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Conformations of tethered polymer chains: a fluorescence energy transfer study

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Abstract

Investigations have been carried out to probe the conformations of tethered poly(ethyleneglycol) (PEG) chains anchored on polystyrene (PS) latex particles in presence of an anionic surfactant, sodium dodecyl sulfate (SDS) and two inorganic salts, sodium and potassium chloride. For this purpose, PS latex particles were labeled with pyrene and mononaphthayl PEG ester. Distance dependent nonradiative energy transfer from naphthalene moieties to pyrene moieties was used as a ruler in this study. Results showed that the separation distance between the acceptor and the donor changes with external stimuli. Analysis of the results suggested that there is considerable contraction of the polymer chain upon interaction with salts and surfactant below its critical micelle concentration. The study also reveals that SDS interacts with PEG chains bellow its critical micellar concentration, probably due to binding of SDS counterions to PEG chains.

Keywords: Conformation; Tethered PEG chains; NRET

1. Introduction

The dynamics of the tethered polymer chains have been studied extensively from both theoretical and experimental points of view [1–15]. In addition to fundamental scientific interest in this area of research, the end tethered polymers have wide technological importance as stabilizer in colloid dispersions, adhesions, wetting and lubrication, etc [16–18]. In the biomedical field, terminally grafted water-soluble polymers have been used for the protection of soft materials, such as liposomes and proteins from the recognition by cell immune systems [19–22]. In spite of a number of studies, the physical basis and the mechanism of the interaction of tethered polymers with cells are not well understood. Our idea behind this work was that a study on the conformation and dynamics of tethered chains might be of great help in finding out the basis of such interactions.

Over the past decade, a number of different techniques have been used to investigate the dynamics of tethered chains, such as ellipsometry [1], small angle neutron scattering [5–7], electron spin resonance [2], light scattering [10,14] and fluorescence spectroscopy [3,4]. Nonradiative fluorescence energy transfer (NRET) is the energy transfer of the excited state from a donor (D) to an acceptor (A) without the appearance of a photon and is primarily a result of dipole–dipole interactions between the donor and acceptor. The rate of energy transfer depends upon the extent of overlap of the emission spectrum of the donor with the absorption spectrum of the acceptor, the relative orientation of the donor and acceptor transition dipoles and the distance between these molecules. The dependence of energy transfer efficiency on the distance has resulted in the use of this technique to measure the distance between the donor and the acceptor. The exciting application of this technique can be seen in the determination of static and dynamic conformational properties of macromolecules in solution [4,23–28].

The chemical inertness, water solubility, low toxicity and low protein adsorption capacity of poly(ethyleneglycol) (PEG) have made it a suitable polymer for use in different biological applications such as drug delivery and gene therapy [29]. It has been demonstrated that the incorporation

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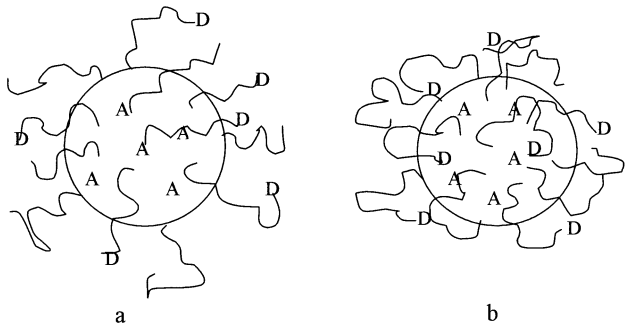

Fig. 1. Schematic representation of possible conformations of PEG chains attached to a PS microsphere (a) stretched out and (b) collapsed state. D: donor (naphthalene), A: Acceptor (Pyrene).

of small amounts of lipids with PEG head groups substantially increases the liposome circulation time [19–21]. This is believed to be due to the steric stabilization of the liposomes by grafted polymer, which prevents their close approach to the cell surface.

