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Conformational transitions and dynamics of thermal responsive poly(N-isopropylacrylamide) polymers as revealed by molecular simulation

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ABSTRACT

Stimuli responsive polymers (SRP) have attracted increasing interest for their unlimited potential of molecular capture, separation, purification and delivery particularly at the cutting edge of bio-nano technologies, as well as for the biotechnological, food and medical industries. However, molecular mechanisms of SRPs and their interactions with target materials are little understood at atomistic levels. Based on poly(N-isopropylacrylamide) (pNIPAAm) and poly(NIPAAm-co-AAc-co-tBAAm) polymers, we examined the SRP operating mechanisms and dynamics by all-atom molecular simulation in varying conditions of temperature and chemistry. The LCST conformational transition predicted by simulation agreed well with experimental results, and simulation results notably leads to elucidate mechanism that torsional energy of isopropyl acryl tethers and H-bond play vital roles in driving the transition in response to temperature changes. These insights are helping molecular design and virtual screening of tailor-made SRPs, and harnessing the responsive control of temperature and other stimuli factors (e.g. ions, hydrophobicity).

1. Introduction

Stimuli responsive polymers (SRP) are new generation of polymers that can undergo structural and physical transitions in response to the changing environmental conditions. For example, changes of temperature, light, or pH can induce SRP to change their phase, conformation, hydrophobicity, solubility and many other properties [1,2]. For the past years, the most widely investigated stimuli responsive polymers are poly(N-isopropylacryamide), namely pNIPAAm, and its various copolymers such as poly(N-isopropylacrylamide-co-acrylic acid-co-tert-butylacrylamide (namely poly(NIPAAm-co-AAc-co-tBAAm)) [1–6]. pNIPAAm in aqueous solution has flexible elongated coiled shape below 303 K, the typical polymer's lower critical solution temperature (LCST, [7]), and has a compact insoluble globule shape above this temperature. The polymer exhibits reversible phase transition when the external temperature is transited from below to above LCST. As a dynamically bonded material, SRPs are the kind of materials capable of self assembly, adaptive and even self healing [1,8]. Thus as homopolymers, constituents of copolymers, gels, hybrid layers and building blocks, pNIPAAm and other SRPs have attracted increasing experimental effort for their potential of molecular capture, separation, purification and delivery, particularly at the cutting edge of bio-nano technologies, as well as in food and medical industries [1,2].

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Selective adsorption, purification and recovery of biological molecules are one of the most promising uses for SRP materials. For example, we have used pNIPAAm copolymers to separate lactoferrin proteins from whey streams [9]. In the reported process, lactoferrin (Lf) is bound onto the pNIPAAm-based SRP resin to form a complex by naturally occurring weak interactions at a temperature above the LCST. When the temperature is lowered beneath the LCST, the pNIPAAm polymers undergo a phase change, weakening the interaction between pNIPAAm and Lf, resulting in the elution of Lf. The reversible pNIPAAm adsorption and desorption of Lf enables the regeneration of the resin without the use of salt as is the case with existing ion exchange resins, this makes pNIPAAm-based SRP resins an environmentally-friendly 'green' technology. Although there have been numerous attempts at characterising and investigating the interactions between pNIPAAm and proteins [10], there are many aspects for the SRP mechanism and operating dynamics details at atomistic level, such as driving forces for LCST transition, competition between hydrophobicity and other forces as per changing chemical groups [11–13], and SRP-protein interactions that need further elucidation. As such, experiments are largely hampered by the technology limits at the molecular scale. Molecular simulation is therefore poised to lead to a more insightful atomistic understanding [14–16]; more promisingly and importantly, leads to *in silico* design and screening for tailor-made SRPs [17,18].

