Characterization and application of the selective strand annealing activity of the N terminal domain of hepatitis delta antigen

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Abstract **We used synthetic DNA oligos to investigate the nucleic acid chaperone properties of the N terminal domain of hepatitis delta antigen (NdAg). We found that NdAg possessed a bona fide chaperone activity. NdAg could distinguish subtle differences in the thermal stability of the base pairing region, and enabled DNA oligos to form a more stable duplex among competing sequences through facilitating strand annealing selectively, stimulating duplex conversion selectively, and stabilizing the more stable duplex. The property of NdAg identified in this study could be applied to improve the efficiency and specificity of dot blot hybridization under conditions of low stringency.**

Keywords: Hepatitis delta antigen; Nucleic acid chaperone; Strand annealing; Hybridization

1. Introduction

RNA chaperones are proteins that aid in the process of RNA folding by preventing misfolding or by resolving misfolded species [1,2]. They belong to different classes of RNA binding proteins and do not share a common structural/sequence motif [2–13]. These proteins facilitate the formation of the more stable RNA structure and resolve folding trap by promoting the annealing of complementary sequences and by catalyzing strand exchange between a preformed duplex and competing sequences until the more stable structure is achieved [1,2,14]. RNA chaperones do not require ATP hydrolysis to function, and once the more stable RNA structure has been reached, their binding is no longer required to maintain the new structure [1,2]. However, the molecular mechanism underlying how chaperone proteins work remains to be elucidated.

Hepatitis delta antigen (HDAg), the only protein coded by hepatitis delta virus (HDV), is essential for viral replication and virion assembly [15–20]. HDAg has an RNA chaperone activity in vivo [21] and in vitro [22,23]. It can modulate the catalytic activity of HDV genome subfragments by altering RNA structure and stimulate hammerhead ribozyme catalysis by facilitating the annealing of ribozymes to cognate substrates in vitro. The RNA chaperone domain of HDAg resides in its N terminal 88 amino acids [22], referred to here as NdAg, which is rich in basic amino acids and interacts with a variety of nucleic acids [24]. NdAg can accelerate the annealing of complementary sequences selectively and promotes strand exchange selectively for the formation of a more extended duplex among competing sequences [23]. Moreover, NdAg acts on HDV RNA, complicated and simple non-HDV RNAs, and even DNA oligos [23,24]. Therefore, NdAg is a nucleic acid chaperone.

In this study, we used complementary DNA oligos to investigate the mechanism underlying the stimulatory effect of NdAg on more stable duplex formation. Factors that may determine the efficiency of the selective strand annealing process, such as the extent of internal mismatches, the relative stability of competing duplexes, the role of single-strand overhangs, and the optimum molar ratio of NdAg and nucleotide, were characterized. The potential application of the nucleic acid chaperone activity of NdAg on DNA hybridization was tested.

2. Materials and methods

2.1. Protein

NdAg that contained the first 88 amino acids of the HDAg and with a His-tag (MGSSH HHHHH SSGLV PRGSH) at the N terminus was expressed in *Escherichia coli* BL21 (DE3) cells and purified by phosphocellulose column chromatography [22]. NdAg was diluted with the protein dilution buffer (50 mM HEPES–NaOH (pH 7.9), 1 M NaCl, 1 mM EDTA, and 20% (v/v) glycerol) and used as 10× stock.

2.2. DNA

Tar(+) and Tar(−) were complementary DNA oligos corresponding to the HIV-1 TAR, their sequences were 5′-GGTCTCTCTT GTTAGACCAG GTCGAGCCCG GGAGCTCTCT GGCTAGCAAG GAACCC-3′ and 5′-GGGTTCCTTG CTAGCCAGAG AGCTCCCGGG CTCGACCTGG TCTAACAAGA GAGACC-3′, respectively [12,13]. Other DNA oligos of this study were listed in Table 1. DNA oligos were gel purified. The 5′ end labeled DNA was made using [γ-^{32}P]ATP and T4 polynucleotide kinase. DNA concentration was determined according to the absorbance at 260 nm, or by the radioactivity of DNA oligo and the specific activity of [γ-^{32}P]ATP. DNA oligos were heated separately, or together with their complementary DNA oligos (only for pre-annealed duplex preparation) at 95 °C for 5 min, cooled to room temperature, and incubated at the reaction temperature for at least 5 min before use.