In this work we take advantage of NRET to determine the conformational change of tethered PEG attached to a polystyrene (PS) microsphere. For this purpose, we have labeled the surface of PS microspheres with pyrene (P) and mono naphthayl (N) ester of PEG ($\bar{M}_n = 4500$, having a root mean square end to end distance ~50 Å) to get the brush polymer of our interest. A schematic representation of the brush polymer system is given in Fig. 1. The characteristic Forster radius, R_0, of this dye pair, N as a donor and P as an acceptor, is 29 Å and the system can be probed within the distance of up to 58 Å (2 R_0) [30], a higher distance than the root mean square end to end distance of PEG used in this study. The influence of inorganic salts, KCl and NaCl, and an anionic surfactant, SDS, in presence and absence of salt, on the conformation of the end attached PEG chain has been investigated. We observed that, SDS interacts with PEG at two different levels. At low SDS concentration, there is an interaction between the counterions of the surfactant and PEG chains which causes contraction of PEG chains. Above a critical concentration of SDS the surfactant molecules bind to the polymer chain in the form aggregates. This interaction causes decoiling of PEG chains. NRET technique could detect the both kind of conformational change of PEG successfully. Interaction of inorganic salts with PEG chains also causes a contraction of PEG chains as revealed by NRET study which confirms that the interaction of SDS at lower concentration with PEG chains occurs through counterion binding.

2. Experimental details

2.1. Materials and methods

Styrene, naphthalene acetic acid, HPLC grade water and methanol were purchased from Merck, Germany. Pyrene, 2,2′-azobis isobutyronitrile (AIBN), hexamethylene diisocyanate (HMDI), dibutyl tin dilaureate (DBTDL) were from Fluka. Isopropanol, PEG 4500 (PEG4500) and dichloroethane (DCE) were from Loba chem. Mumbai. DCE was dried by distillation over P_2O_5. We followed our earlier procedure for the synthesis, modification and characterization of cross-linked PS microspheres [31]. PS microspheres were of narrow dispersion with an average diameter of 1.4 μm and polydispersity index 1.011. Surface amino groups were estimated as 0.587 mM/g of the microspheres. α-acetoxy ω-hydroxy PEG (Aco-PEG-OH) was synthesized by following a reported procedure [32]. Molecular weight of PEG was estimated (M_n 4500 and $M_w/M_n = 1.11$), against polyethylene oxide standards using Shimadzu GPC. ^{1}H NMR was recorded on Gemini 200 MHz unit and UV measurements were carried out on a Perkin Elmer multi cell automatic UV spectrometer.

2.1.1. Synthesis of pyrene-1-carboxaldehyde

Pyrene-1-carboxaldehyde was prepared from pyrene [33]. Yield: 25%, Melting point: 122°C.

IR: 3045, 2933, 2862, 1749, 839 cm^{-1}.
^{1}H NMR: 10.4 ppm (s, 1H), 9.75 ppm (d, 1H), 8–8.35 ppm (m, 8H).
Mass spectra: *m*/*z* 230.

2.1.2. Synthesis of 1-pyrenyl methanol

1-pyrenyl methanol was synthesized by the reduction of pyrene-1-carboxaldehyde [34]. Yield: 82%. Melting point: 132°C.

IR: 3200–3400 (broad absorption), 3040, 2894, 2862, 839 cm^{-1}.
^{1}H MNR: 7.95–8.45 ppm (m 9H), 5.4 ppm (s, 2H).
Mass spectra: *m*/*z* 232.

2.1.3. Synthesis of naphthalene labeled PEG

One hydroxyl group of the α,ω-dihydroxy PEG chain was selectively modified with naphthalene acetic acid by an esterification reaction [32]. PEG (10 g), boric acid (0.0462 g), naphthalene acetic acid (NAA) (0.410 g), *p*-toluene sulphonic acid (PTSA) (0.100 g), toluene (100 ml) were used. Borate ester was prepared by the reaction of boric acid and PEG at 110°C under suction (vacuum) for 2 h with stirring. The borate ester of PEG, NAA, PTSA and toluene were taken in a 250 ml RB flask attached with a Dean–Stark condenser and maintained at refluxing temperature for 12 h, cooled, and the solvent was removed by rotary evaporator. To the residue, 1 ml of water was added and heated for 1 h at 100°C. Excess water was removed by an azeotropic distillation with toluene. After the removal of toluene the residue was washed several times with hexane and cold ether (0°C) to remove any unreacted NAA. Finally the product was precipitated from chloroform solution with cold ether and dried in vacuum at

room temp. The product was semi solid and was characterized by IR, ^{1}H NMR and UV spectroscopy. The degree of substitution of NAA was calculated from UV absorption and is found to be 1.1 per chain. Yield: 93%.

