Probing Intramacromolecular Forces by Pyrene Excimer Fluorescence

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INTRODUCTION

Intramacromolecular forces (IMFs) of a macromolecule control its conformation and deformability under stress. For instance, shear will induce the elongation of a macromolecule only if it is subject to a stress that overcomes its IMFs. Since changes in the conformation of a macromolecule are usually associated with changes in the viscoelastic properties of its solution, the characterization of the IMFs has been the object of intense scientific scrutiny. Numerous procedures have been implemented to probe IMFs but they all involve tethering a nanometer-scale macromolecule of interest to two macroscopic surfaces which can be micron-sized latex or silica particles or 1 cm×1 cm mica plates, prying the two surfaces apart, and monitoring the reaction force exerted by the macromolecule onto the two surfaces.[1] In these experiments, great care must be applied to ensure that the molecular linker tethering the macromolecule to the macroscopic surfaces does not affect the behavior of the macromolecule as well as prevents interactions between the macromolecule and the surfaces.[2] Also the macroscopic surfaces are expected to have no effect on the IMFs of the macromolecule being investigated.

By contrast, the present study investigates how excimer formation between the pyrene-labeled ends of a series of poly(ethylene oxide)s (Py_2-PEO) can yield quantitative information on the IMFs experienced by these macromolecules. Instead of being tethered to macroscopic surfaces, the pyrenyl end groups are encapsulated inside nanometer-size sodium dodecyl sulfate (SDS) micelles whose electrostatic repulsion can be investigated by monitoring pyrene excimer formation. These experiments provide a first example where the IMFs of macromolecules freely floating in solution are being probed quantitatively without being tethered to a macroscopic surface.

EXPERIMENTALS

Materials: SDS and NaCl were purchased from Sigma-Aldrich. The Py_2-PEO samples were synthesized earlier or prepared according to a well-established protocol.[3]

Steady-state Fluorescence: Steady-state fluorescence spectra were acquired on a Photon Technology International (PTI) spectrofluorometer using an Ushio UXL-75Xe Xenon lamp and a PTI 814 photomultiplier detection system. The Py_2-PEO solutions were excited at 344 nm and their fluorescence intensity was scanned from 350 to 600 nm.

Time-resolved fluorescence: The fluorescence decays of the pyrene monomer and excimer were acquired on an IBH time-resolved fluorometer using a *nano*LED 340 to excite the Py_2-PEO solutions at 344 nm and the fluorescence intensity was monitored at 375 and 510 nm, respectively. The monomer and excimer fluorescence were fitted globally according to the Model Free Analysis (MFA).

RESULTS AND DISCUSSION

Time-resolved fluorescence carried out on a series of 1-pyrenemethoxy end-labeled PEOs (Py_2-PEO(X) where X equals 2.0, 3.4, 5.0, 6.0, 8.0 and 10K) provided a quantitative measure of their IMFs which were found to equal 1-2 pN, a range of IMFs that seems ideally suited to probe the elastic strength of a macromolecule or H-bond interactions within macromolecules in solution. In

the case of the Py_2-PEO(X) series, PEO in water adopts a conformation that results in a well-defined end-to-end distance (r_{EE}) that increases with increasing chain length. But the strong hydrophobicity of the pyrenyl end-groups of Py_2-PEO led to their encapsulation inside surfactant micelles that were added to the solution. In our experiments, sodium dodecyl sulfate (SDS) was used as SDS is known not to interact with PEO at the extremely low polymer concentrations used in our experiments (less than 13 mg/L) so that the SDS micelles would specifically target the pyrenyl end groups of the Py_2-PEO constructs.[4] Furthermore, the use of a large molar excess of 15 mM SDS ensured that only 1% of all SDS micelles interacted with 1 molecule of Py_2-PEO, and that the fluorescence experiments reported on the interactions between isolated Py_2-PEO molecules and SDS micelles.