2.3. Strand annealing and duplex conversion assays

Ten microliter reaction was performed in 1× reaction buffer (40 mM Tris–HCl (pH 7.5), 0.1 M NaCl, 0.1 mM EDTA, and 2% glycerol). Reactions were terminated by the addition of 2.5 μL of stop solution (50 mM EDTA (pH 8.0), 2.5% SDS, 25% glycerol, 0.01% xylene

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Table 1
DNA oligos used in this study

Name	Sequence	T_m of cd18 duplex (°C)
cd18	3′-TCTCGCGTCATGGTTTAT-5′	
d18	5′-AGAGCGCAGTACCAAATA-3′	57
$d18T_{5/10/15}$	5′-AGAGCGCAGTACCAAATA $(T)_{5/10/15}$-3′	
$T_{10}d18T_{10}$	5′-$(T)_{10}$AGAGCGCAGTACCAAATA $(T)_{10}$-3′	
d18m1	5′-AGtGCGCAGTACCAAATA-3′	52
d18m2	5′-AGAGCGCtGTACCAAATA-3′	52
$d18m2T_5$	5′-AGAGCGCtGTACCAAATA$(T)_5$-3′	
d18m3	5′-AGAGCGCAGTtCCAAATA-3′	52
d18m4	5′-AGAGCGCAGTACCtAATA-3′	52
d18m5	5′-AGtGCGCtGTACCAAATA-3′	48
d18m6	5′-AGtGCGCAGTtCCAAATA-3′	47
d18m7	5′-AGtGCGCAGTACCtAATA-3′	47
d18m8	5′-AGAGCGCtGTtCCAAATA-3′	46
d18m9	5′-AGAGCGCtGTACCtAATA-3′	47
d18m10	5′-AGAGCGCAGTtCCtAATA-3′	46
d18m11	5′-AGtGCGCtGTtCCAAATA-3′	<37
d18m12	5′-AGtGCGCtGTACCtAATA-3′	<37
d18m13	5′-AGtGCGCAGTtCCtAATA-3′	<37
d18m14	5′-AGAGCGCtGTtCCtAATA-3′	<37
d18m15	5′-AGtGCGCtGTtCCtAATA-3′	<37

cyanol, and 0.01% bromophenol blue). Different DNA species were resolved on a 8% (for Tar(+) and Tar(−) annealing assay) or 15% polyacrylamide gel (for other assays) in TBE buffer (89 mM Tris–HCl, 89 mM boric acid, and 1 mM EDTA) with 0.1% SDS at 4 °C. Gels were dried and autoradiographed.

2.4. T_m analysis

T_m analysis was carried out on a spectrophotometer (Beckman) equipped with a T_m analysis accessory. 0.5 μM of pre-annealed duplex in 40 mM Tris–HCl (pH 7.5) and 0.1 M NaCl was heated from 25 to 70 °C at the rate of 1 °C/min. The normalized $A_{260\ nm}$ values were plotted as a function of temperature for T_m estimation.

2.5. Dot blotting

DNA oligo resuspended in ddH_2O was heat denatured, chilled on ice, and applied onto 1.5 μL nylon membrane (Gene Screen), and then fixed by UV-cross linking at 0.225 J. The blot was soaked in 10 mM Tris–HCl (pH 8.0), 1 mM EDTA, 0.15 M NaCl, and 0.1% Triton X-100 at room temperature for 30 min and then rinsed with H_2O twice. Two hybridization buffers were prepared: the high ionic strength one contained 5× Denhardt's solution, 6× SSC (0.09 M NaOAc and 0.9 M NaCl), 0.5% SDS, and 100 μg/mL salmon sperm DNA; and the low ionic strength one contained 5× Denhardt's solution, 40 mM Tris–HCl (pH 8.0), 0.1 M NaCl, and 0.01% SDS. Hybridization reactions were performed in 3 cm diameter dishes for 60 min. The blot was washed with 6× SSC and 0.1% SDS for 15 min and autoradiographed.