IR: 3200–3600 (broad absorption), 2780–3000, 1725, 1100 cm^{-1}.
^{1}HNMR: 7.25–8.1 ppm (m), 4.2 ppm, 4.05 ppm (s), 3.6 ppm (s), 3.5 ppm (s).
UV λ_{max}: 292, 282, 274 nm.

2.1.4. Synthesis of fluorophore labeled brush polymers

Amine modified PS microspheres (0.250 g) were first dispersed in dry DCE for 5 min using a magnetic stirring bar. HMDI (0.5 g) and DBTDL (0.025 g) were added to the mixture and stirred under nitrogen for 3 h at room temperature (25 ± 1°C). The dispersion was filtered and washed several times with dry DCE. The diisocyanate modified beads were transferred to another RB flask containing a solution of Aco-PEG-OH (*E*) and Nph-PEG-OH (*F*) in the ratio 10:1 ($E = 0.750$ g and $F = 0.075$ g) and DBTDL (0.025 g) and 1-pyrenyl methanol (1 mg) in dry DCE (9.506 g). Stirring was continued for 24 h in nitrogen atmosphere at room temperature. The grafted microspheres were filtered, washed several times with DCE, methanol, and water and then with methanol and dried under vacuum at room temperature. The product was characterized by UV and fluorescence spectroscopy. The amount of PEG and pyrene attached to microspheres was calculated from the difference in the absorbance of the supernatant of initial and final reaction mixture (λ_{max}: −292 and 340 nm respectively for naphathalene acetic acid and pyrene).

We followed same method (1-pyrenyl methanol was omitted from the reactants) to prepare PS-*g*-PEG containing only naphthalene labels. Amount of PEG attached was estimated as in the previous case.

2.1.5. Steady state fluorescence measurements

Fluorescence measurements were carried out on a Spex Fluororog spectrophotometer in right angle geometry using slit opening 5 mm. The emission spectra were accumulated with an integration time of 1 s/0.5 mm. The excitation wavelength chosen was 290 nm. Emission spectra were recorded at room temperature in the range 300–500 nm. Fluorescence spectra of the aqueous dispersion (0.2 wt% of the medium) of PS-*g*-PEG latex particles (both donor only and donor–acceptor labeled) were recorded as function of the concentration of SDS, SDS + NaCl, NaCl, and KCl.

3. Results

3.1. Synthesis

The study of latex particles is of current interest of science due to their versatile application in the biomedical filed. Several methods such as transmission electron microscopy (TEM) [35], laser light scattering [36], small angle neutron scattering (SANS) [37], atomic force microscopy (AFM) [38] have been used to study these systems. Fluorescence probe method is also one of the finest and simplest tools to characterize latex particles [3,39]. For the successful application of this technique, the particles have to be labeled with suitable flurophores at appropriate concentrations. Here we describe a simple synthetic route for labeled PS latex particles by an isocyanate coupling reaction. This reaction is proved to be efficient for the successful attachment of PEG onto PS microspheres [31]. The characteristics of the brush polymer systems (donor only and donor–acceptor labeled) are given in the Table 1. Unlabeled PEG is used as a statistical dilution for donor molecules around PS microspheres. This could minimize the possibility of energy transfer between donor molecules. These systems could be used as a model for several problems, including interaction of tethered polymers with liposomes or other organized media and proteins.

