Simple Basic Peptides Activate DNA Strand Exchange

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(Received March 3, 2003; CL-030176)

The simple cationic peptides containing arginine moieties were proved to accelerate DNA strand exchange reaction.

Molecular chaperones are the functional proteins that support precise folding and assembling of biopolymers. Among the molecular chaperones, nucleic acid chaperones catalyze the folding of nucleic acids into conformations that have the maximal number of base pairs.[1] Lowering the energy barrier for breakage and re-formation of base pairs has been considered a thermodynamic role of the nucleic acid chaperones. Retroviral nucleocapsid (NC) proteins, such as HIV-1 NCp7, have the nucleic acid chaperone activity and play a crucial role in proviral life cycle. The dimerization of viral RNA, the formation of the primer tRNA-viral template hybrid, the several strand transfer steps that occur subsequently to the initiation of reverse transcription, and reverse transcription at stem-loop domains in the template RNA were described to be assisted by the chaperone activity of the NC proteins.[1,2] The NC proteins are very small, highly basic proteins and contain one or two zinc fingers of the form CX2CX4HX4C. NCp7 was reported to destabilize double stranded (ds) DNA to decrease the energy barrier for the strand displacement.[3] The zinc-finger architecture was required for NCp7 to exhibit some of nucleic acid chaperone functions,[4,5] whereas it was not critical for primer/template annealing.[6] The role of highly basic and cationic characters of the protein upon strand displacement has, however, remained unclear.

We have been interested in the spontaneous complex formation between the polycationic polymer, cationic comb-type copolymers (CCCs), having hydrophilic side chains of dextran and DNA as a model of nucleic acid-acting proteins that ubiquitously possess highly basic domains.[7,8] In the course of our studies, CCCs are found to stabilize DNA duplexes and triplexes of either Hoogsteen or reverse-Hoogsteen types.[9] Acceleration of association rate rather than decrease in dissociation rate was identified as the major kinetic role of CCC upon the hybrids stabilization.[10] Furthermore, we have demonstrated that CCCs stimulated the strand displacement between double stranded (ds) DNA and its homologous single stranded (ss) DNA by 4–5 orders.[11] Of interest, unlike NCp7, CCCs accelerate the strand displacement rate while stabilizing ds DNA. The CCC-mediated stabilization of the transitional state, that is a branched nucleation complex consisting of the initial duplex and a homologous strand, to reduce the activation energy of the strand displacement is considered an acceleration mechanism.[12]

Although mechanisms involved in the chaperone activity of NC proteins seem partly different from those of CCCs, it was speculated that the electrostatic or ionic interaction with DNA plays a central role on the strand displacement acceleration. In this study, we examined varieties of simple cationic peptides lacking zinc finger motif as a common chemical ingredient of the NC proteins and CCCs. We found that highly basic oligopeptides facilitated the strand displacement between 20 bp duplex and its homologous ss DNA with concentration dependent manner. Further, arginine-rich peptides showed considerably higher activity than a lysine-rich oligopeptide or polymer.

Figure 1a shows the experimental format for the strand exchange detection with fluorescence resonance energy transfer (FRET) method[3,12] using duplex which was labeled with the energy donor, fluorescein isothiocyanate (FITC; $E_x = 490$ nm, $E_m = 520$ nm) and energy accepter, carboxytetramethyl-rhodamine (TAMRA; $E_x = 530$ nm, $E_m = 570$ nm) (FT-duplex). In FT-duplex, donor's emission energy is transferred to the adjacent accepter, resulting in quenching of FITC emission. Donor's emission energy can recover only when FT-duplex was dissociated. Progress in the strand exchange reaction can be monitored by observing recovery of FITC emission. Cationic peptides used in this study are listed in Figure 1b.

Firstly, interpolyelectrolyte complexes (IPECs) formation between F-duplex and cationic peptides were observed. As

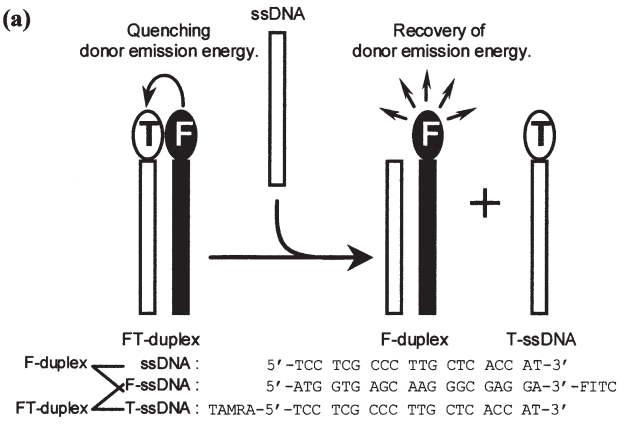

Figure 1. (a) Schematic representation of the FRET assay for strand exchange detection and DNA sequences used in the assay. F-Duplex and FT-duplex were prepared by annealing equal amount of corresponding ss DNA, respectively. (b) Cationic peptides used in this study. R16 is a synthetic peptide consisting of 16 amino acids. PLR50, PLL16, and PLL40 were poly(L-arginine) and poly(L-lysine) having degree of polymerization (Dp) of 50, 16, and 40, respectively.

