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Single Molecular Study of interaction between DNA and repetitive peptides

Yaning Xing, Gaowei Lin, Huifang Sun and Yanwei Wang*

College of Mathmatics and Physics, Wenzhou University, China

wangyw@wzu.edu.cn

Abstract: The interaction between cationic polypeptides and deoxyribonucleic acid (DNA) is of great importance for basic research in biology and in the pathogenesis of numerous diseases. In this paper, we focus on the complexation of a series of peptides with repeating proline and arginine (poly PR) sequences with DNA in aqueous solution using magnetic tweezers (MT), dynamic light scattering (DLS), and optical inverted microscopy. Through the study of magnetic tweezers, we found that the critical condensing force (Fc) of poly PR would increase with the increase of concentration. However, after the concentration reaches a certain level, the critical condensing force begins to decline. The electrophoretic mobility of poly PR was measured by dynamic light scattering. Poly PR induces charge reversal of DNA and is very effective in neutralizing the positive charge of DNA. Related studies have found that poly PR is a positively charged polypeptide that binds to RNA to undergo liquid-liquid phase separation (LLPS) and further coagulation of the polypeptide, there by inducing the development of related diseases. We hypothesize that the high efficiency of charge neutralization is due to poly PR being positively charged.

Keywords: Poly peptides, DNA compaction, LLPS

1. Introduction

DNA (deoxyribonucleic acid) is a long chain of nucleic acids that plays an important role in the storage and transmission of genetic information in cells. DNA is made up of four bases (adenine, guanine, thymine and cytosine), and the order in which these bases are arranged determines the specific content of genetic information. The primary role of DNA is to store an organism's genetic information, which is used to guide cell growth, differentiation, and function. During cell division, DNA copies itself, ensuring that the new cell has the same genetic information as the mother cell. At the same time, DNA can also control protein synthesis through gene expression regulation, and then affect various physiological functions of the organism. DNA binding polycationic molecules to form multistranded bodies is widely used in biotechnology applications[1], such as non-viral gene delivery, gene expression regulation, and DNA replication[2, 3]. Research has shown that in aqueous solutions, without a denaturing agent, DNA chains usually exhibit a long, curled state. However, DNA molecules exist in highly compacted states in living cells. It has been found that as the concentration of the denaturant increases in the solution, the separated DNA strands undergo a transition from helices to spheres [4-6]. Chemical reagents induce DNA condensation through various effects such as altering electrostatic interactions between DNA segments, changing the DNA-solvent interaction, excluding worm-like loops (and/or counterions) from volume, causing local bending or deformation of helices, or by a combination of these effects. By adding compounds such as polyamines [7], multivalent metal ions[8], hydrophilic polymers[9], polycations[10, 11], anionic liposomes[12], cations [13], and nonionic surfactants[14], in vitro DNA condensation can be induced.

Polypeptide (poly PR) composed of proline and arginine residues, are a highly toxic peptide that plays a crucial role in C9ORF72 chromosome abnormal translation in humans[14-19]. Studies have shown that the abnormal expansion of the C9ORF72 chromosome in humans results in the production of large amounts of poly PR and poly glycine (poly

GR) peptides, which generally have low complexity structures and can undergo LLPS in vivo. These peptides are ubiquitously toxic to cellular RNA components and damage neurons in patients with amyotrophic lateral sclerosis (ALS). In contrast, normal individuals in this population have very little of these peptides. Therefore, the abnormal aggregation of these peptides is directly linked to the occurrence of ALS. Relevant studies have found that poly PR is positively charged and can bind to RNA to form LLPS and further aggregate, thereby leading to disease progression[20-22] .