Encapsulation of the pyrenyl end groups of a same Py_2-PEO chain inside two SDS micelles resulted in two possible outcomes. The first outcome was when the electrostatic repulsive force (f_{elec}) between the two SDS micelles was so strong that it led to an extension of the PEO chain that overcame the elastic restoring force (f_{elas}) of the chain resulting in one pyrenyl end group disengaging itself from the SDS micelle and entering the SDS micelle occupied by the other pyrenyl end. Since this outcome resulted in two pyrene moieties being present inside a same SDS micelle, excimer formation took place (Figure 1). This outcome was observed mostly for the shorter PEO chains which had a larger spring constant. The second outcome was detected for the longer chains whose much smaller spring constant resulted in a weaker f_{elas} that allowed them to adopt an extended conformation where the two pyrene moieties could occupy two different SDS micelles thus preventing excimer formation (top and middle panels in Figure 1). Interestingly, some of the shorter Py_2-PEO constructs that formed excimer due to their larger spring constant that prevented full extension of the chain could be induced to bridge two micelles by increasing the ionic strength of the solution. Addition of increasing amounts of NaCl to the solution led to a sharp reduction in f_{elec} between two SDS micelles located at the two ends of a PEO chain, thus reducing the elastic extension of the chain, and allowing the pyrenyl moieties to occupy two different micelles where they did not form excimer.

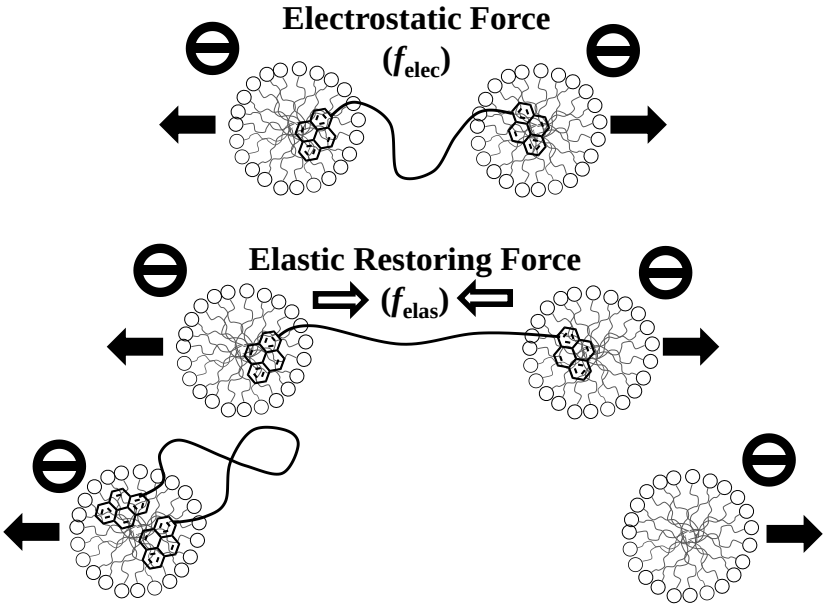

Figure 1. Depiction of the effect of f_{elas} and f_{elec} on the conformation of a PEO chain end-capped with two pyrenyl units encapsulated inside SDS micelles.

In order to draw such conclusions however, several precautions needed to be taken. Since pyrene excimer fluorescence depends on both the number of pyrenes forming excimer and their efficiency at forming excimer, a same excimer signal can be observed in a steady-state fluorescence spectrum acquired for a macromolecule where very few pyrenes form excimer efficiently with a large excimer formation rate constant (k_{exci}) or for a macromolecule where many pyrenes form excimer with a small k_{exci} rate constant. Since the size and viscosity of SDS micelles increase with increasing salt concentration,[5] both effects contributed to decreasing k_{exci} when two pyrenyl labels were present in a same SDS micelle and consequently, excimer fluorescence in the fluorescence spectrum of the Py_2-PEO constructs. To distinguish between the effects induced on the excimer fluorescence intensity resulting from changes in the number of pyrenyl labels forming excimer and the rate constant k_{exci}, the recently developed Model Free Analysis (MFA) was applied to fit globally the monomer and excimer decays.[6] MFA of the decays yielded k_{exci} and f_{free}, the molar fraction of pyrene labels that were isolated inside a same micelle, did not form excimer, and emitted as if they were free in solution. A plot of f_{free} is shown in Figure 2A as a function of salt concentration and will now be discussed.