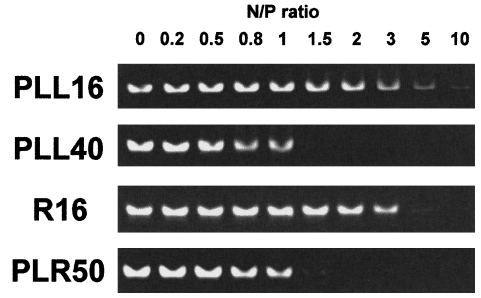

Figure 2. Electrophoresis assay evaluating IPECs formation. F-Duplex (1.5 μM, 14 μL) was mixed with 8 μL of each cationic peptide solution at various N/P ratios (0–10) and incubated at 37 °C for 30 min in 10 mM phosphate buffer (pH 7.2, 150 mM NaCl). After incubation, the mixtures were separated on 15% native polyacrylamide gel and FITC emission of free F-duplex was detected.

shown in Figure 2, with increasing polycation/DNA charge ratio (N/P ratio) the amount of free F-duplex decreased owing to IPEC formation. The titration points (the N/P ratios where free DNA disappeared) for PLR50 and PLL40 were nearly one, while those for oligopeptides, R16 and PLL16 were more than 3 and 5, respectively.

The strand exchange reaction in the presence of the cationic peptides was, then, performed according to the following procedure;

Step 1) Addition of peptides to the mixture of FT-duplex and homologous ss DNA,
Step 2) Incubation of the mixture at 37 °C for a proper time period (arginine peptides for 30 min and lysine peptides for 60 min) with stirring,
Step 3) Quenching the reaction and dissociation of the IPECs by sodium dodecyl sulfate (SDS) addition,
Step 4) Measurement of fluorescence intensity of F-duplex ($E_x = 490$ nm, $E_m = 520$ nm).

Because fluorescence of F-duplex was considerably quenched upon IPECs formation, fluorescence measurements (Step 3) were carried out after dissociation of the IPECs by SDS addition.

The value of % exchange degree was calculated with following equation:

$$\%\ \text{Exchange Degree} = (FI_t - FI_0)/(FI_\infty - FI_0) \times 100$$

(FI_0 is the initial fluorescence intensity, FI_t is that at time t, and FI_∞ is that after the reaction reached equilibrium. FI_∞ was practically obtained by measuring the mixture that had been annealed (heating at 90 °C for 5 min, followed by slow cooling to 37 °C)).

Figure 3 shows the results of the strand exchange performed at the N/P ratio of 2. PLR50 and R16 exhibited the strong accelerating effect, leading to the exchange degree of 91.9% and 76.8% with 30 min incubation. On the other hand, the presence of PLL40 or PLL16 resulted in a slight increase in the exchange degree despite of the longer incubation (60 min). Comparing at similar Dps (PLR50 vs PLL40 and R16 vs PLL16), the arginine-rich peptides were found to show higher acceleration activity than the lysine peptides. Peptide R16 showed approximately 1/50 accelerating activity of CCCs.

Under the higher salt concentration both peptides lost the accelerating activity. The ionic interaction between the peptides

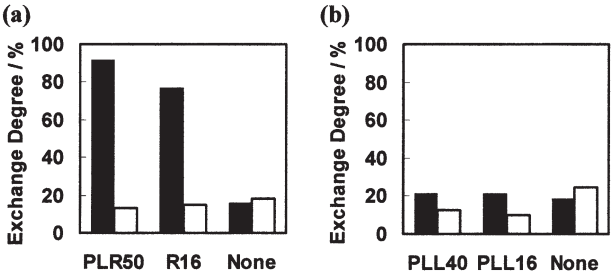

Figure 3. Strand exchange reaction with or without cationic peptides (N/P ratio = 2) in 10 mM phosphate buffer (pH 7.2) containing 150 mM NaCl (black bar) or 1000 mM NaCl (white bar). FT-Duplex (12 nM) and 5 molar excess ss DNA (60 nM) were incubated for (a) 30 min (arginine peptides) and (b) 60 min (lysine peptides).

and DNA is seemingly essential for exhibiting the acceleration activity. This consideration was further supported by the fact that polyhistidine (Dp ca. 86), a weakly basic peptides, showed slight activity under the same experimental condition (data not shown).

In summary, highly basic peptides were demonstrated to stimulate the strand exchange reaction. The arginine peptides showed considerably higher accelerating effect than the lysine peptides though both peptides similarly formed IPECs with DNA. Difference in actions between arginine and lysine peptides upon strand exchange acceleration has remained unsolved. Guanidium moieties of arginine peptides were reported to interact with DNA through not only electrostatic interaction but also hydrogen bonding interaction.[13] We speculate that the hydrogen bonding interaction between arginine peptides and DNA in the IPEC may influence the strand exchange acceleration.

We thank Profs. Hisakazu Mihara and Mitsuo Sekine for valuable discussion and assistance of MALDI-TOF mass spectrometry measurement. This work was supported in part by grant-in-aids (11167225 and 12480260) for scientific research from Ministry of Education, Culture, Sports, Science, and Technology of Japan.

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Published on the web (Advance View) April 25, 2003; DOI 10.1246/cl.2003.470