2. Materials and Methods

2.1 Materials

In this study, double-stranded λ-DNA (48502 bp) was purchased from New England Biolabs Co. (Ipswich, MA, USA) and used without purification prior to use. Salmon sperm DNA was purchased from Thermo Fisher Scientific US Company. Different lengths of poly PR (PR2, PR4, PR8, PR12, PR15, and PR35) were synthesized powder obtained from Jiangtai Peptide Biotechnology Co., Ltd., Suzhou, China, with purity of 95%. TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH = 8.0) was used for storing DNA solution, and the initial concentration of DNA was 500 ng·μL−1. Other biochemical reagents including Tris (hydroxymethylaminomethane) and BSA (bovine serum albumin) were purchased from Sigma-Aldrich (St. Louis, Missouri, USA) and used as received. Deionized water of resistivity of 18.2 MΩ·M was purified through Milli-Q system (Millipore Corporation, Burlington, MA, USA) and used for preparation of all solutions. A reserve solution containing 1 mg·mL-1 of poly-proline-arginine was prepared in 1 mM Tris-HCl buffer (pH = 7.4). All chemicals were used as received. Our experiments will be repeated at least three times to ensure the accuracy of the results.

2.2 Magnetic Tweezers Experiment

A lateral MT setup was established on an inverted microscope (Nikon TE2000U, Tokyo, Japan) for studying the dynamic process of DNA binding, as shown in Figure 1A. The detailed description of the magnetic tweezers setting has been introduced in previous works. The procedure for measuring force can be briefly described as follows: one end of the DNA molecule is connected to an immobile substrate (the glass sidewalls) and the other end is connected to a small magnetic sphere. When the two ends of the DNA are firmly attached, we begin to use magnets controlled by a micromanipulator system（MP-285，Novato，CA，USA） to approach the small magnetic ball, and then pull the DNA strands connected by the small magnetic ball to apply force to the DNA molecule. We use a CCD camera to record the movement of the small magnetic ball in real time. Fast Fourier Transform based software captured video of the small magnetic sphere, and Mathcad software was used to analyze the video. We calculate the cohesion of DNA molecules by fitting the data to a worm-like chain (WLC) model. We call the force at the time of the first stepped contraction in the tension-time curve of DNA critical condensing force.

The DNA binding scheme can be briefly described as follows: We linked the ends of λ-DNA chemically labeled, single-stranded, 12-base oligonucleotide（3′-digoxigenin-tccagcggcgggand 3′-biotin-cccgccgctgga). Then, we gently incubated a reserve solution of 0.5μL streptavidin coated small magnetic spheres and a 0.5μL modified λ-DNA solution in 150μL buffer for 30 minutes. After the incubation, we observed the formation of DNA-bead structure.

Next, we used an injection pump to inject different concentrations of poly PR solution into the sample pool. At first, we had to find a suspended λ-DNA and use a force of more than 10 pN to pull the DNA to its maximum length, which was achieved by placing a magnet close to the small magnetic ball. Then, we slowly remove the magnet and reduce the force to the desired value. Due to the presence of poly PR in the solution, bound DNA usually shrinks when the applied force is reduced to a small enough value. Finally, we monitor and record conformational changes of the DNA. Figure 1B, C show the typical elongation time curve of DNA compression induced by poly PR, where (B) corresponds to the case without DNA concentration and (C) shows the DNA concentration curve.

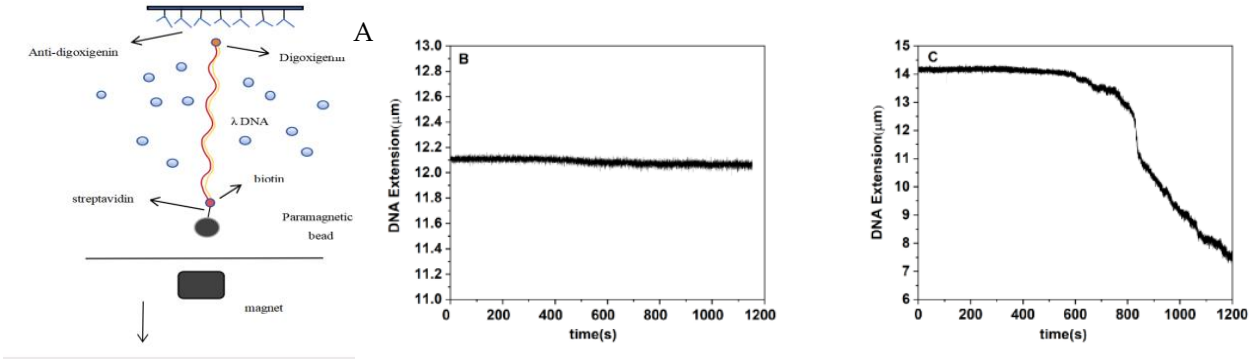

Figure 1. (A) A schematic diagram of the experiment setup; The curves of condensing forces are shown in (B) a non-condensing curve and (C) a condensing curve. DNA extension-time curve measured by MT in DNA compaction process with poly PR.