Table 1
Properties of the brush polymer systems (A: acceptor (pyrene), D: donor (naphthalene), *M*: concentration in moles)

Sample	PEG attached g/g of PS	A attached g/g of PS	$[M]_D/[M]_A$
PS(A)-*g*-PEG(D)	0.1377	5.19×10^{-4}	0.9761
PS-*g*-PEG(D)	0.2604	–	–

3.2. Conformational change of tethered PEG chain monitored by NRET

As we are working with a dispersion of microspheres, it is very much difficult to measure the absolute intensities of the fluorescence from fluorophore attached on or to the particles. One problem is light scattering from the dispersed particles and the second problem is the difficulty in determining accurate concentration in terms of number of particles of the dispersed systems. To avoid these problems, we measured intensities of donor (at 340 nm) and acceptor (at 400 nm) emission in each spectrum and the ratio of these (I_{400}/I_{340}) is used as a ruler for the present study [30]. This ratio is independent of the total number of microspheres, but sensitive to the donor–acceptor distance. We relate the I_{400}/I_{340} ratio to the process occurring in the system at the molecular level. The ratio of the acceptor to the donor fluorescence emission intensity, I_A/I_D, can be written in the form [4]

$$I_A/I_D \propto (Q_A/Q_D)k_{DA}\tau_D[A] \quad (1)$$

where Q_A and Q_D are the quantum yields of the donor and acceptor, respectively. k_{DA} is the rate coefficient of energy transfer, τ_D is the intrinsic lifetime of the donor, and $[A]$ is the acceptor concentration. The rate coefficient k_{DA} reflects

the distribution of acceptors around a donor, via the spatial dependence of probability of energy transfer, $\omega(R_{DA})$ for a donor–acceptor pair separated by a distance of R_{DA}. For the dipole–dipole interaction, the probability of long-range non-radiative energy transfer is given by the Forster equation

$$\omega(R_{DA}) = (1/\tau_D)(R_0/R_{DA})^6 \quad (2)$$

where R_0 is Forster critical energy transfer radius, R_{DA} is the distance between donor–acceptor pair, τ_D is lifetime of the donor. Eq. 2 emphasizes that the smaller the donor–acceptor distance R_{DA}, the higher the probability of energy transfer $\omega(R_{DA})$. As a result k_{DA} is larger for small average separation between donor and acceptor. This value is directly related to the I_A/I_D ratio. In the present case, the fluorescence intensity ratio I_{400}/I_{340} should increase with decrease in the separation of donor–acceptor pair, for a given concentration of donor–acceptor molecules on each particle. Since the donor and acceptor are covalently attached to the chain end and polymer surface, respectively, the distance between the two will be determined exclusively by the conformation of the tethered PEG chains, i.e. change in the end to end distance. A large value of the I_{400}/I_{340} indicates polymer chain contraction, whereas a small value suggests chain expansion. Thus a comparison of the dependence of I_{400}/I_{340} with different experimental conditions at constant value of I_A/I_D, provides information about the donor–acceptor separation distance, and hence the conformation of PEG chains tethered to PS microspheres.

We carried out energy transfer experiments to understand the influence of ambient conditions, especially the effect of SDS, in the presence and absence of salt, as well as effects of salts on the conformation of PEG chains tethered onto the PS microspheres dispersed in aqueous medium. Fig. 2 shows the effect of SDS concentration on the I_{400}/I_{340} ratio. Initially with increasing concentration of SDS, there is an increase in the value of I_{400}/I_{340}. Above an SDS concentration of 7.5 mM, (in the absence of salt) I_{400}/I_{340} decreases. Under similar experimental conditions particles labeled only with PEG naphthyl (donor) ester does not record any change in the I_{400}/I_{340} values (Fig. 2). Fig. 2 also shows the variation of I_{400}/I_{340} with [SDS] in the presence of NaCl of two different concentrations, 0.1 and 10 mM. We observed that the variation of I_{400}/I_{340} on [SDS] in presence of NaCl at two different concentrations is qualitatively same as that of SDS alone. There is also a change noticed in the concentration of SDS which I_{400}/I_{340} reaches its maximum value. The corresponding SDS concentrations are 7.5, 4.6 and 0.6 mM in the presence of 0, 0.1 and 10 mM NaCl, respectively.