Our motivation was to improve the fundamental and molecular understanding of SRP conformational transitions and dynamics. In addition, by utilising molecular simulation we expect to speed up experimental development of SRP, for example, for characterisation and optimisation of protein separation using SRP-based chromatographic resins. The initial and crucial aims of molecular simulations were to derive the conformational changes and LCST transition of pNIPAAm and copolymers in designed solution, and further investigate its interaction with targeted proteins and other molecular systems. We made a series of pNIPAAm and its copolymers with different monomer concentration and radical groups, and conducted a complete set of all-atom explicit dynamics simulation at wide range of temperatures and other conditions. From dynamics analysis, we tried to deduce the dynamics descriptors for the conformational transitions of pNIPAAm and copolymer SRPs. We also examined the driving forces for LCST transition and key mechanism in pNIPAAm and copolymers regulated by dynamic torsion and H-bond networking.

2. Materials and methods

2.1. SRP molecular models and dynamics simulations

This work is focused on pNIPAAm and its copolymers with poly acrylic acid (AAc) and/or *tert*-butylacrylamide (tBAAm), pNIPAAm-co-AAc-co-tBAAm. Fig. 1 shows the molecular CPK models of NIPAAm, AAc and tBAAm monomers and polymers in water solutions. We set up the SRP systems by using at least 25 single chain and double chains of poly(NIPAAm$_x$-co-AAc$_y$-co-tBAAm$_z$), which have enough accuracy for predicting the bulk properties [19,16]. During initializing the systems, an pNIPAAm or pNIPAAm-co-AAc-co-tBAAm in water solution was chosen as 1.020 g/ml (at 293 K) by AmorphousCell method [20]. The pNIPAAm and copolymers were typically solvated in a $35 \times 35 \times 42$ Å^3 water box, giving a total system of about 1800 water molecules plus 25 or 50 units of polymers. The systems were heated or cooled accordingly to the designed temperature (i.e. 288 K, 292 K, 296 K, 299 K, 303 K, 308 K, 314 K, 320 K and 330 K). Three or more conformers were generated as ensembles of different possible starting states and the results and the property analysis was averaged over multiple conformers.

All-atom molecular dynamics (MD) simulations of pNIPAAm and copolymer were performed using the COMPASS force field [21,22], Particle Mesh Ewald (PME) [23] electrostatic interactions, the SHAKE algorithm [24] constraining the bonds with H-atoms. Molecular visualisation and trajectory analysis were processed using the Materials Studio [25] or VMD [26] program. More details please see the supplementary A: Materials and Methods, sections of s1–s4.

For understanding the conformational changes and transitions at given temperature, a complete set of dynamics trajectories of SRPs were measured and analysed. From dynamics analysis, we derived the following properties as the essential molecular descriptors to elucidate the atomistic origin of LCST transition:

- Radius of gyration, which is a measure of the size of a molecule (distance from its centre of mass) and was measured as the geometry of the polymer.
- Solvent accessible surface area, which was measured on the polymer.
- Intermolecular (i.e. between SRP and water molecules) and intramolecular hydrogen bonds of the SRP polymer
- Torsional energy and side chain angle profile for the dynamics runs.

2.2. Synthesis and LCST transition characterisation of SRP polymers

We used the polymerisation procedure as outlined in previous work [9]: The precursor solution consisted of NIPAAm (112.5 mmol), tBAAm (6.25 mmol), AAc (6.25 mmol)) and N,Nmethylenebisacrylamide [MBBA (1.25 mmol), cross-linking agent] (they were dissolved in ethanol, 4,4-azobis(4-cyanovaleric acid) (ACVA) immobilised gel). The polymerisation reaction was proceed in an argon atmosphere at 80 °C for >16 h with continuing stirring. In order to obtain a sample of the ungrafted free poly(N-isopropylacrylamide-co-acrylic acid-co-tert-butylacrylamide) copolymer [poly(NIPAAm-co-AAc-co-tBAAm)] formed by this polymerisation were collected by vacuum filtration and evaporated using a rotary evaporator (Buchi Labortechnik, Flawil, Switzerland). The dried polymer was dissolved in tetrahydrofuran (25 mL) and then precipitated in diethyl ether (250 mL). The recovered polymer was washed with diethyl ether and dried under vacuum condition. Three different concentrations of monomers were examined and the optimal monomer composition