2.3 Electrophoretic Mobility Measurement (EM) by DLS

Electrophoretic mobility (EM, μ) measurements were performed using the Malvern Zetasizer nano ZS90 (Malvern Instruments Limited Company, Malvern, UK) and M3-PALS technology. The light source used in DLS was a He-Ne gas laser (*λ*=633 nm). We received light scattering signals from an avalanche photodiode installed vertically on the angular arm. We diluted DNA samples to a concentration of 2 ng·μL−1 in 1 mM Tris (pH = 7.4) by adding buffer solution. Then, we added poly PR to the solution and obtained samples with different concentrations for measurement. Before starting the measurement, we incubated the mixed solution at room temperature for 10 minutes. We measured DNA solutions of 1 mL volume and controlled the sample cell temperature to 25℃.

2.4 Optical microscopy experiment

Place the cleaned coverslip on the sample stage of the microscope, adjust the light source, mix the required solution in a cleaned 1mL centrifuge tube, use a pipette to extract 15-25 μL of the solution onto the coverslip, allow the sample solution to settle for 2 minutes to ensure stability, adjust the focus of the microscope until the field of view is clear, and take pictures for record.

3. Results

3.1 Condensing Force of DNA-poly PR Complex

Poly PR *has a* condensation effect with *λ*-DNA: Magnetic tweezers were used to study the condensation effect of Poly PR with DNA, and PR2 and PR4 had no condensation effect with *λ*-DNA. (PR)8, (PR)12 and (PR)15 have a condensation effect with DNA, and the critical condensing force increases with the maximum concentration of poly PR. The critical condensing force of (PR)12 increased from 3.6 pN to 16.3 pN and then decreased to 3.58 pN. Figure 2 (a)、(b)、(c).The critical condensing force of PR15 increased from 3.16 pN to 7.7 pN. Figure 3 (a)、(b)、(c).

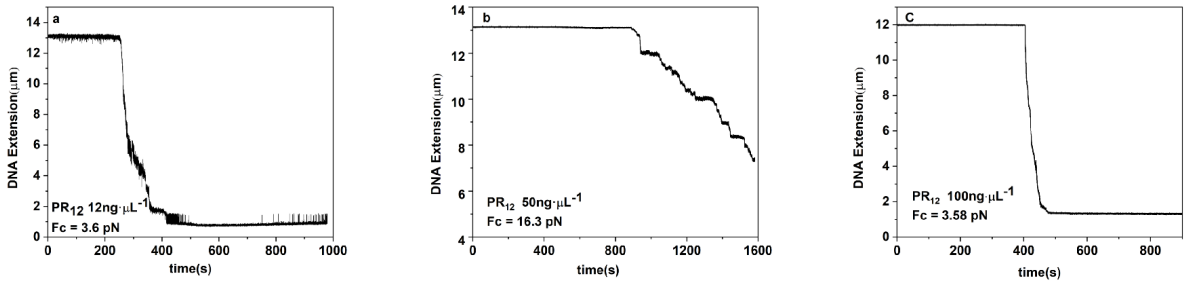

Figure 2. DNA contraction curves by MT at different concentrations of poly (PR)12 in 10 mM Tris-Cl, pH=7.4. (a) 12 ng·μL-1 (b) 50 ng·μL-1 (c) 100 ng·μL-1