3. Results and discussion

3.1. The competing duplex formation assay

We performed competing duplex formation reactions with DNA oligo cd18 and its complementary sequences d18 and d18 substitution mutants (Table 1) to investigate whether NdAg could promote more stable duplex formation among competing sequences.

The thermal stability of individual cd18 duplex was determined by measuring the melting temperature (T_m) of each pre-annealed duplex. The T_m analysis showed that d18/cd18 duplex had a T_m of ~57 °C in 40 mM Tris–HCl (pH 7.5) and 0.1 M NaCl; the disruption of one base pair at different locations decreased the T_m by ~5 °C; duplexes with two mismatched base pairs had a T_m ~10 °C lower than that of d18/cd18 duplex; and duplexes with more than two mismatched base pairs were not stable at all at 37 °C (Table 1).

To analyze the competing ability of different DNA oligos for cd18 duplex formation, we incubated limiting amounts of cd18 (1 nM) with two species of cd18 complementary DNA oligos (d18, d18m1–d18m7), 2 nM of each with one species labeled, for 1 h at 37 °C, which was below the T_ms of all potential duplexes. For reactions performed in the absence of NdAg, the formation of the labeled duplex varied only slightly by the competing, unlabeled DNA oligo for each species of labeled DNA oligo (the studies with d18m6 as the labeled species were shown in Fig. 1A and B). The result indicated that complementary DNA oligos annealed spontaneously and randomly at the nM level, and a d18 double mutant competed well with d18, d18 single mutants, and other d18 double mutants in annealing to cd18. However, in the presence of NdAg, the formation of labeled duplex varied significantly depending on the species of competing DNA oligos for each species of labeled DNA oligo. Competition reactions with labeled d18 showed that d18 was better than all d18 mutants in annealing to cd18, and competition reactions with labeled d18m3 or d18m6 showed that d18 and d18 single mutants were dominant over d18 double mutants in competition for annealing to cd18 (Fig. 1A and B). Therefore, the competing ability to form cd18 duplex in the presence of NdAg in general could be divided into three

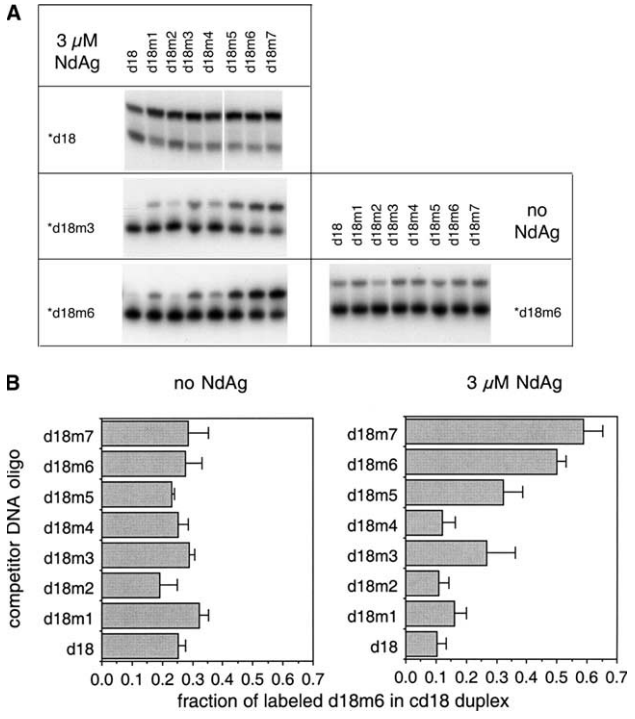

Fig. 1. The competition of DNA oligos for annealing to cd18. (A) 2 nM of a labeled DNA oligo and 2 nM of a DNA oligo of the same or another species were mixed with 2 nM of cd18 in the absence or presence of 3 μM NdAg at 37 °C for 60 min. Top bands are the duplex and bottom bands are free DNA oligo. (B) The fraction of labeled d18m6 participating in duplex formation of $\geqslant 3$ experiments are summarized.

