Effect of poly(L-lysine)-g-dextran copolymers on DNA hybridization

Longliang Wu¹, Naohiko Shimada¹, Arihiro Kano¹, Atsushi Maruyama¹,²

¹Institute for Materials Chemistry and Engineering, Kyushu University, Motooka 744, Nishi-ku, Fukuoka 819-0395, Japan and ²CREST, Japan Science and Technology Agency, 4-1-8 Honcho, Kawaguchi, Saitama 332-0012, Japan

ABSTRACT

We have focused on design of biomaterials that are capable of engineering nucleic acids folding. In this study, we explored the effect of poly(L-lysine)-g-dextran, PLL-g-Dex, copolymer on hybridization kinetics of DNA. The hybridization kinetics was studied by using a fluorescence resonance energy transfer (FRET) assay in the absence and presence of the copolymer. The copolymer at nano molar concentration significantly accelerated the hybridization rate over 200-fold under physiologically relevant ionic conditions.

INTRODUCTION

Studies on the interaction between nucleic acids and cationic polymers, especially basic peptides as a model of nucleic acid binding protein, began in the 1960s. In the past few decades, various types of cationic polymers have been studied with the aim of transferring genetic materials into cells for biological and medical purposes. However, studies on the interaction between polycations and nucleic acids have been hampered by the aggregation, precipitation, and condensation (or compaction) of the resulting interpolyelectrolyte complexes (IPECs).

We have studied interactions between DNA and cationic comb-type copolymers, poly(L-lysine)-graft-dextran (PLL-g-Dex, Fig.1A), which have abundant water-soluble side chains (>80 wt %).₁,₂ We showed that the copolymer does not induce coil-globule transition of DNA and forms a totally soluble inter-polyelectrolyte complex with DNA. We have investigated thermodynamic and kinetic effects of cationic copolymer. Interestingly, the copolymers considerably accelerate DNA strand exchange reaction while stabilizing DNA hybrids.₁,₃ The cationic copolymer is also expected to accelerate duplex formation. But the kinetic effect of the cationic copolymer on duplex formation has not been properly estimated, because of extremely higher rate of DNA hybridization in the present of the copolymer. In this study, we carefully optimized the experimental conditions such as DNA concentration and temperature. Finally, we estimated that the cationic copolymer at nano molar amino group concentrations accelerated DNA duplex formation by two orders under physiologically relevant conditions.

RESULTS AND DISCUSSION

Duplex formation of complementary DNA strands was traced by using a fluorescence resonance energy transfer (FRET) assay. For FRET assay, the DNA strands labeled with TAMRA or DABCYL (Fig.1B) were used. When a TAMRA-labeled DNA probe strand associates to the complementary DNA DABCYL-labeled target, the fluorescence emission from TAMRA is quenched by DABCYL. The DNA sequences were chosen to have approximately equal AT and GC contents and were designed to minimize self-complementary target-target and probe-probe interactions. Fig. 2 shows change in fluorescence intensity of TAMRA-labeled probe (T25 final conc. = 0.5 nM) by the addition of a DABCYL-labeled targets (final conc. = 0.5 nM).

Fig. 1: Materials used in this report. (A) Structural formula of poly(L-lysine)-graft-dextran (PLL-g-Dex) copolymer. (B) DNA sequences used.

The fluorescence intensity quickly decreased in the presence of PLL-g-Dex (N/P), while that gradually decreased in the absence of the copolymer. The results obviously indicated that the copolymer increased the hybridization rate. Change in fluorescence intensity was not obviously observed either in the absence or presence of the copolymer when a scramble sequence, S25, instead of D25 was used, implying that the decrease in the fluorescence intensity was owing to sequence-selective DNA interactions.

Next, we investigated the effect of ionic strength on DNA hybridization in the absence of 3.56 mM
PLL-g-Dex ([PLL-g-Dex]_{amino~group} = 0.26 μM). The results are shown in Fig. 3. In the absence of PLL-g-Dex, the hybridization rate increased with increasing [NaCl], in agreement with the previous report. A linear relationship between log k and log [Na+] with a slope of 1.3 was observed, indicating that 1.3 sodium ions associated to the DNA at the rate-limiting step of duplex formation. The value is almost consistent with that previously reported for the hybridization of 8-mer DNA.

The [NaCl] dependency in the presence of PLL-g-Dex was totally different from that observed in the absence of the copolymer. At [NaCl] < 200 mM drastic acceleration effect of PLL-g-Dex was observed, where no [NaCl] dependency was seen. At [NaCl] > 200 mM, the effect of the copolymer was reduced, and was diminished at [NaCl] > 300 mM. The result strongly suggested that ionic interactions between the copolymer and DNA plays a central role in the hybridization acceleration. The [NaCl] independence observed at the lower [NaCl] implied that the copolymer reduces the counterion condensation effects at the rate-limiting step of duplex formation. With increasing [NaCl] > 200 mM, the copolymer/DNA interaction was weakened, resulted in decrease in the accelerating effect of the copolymer. Similar result was also obtained from our kinetic study with different DNA sequences (data not shown).

It was reported that cationic surfactants, such as cetyltrimethylammonium bromide, CTAB, at mM level concentration, considerably promote DNA hybridization. Here, we found that the cationic copolymer at mM level concentration promotes DNA hybridization, suggesting distinguished effects of the copolymer on DNA hybridization.

**ACKNOWLEDGEMENT**

This work was supported in part by Grant-in-aid (16200034) for scientific research from Ministry of Education, Culture, Sports, Science and Technology of Japan.

**REFERENCES**


*Corresponding Author. E-mail: maruyama@ms.ifoc.kyushu-u.ac.jp*