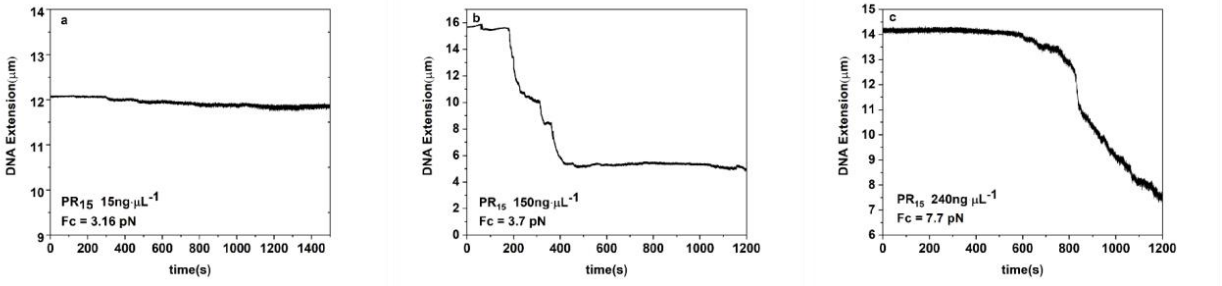

Figure 3. DNA contraction curves by MT at different concentrations of poly (PR) 15 in 10 mM Tris-Cl, pH=7.4. (a) 15 ng·μL-1 (b) 150 ng·μL-1 (c) 240 ng·μL-1

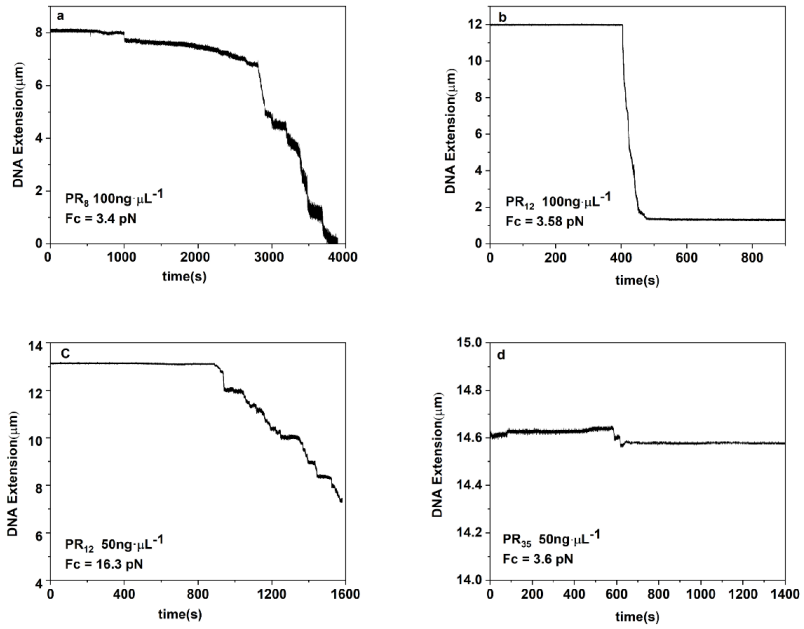

Figure 4. DNA contraction curves by MT at different concentrations of poly PR in 10 mM Tris-Cl, pH=7.4. (a) (PR)8 100 ng·μL-1 (b)(PR)12 100 ng·μL-1 (c) (PR)12 50 ng·μL-1 (d) (PR)35 50 ng·μL-1

3.2 Separation of PolyPR from salmon extract DNA in LLPS

Mutated C9ORF72 gene translation *o*f the rich arginine repeat protein (DRPs) is one of the main causes of ALS [23-26]. Poly PR, composed of proline and arginine, is one of the DRPs. The large positive charge of arginine makes poly PR capable of forming liquid-liquid phase separation (LLPS) droplets through electrostatic interactions and cation-π interactions at multiple sites, thus forming LLPS[27]. Their LLPS is sensitive to the valence of Poly PR, with a length range of 6 to 60 repetitions. Longer poly PR (poly PR) significantly reduces the formation and solubility of droplets and lowers the molar ratio of phase separation[28]. In addition, the repetition length is also related to the cytotoxicity of Poly PR, especially when the repetition length is longer, the inhibition rate of protein synthesis is higher. The interaction between Poly PR and DNA becomes more significant with the increase of Poly PR concentration, and the LLPS phenomenon becomes more prominent, as shown in Figure 5. The phase diagram of (PR)12 interaction with salmon extract DNA was also made. It can be seen that salmon extract DNA has a promoting effect on LLPS,as shown in Figure 6.When the concentration of (PR)8 reaches 10000 ng·μL-1, the interaction with salmon sperm also changes from a liquid droplet state after two minutes to a gel state, as shown in Figure 7.

