAA-CD as pyrene complex (*vide infra*)[11]. However, this was rebutted by *Martel et al.*[2] on the basis of the dependence of the binding constant on DS and structural point of the polymer chain. Though the curves in Fig. 8 are well fitted to a scheme of 1:1 binding for the polymers without CD groups, the data of PAA-CD are fitted better to a model of successive 1:1 and 2:1 binding. This indicates that the 1:1 TNS/CD complex binds with another CD group to form a 2:1 complex in the polymer. In this case, the high stability of the 1:1 complex can be explained by the involvement of electrostatic interaction with ammonium groups. As with the native β-CD complexes, the 2:1 complexes exhibit much higher fluorescence quantum yield than the 1:1 complexes.

Conclusion

Complexation behaviour of a cyclodextrin polymer can be controlled by varying entropy contributions of the chain situation. Control parameters are temperature, pH, salt and urea concentration. Some of these parameters lead to additional reaction channels for pyrene. In these cases, qualitative comparison of complexation entropy with that in neutral, salt-free polymer solutions is difficult. Poly(allylamine) with co-pendant decyl and β-CD side groups adapts compact structure *via* intra-chain association of the decyl group with β-CD. The association is disrupted by a guest of high binding affinity to β-CD.

Derivatization of PAA with decyl and β-CD groups results in high affinity to TNS and large fluorescence intensity of the polymer bound TNS. The environment of TNS in the domain of PAA-decyl depends little on the dye / polymer ratio, but stoichiometry of CD/TNS complexes with PAA-CD changes to 2:1 from 1:1 as the concentration of polymer is higher.

Acknowledgement

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