We investigated the effect of two different inorganic salts (KCl, NaCl) on the conformation of tethered PEG chain by NRET. The variation of I_{400}/I_{340} on the concentration of KCl and NaCl are shown in Fig. 3. Donor only labeled particles do not change the ratio under similar experimental

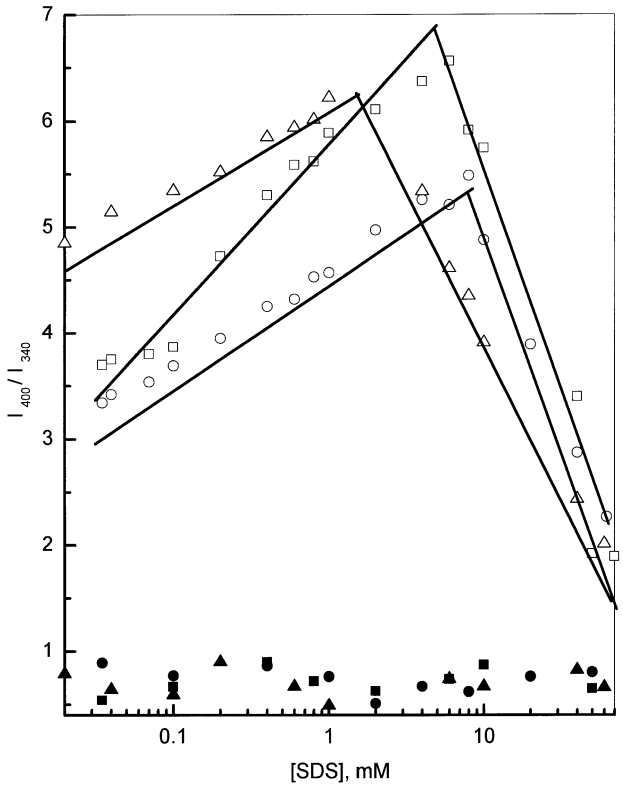

Fig. 2. Variation of I_{400}/I_{340} for donor and acceptor labeled and donor only labeled (Filled Symbols (▲ ■ ●)) brush polymer system on its interaction with SDS in presence of (-○-) 0.0 mM, (-□-) 0.1 mM and (-△-) 10 mM NaCl.

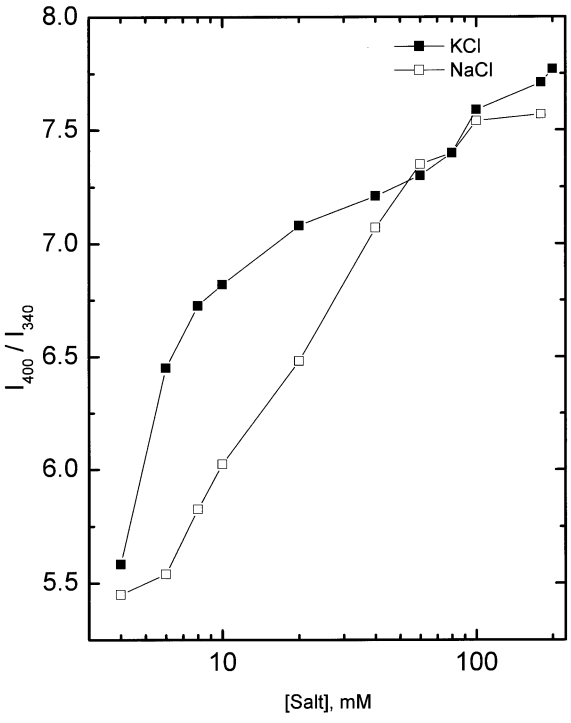

Fig. 3. Variation of I_{400}/I_{340} for donor and acceptor labeled brush polymer system with salt concentration.

conditions (data are not shown). As can be seen in Fig. 3, the values of I_{400}/I_{340} initially increase with increase in the salt concentration and then become more or less invariant with salt concentration. The interesting observation here is that the concentration of salt needed to achieve this constant I_{400}/I_{340} level is less in case of KCl than that in case of NaCl.