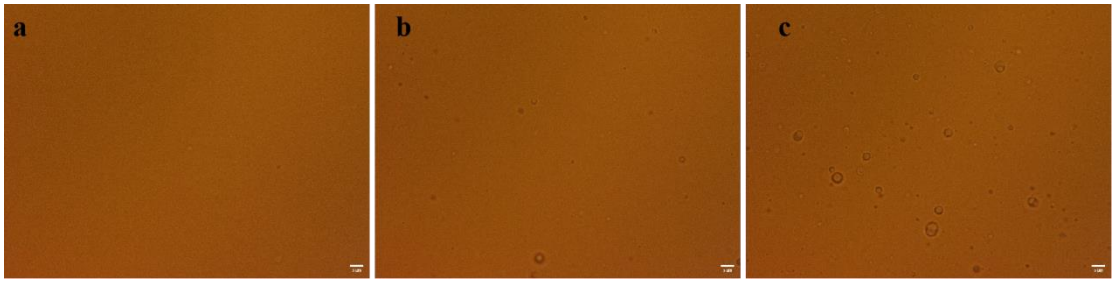

Figure 5. Droplet diagram of interaction between poly PR and salmon extract DNA (a) (PR)8 1000 ng·μL-1 (b) (PR)8 5000 ng·μL-1 (c) (PR)8 8000 ng·μL-1

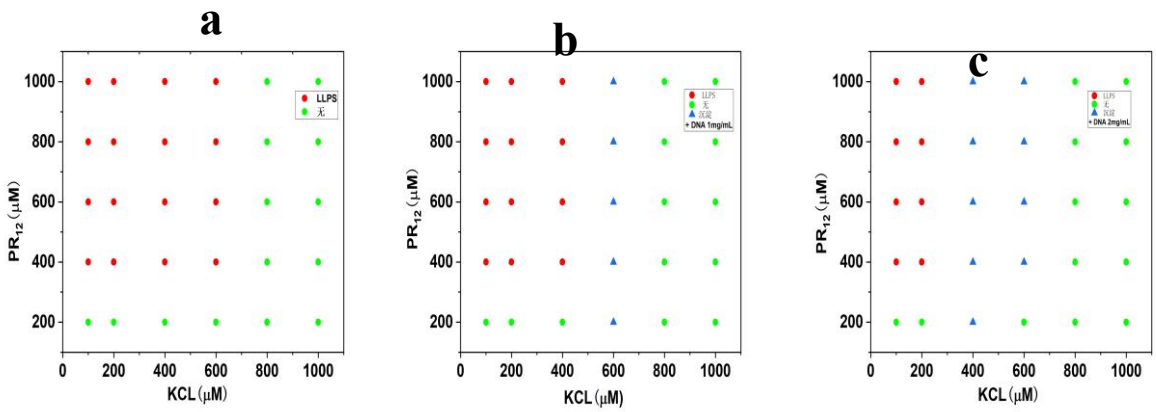

Figure 6. Droplet diagram of interaction between poly PR and salmon extract DNA (a) DNA 0.5 mg·μL-1 (b) DNA 1 mg·μL-1 (c) DNA 2 mg·μL-1