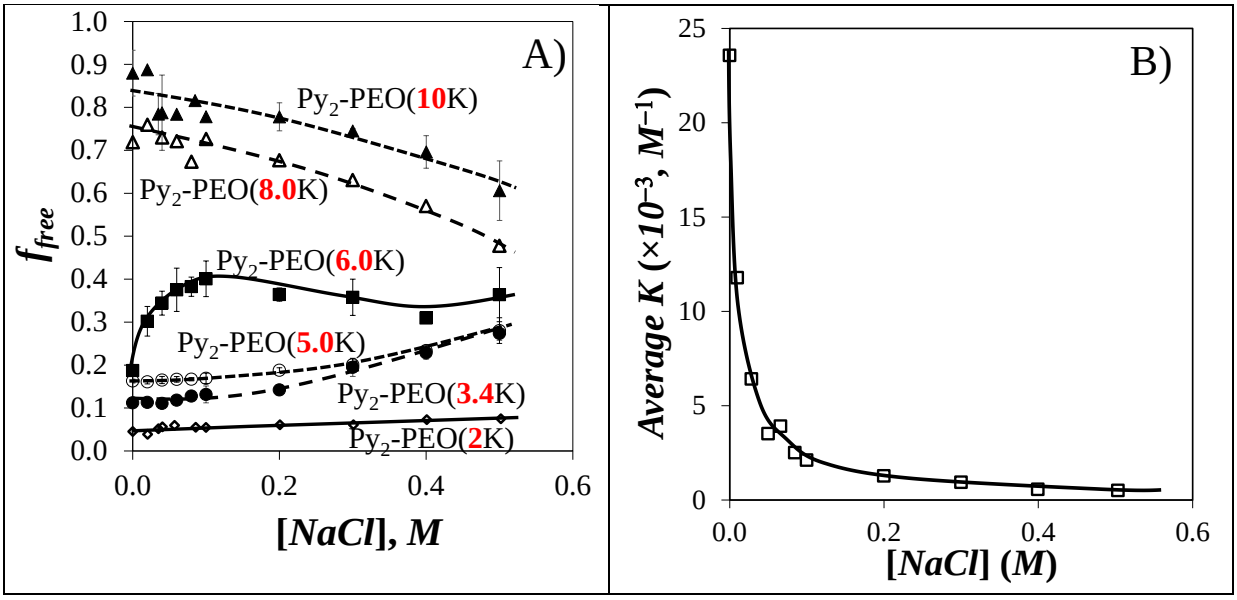

Figure 2. A) Plot of f_{free} as a function of salt concentration for a series of Py_2-PEO constructs. B) Plot of K for the binding of Tl^+ cations to SDS micelles as a function of salt concentration.

For the shortest polymer, Py_2-PEO(2K), f_{free} remained small, constant with salt concentration, and equal to 0.06 ± 0.01, equivalent to the fraction of monolabeled Py_1-PEO(2K) in the sample. Consequently, regardless of the salt concentration, all Py_2-PEO(2K) chains had their two pyrenyl end groups in a same SDS micelle reflecting the large spring constant of this short chain which prevented its full extension and ability to bridge two micelles. The next two polymers, Py_2-PEO(3.4K) and Py_2-PEO(5K), yielded higher f_{free} values that increased with increasing salt concentration in Figure 2A. The higher f_{free} values were due to a small but increasing fraction of the Py_2-PEO(3.4K) and Py_2-PEO(5K) chains able to bridge two SDS micelles with increasing PEO chain length and salt concentration. The most striking behavior was observed with the Py_2-PEO(6K) sample which was ready to undergo extensive bridging upon addition of a small amount of salt. The increase in f_{free} with increasing salt concentration stopped after 0.1 M NaCl because

the size of the SDS micelles increased substantially thus allowing the shorter chains of the molecular weight distribution (MWD) of Py_2-PEO(6K) to be accommodated inside a same SDS micelle. Finally the two longest constructs, Py_2-PEO(8K) and Py_2-PEO(10K), underwent extensive bridging regardless of salt concentration. The f_{free} trends in Figure 2A drifted downwards with increasing salt concentration as the shortest chains of the MWDs of these two longer constructs could be accommodated by the larger SDS micelles obtained at larger salt concentration.

The ability to probe the conformation of the Py_2-PEO constructs as a function of PEO chain length and salt concentration as demonstrated by Figure 2A is quite remarkable, but more impressive is the ability to infer quantitatively the IMFs being applied to the Py_2-PEO constructs. To this end, the aggregation number (N_{agg}) and thus size of the SDS micelles and the binding constant of monovalent counterions (Tl^+) to SDS micelles was determined as a function of NaCl concentration. The results describing the size of SDS micelles have already been published[5] and a plot of the binding constant, K, of Tl^+ is shown in Figure 2B as a function of salt concentration. As expected, K decreases continuously with increasing salt concentration as a stronger ionic strength screens the electrostatic interactions between Tl^+ and the SDS micelles. But K also provides us with the fraction of SDS surfactants in a given SDS micelle whose counterions are not tightly associated with the micelle and thus contribute to the overall charge of the SDS micelles. Since N_{agg} is known, the actual electric charge of the SDS micelles can be determined from the K value as a function of salt concentration, and thus their electrostatic repulsive force.