4. Discussion

The PEG–SDS system is by far the most studied polymer–surfactant system [40–47]. Generally, most of the studies were focused only on the adsorption of surfactant micelles on the polymer chains close to the micellization of the surfactants [40,42,43,47]. However, the conformation of the polymer itself in presence of SDS has rarely got attention, though there are lot of information available on the overall structure of PEG–SDS complex itself [48–50]. The most striking feature in Fig. 2 is that the increase in I_{400}/I_{340} upon the initial addition of SDS indicates that the tethered polymer chain is coiling. Upon further addition of SDS, I_{400}/I_{340} starts to decrease after attaining a maximum. The exact mechanism of interaction between PEG and SDS is still open to debate. In our opinion, on initial addition of SDS, the counterion, Na^+, of the surfactant binds to the PEG directly onto the ether oxygen atoms along the polymer chain. This forces the polymer to adopt a more coiled conformation, giving lesser separation distance between donor and acceptor and thus an increased the I_{400}/I_{340} value. This binding also brings a slight positive charge along the chain, favors the strong electrostatic interaction of the negatively charged ionic head of the surfactant and positively charged PEG chains. As the concentration of SDS increases, the binding increases and reaches a stage were the electrostatic repulsion between the adsorbed surfactant aggregates forces the polymer chain to uncoil. This results in a larger separation between donor and acceptor give rise to a low I_{400}/I_{340} value. Dubin et al. [51] and Maltesh et al. [44] also reported a similar kind of behavior on SDS–PEG system. The SDS concentrations at which the tethered PEG chain is most coiled — [SDS] at $(I_{400}/I_{340})_{max}$ — are 7.5, 4.6 and 0.6 mM in presence of 0, 0.1 and 10 mM NaCl which are the cmc's of SDS alone in these conditions measured by surface tension method (data are not shown). We did not notice any change in cmc's in presence of brush polymer (as found in the other studies of SDS–PEG interaction [44]). This could be due to the presence of very low concentration of PEG in the dispersion (concentration of PEG in the dispersion medium is 0.0242% (*w*/*v*) PEG), which has very little noticeable effect. Moreover, the PEG is anchored to a solid support, which prevents it to form an intimate solution.

Up on adding the SDS along with NaCl to the tethered PEG system, the concentration of SDS needed to reach maximum I_{400}/I_{340} decreases with increasing in the concentration of salt. The reason for this may be the cation binding to the PEG. The whole process could be explained in this way. In presence of salt even at lower concentration of SDS itself, the polymer is in coiled state due to the binding of Na^+ to PEG chains. So it is reasonable to state that the amount of surfactant needed to saturate it and force it to stretch out will be less than that in the absence of cation binding agent to PEG [44].

Association of inorganic salts with PEG in aqueous medium has been studied by a variety of techniques including viscometry, conductometry, calorimetry, light scattering etc. [52–57]. Such associations are of much interest in chemistry as well as biology [58]. In most of these studies, the binding affinity of ions to PEG chain and mechanism of such interaction have received more attention rather than the conformational change of PEG chain in the presence of such salt. Fig. 3 indicates that there is a considerable effect on the conformation of PEG chain due to this interaction. Upon addition of inorganic salt to this tethered PEG system, a sharp rise in I_{400}/I_{340} was observed, which indicates the decoiling of the chain. This is quite reasonable because upon formation of complex with cation, PEG chain gets dehydrated which allows the chain to contract. From Fig. 3 it can also be seen that I_{400}/I_{340} increases more rapidly upon addition of KCl than the addition of NaCl. From the ultra centrifugation studies on PEG in aqueous salt solutions, Sartori et al. reported that extend of potassium ion binding to PEG chains is more than that of sodium ions [54] which reflects in Fig. 3. The difference in the absolute value of I_{400}/I_{340} in Fig. 2 (at small concentrations of SDS with 10 mM NaCl) and Fig. 3 (at 10 mM NaCl) may due to the slight difference in the donor/acceptor ratio of the samples used in these studies.

Here we would like to make a comment. The similar effect, coiling of tethered PEG chain on the interaction with cations (Na^+ and K^+) and coiling of PEG at low SDS concentration confirm that, the counterion of the surfactant plays a major role in the interaction of SDS with PEG at low SDS concentrations.

5. Conclusions

The brush polymer, described in this study, can be used as a model system to study other biological problems such as interaction of polymers with proteins, liposomes or drug carriers. NRET technique used in this study is a powerful one and can serve as a better tool for these purposes. There are two opposite kind of conformational changes that occur in the PEG chains upon interaction with SDS: contraction of the chain below the cmc and expansion of the chain above the cmc. The report also gives further evidence on the role of surfactant counterions at the initial stages of SDS–PEG interaction.

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