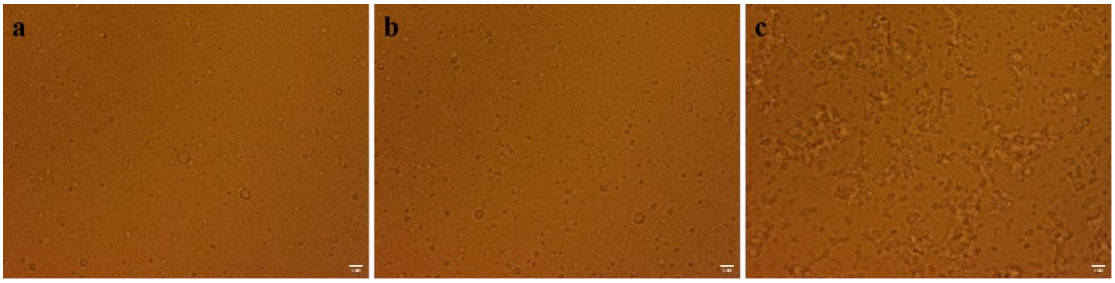

Figure 7. Droplet diagram of interaction between poly PR and salmon extract DNA (PR)8 10000 ng·μL-1

3.3 DNA Electrophoresis Mobility in poly PR Solutions

DNA is a negatively charged deoxyribonucleic acid, so it exhibits a negative Zeta potential and electrophoretic mobility in aqueous solutions. The electrophoretic mobility of DNA and PR complex is related to its net positive charge, which reflects the degree of DNA neutralization by counter-ions in solution. We measured the electrophoretic mobility (EM) of DNA at different concentrations of PR to calculate the proportion of charge neutralized by PR. Figure 8 shows that the electrophoretic mobility of DNA in aqueous solution with PH=7.4 is about -2.8*10−4 cm 2V−1S (10−4 cm 2V−1S is the unit of electrophoretic mobility, the following are the same and will not be repeated). The electrophoretic mobility also changes with the increase of PR concentration, increasing gradually from −2.8 at the beginning. The electrophoretic migration of PR8 at 100 ng·μL−1 is close to zero, which means that the DNA has been neutralized, and the electrophoretic mobility becomes positive as the concentration continues to increase. The electrophoretic migration of PR12 is close to zero at a concentration of 50 ng·μL−1, and is close to zero at a concentration of 30 ng·μL−1. It can be inferred that the concentration required to neutralize the charge carried by DNA decreases with the increase of strand length.

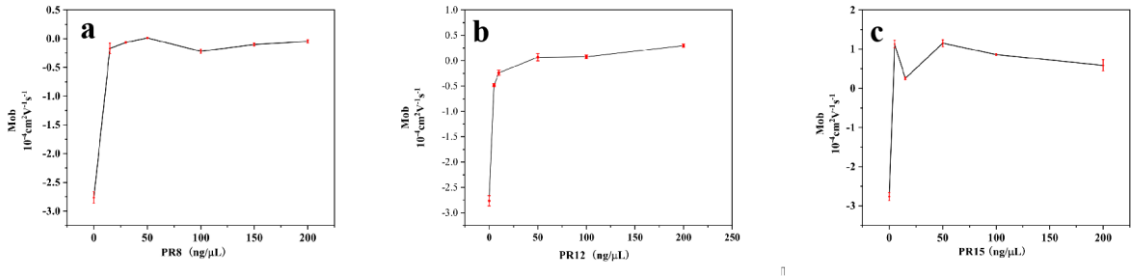

Figure 8. DNA Electrophoresis Mobility in poly PR Solutions (a) (PR)8 (b) (PR)12 (c) (PR)15

4. Discussion and Conclusion

When the concentration of poly PR in solution is low, only a small part of the negative charge of DNA is neutralized, so the electrostatic repulsion force between the DNA fragments will be strong, and no DNA condensation will occur. The drawing in Figure 1B is illustrated. In the figure, there is no tendency for the DNA to shrink, in which case the corresponding DNA cohesion is 0 pN. As the PR concentration and chain length increase, more negative charges are neutralized in the solution, and the Coulomb repulsion barrier weakens, leading to DNA condensation. As shown in Figure 1C. As we continue to increase the concentration of PR, the negative charge of DNA in solution will be completely neutralized by PR. DNA attracts a positive charge so the poly PR-DNA water-soluble complex becomes positively charged, a charge reversal. Charge neutralization is why PR binds DNA together to overcome electrostatic repulsion between DNA strands.

Acknowledgements

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