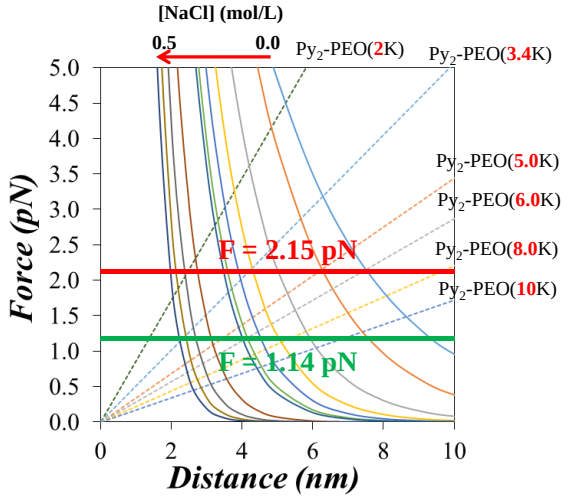

Figure 3. Plot of f_{elas} and f_{elec} experienced by the Py_2-PEO constructs as a function of PEO end-to-end distance and ionic strength.

Similarly, the mathematical expression of the spring constant of linear chains in general and PEO in particular is well known from its established r_{EE} in water so that the elastic restoring force of the Py_2-PEO constructs can be determined quantitatively. Both f_{elas} of the Py_2-PEO constructs and the f_{elec} of the SDS micelles were calculated according to well-established mathematical expressions[1,7,8] and they are represented in Figure 3. Since the spring constant of a chain increases with decreasing chain length, f_{elas} increases linearly with increasing separation distance between the two ends but with a slope that increases with decreasing chain length. Consequently, the largest f_{elas} values are obtained for longer r_{EE} distances.

By contrast, f_{elec} is strongest when two pyrenyl end groups are encapsulated inside SDS micelles that are separated by the shortest r_{EE} distances. Consequently, f_{elec} takes its largest value at the shortest r_{EE} distances, but f_{elec} decreases substantially with increasing salt concentration due to the screening effect. The resulting plot is shown in Figure 3.

Figure 3 can then be used in conjunction with Figure 2A to determine the minimum or maximum force required to, respectively, enable or prevent bridging of a Py_2-PEO construct between two SDS micelles. An increase in f_{free} for a given salt concentration in Figure 2A indicates the onset of bridging where f_{elas} must equal f_{elec} at this salt concentration. By conducting this exercise for each PEO chain length, Table 1 was constructed where the areas in orange or green

indicate where bridging does not or does happen, respectively. The interface between the orange and blue areas in Table 1 represents the boundary where bridging becomes possible. This analysis led to the conclusion that forces greater than 2.15 pN always prevented bridging whereas forces smaller than 1.15 pN always allowed bridging.

Table 1. Value of the force experienced by the Py_2-PEO constructs when f_{elas} equals f_{elec} for a given salt concentration.

[NaCl] (M)	0	0.01	0.03	0.05	0.07	0.09	0.1	0.2	0.3	0.4	0.5
2K	4.5	4.17	3.62	3.25	3.02	2.84	2.77	2.3	2.03	1.85	1.73
3.4K	3.19	2.86	2.43	2.17	2	1.87	1.82	1.49	1.31	1.19	1.11
5K	2.47	2.19	1.8	1.58	1.49	1.41	1.39	1.14	0.97	0.86	0.75
6K	2.15	1.88	1.57	1.39	1.27	1.19	1.15	0.93	0.82	0.74	0.68
7.8K	1.8	1.53	1.31	1.21	1.06	0.97	0.9	0.79	0.63	0.58	0.55
10K	1.49	1.27	1.05	0.92	0.84	0.78	0.76	0.61	0.53	0.48	0.44

CONCLUSIONS

Consequently, the experiments presented above describe a novel means to measure IMFs for macromolecules quantitatively. Contrary to all other experiments carried out thus far, they do not involve the tethering of two specific points of a macromolecule to two macroscopic surfaces but rather use molecular objects (surfactant micelles) of size similar to that of the macromolecule being studied. Most importantly, these experiments are conducted in the solvent where the macromolecules adopt their native conformation, not at the interface between a macroscopic surface and the solvent as is currently being done.[1,2] They open a new field of research where pyrene excimer fluorescence can be applied to probe the IMFs of many macromolecules.

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