groups with d18 > d18 single mutants > d18 double mutants. Moreover, if one looks in detail at the competition reactions involving labeled d18m3 as well as d18m6, one can see that the competing ability to form cd18 duplex of d18m2 and d18m4 was greater than that of d18m1 and d18m3, and the competing ability of three d18 double mutants had an order of d18m5 > d18m6 > d18m7 (Fig. 1A and B). These findings disclose that NdAg can stimulate more stable duplex formation among competing sequences, although the subtle difference in the thermal stability of different duplexes may not be obvious as assayed by T_m analysis.

3.2. Effect of NdAg on dot blot hybridization

We then studied whether the activity to promote more stable duplex formation of NdAg could be applied to improve the specificity and efficiency of dot blot hybridization. DNA oligos previously used in competition experiments (d18, d18m1–d18m7) together with more d18 mutants (d18m8–d18m15) were individually fixed on nylon membrane, and DNA blots were probed with limited amounts of labeled cd18 for 1 h in the presence of NdAg. Since NdAg bound nucleic acids with significantly lower affinity in buffer of high ionic strengths, i.e., >0.1 M NaCl [23] and proteins in general would be denatured at high temperatures, the dot blot hybridization reactions were performed in a low ionic strength buffer similar to the ones used for competition reactions that contained 0.1 M Na^+ (Section 2) (rather than in the traditional high ionic strength buffer that contained 1 M Na^+ (Section 2)) in the presence of NdAg at room temperature. The blots were then rinsed at 30 °C. In addition, because cross-linking of the DNA oligo to the nylon membrane might have affected the annealing of complementary sequences, we evaluated the relative thermal stability of different cd18 hybrids on the nylon membrane by performing dot blot hybridization reactions at elevated temperatures in hybridization buffers of low as well as high ionic strength, or alternatively by washing the nylon membrane at elevated temperatures post room temperature hybridization in either buffer, and then compared the radioactivity of different cd18 hybrids.

For the blot containing d18 single mutants only (22.5 pmol per dot), the probe (1.3 μM of labeled cd18, 1.3 pmol in 1 mL) hybridized predominantly to d18m2 and d18m4 when the hybridization reaction was performed at room temperature in both the high and the low ionic strength buffer in the absence of NdAg and when the DNA blot was washed at 30 or 40 °C post room temperature hybridization (Fig. 2A). However, with a higher hybridization temperature (40 °C) or/and a higher wash temperature (50 °C), d18m1 and d18m4 gave stronger hybridization signal than the other two DNA oligos (Fig. 2A). The results revealed that the cd18 hybrids of d18m1 and d18m4 were more stable than the cd18 hybrids of d18m2 and d18m3 on the nylon membrane. Therefore, in the case of d18 single mutants, the more stable cd18 hybrids identified by dot blot hybridization were not the same as the more stable cd18 duplexes identified by the competition assay (Fig. 1A and 1B). We speculate that the cross-linking of short DNA oligos to nylon membrane may slightly perturb the interaction between complementary sequences resulting in the differences in the relative thermal stability of cd18 hybrids on nylon membrane and cd18 duplexes in solution. It is notable that the probe hybridized predominantly to d18m1 and d18m4 when the hybridization reaction was carried out in the presence of NdAg in the low ionic strength buffer at room temperature followed by washing at 30 °C (Fig. 2A). Increase in the difference between the stronger and the weaker hybridization signals by the addition of NdAg to the hybridization reaction (Fig. 2A) suggested that NdAg could increase the specificity of hybridization reaction.

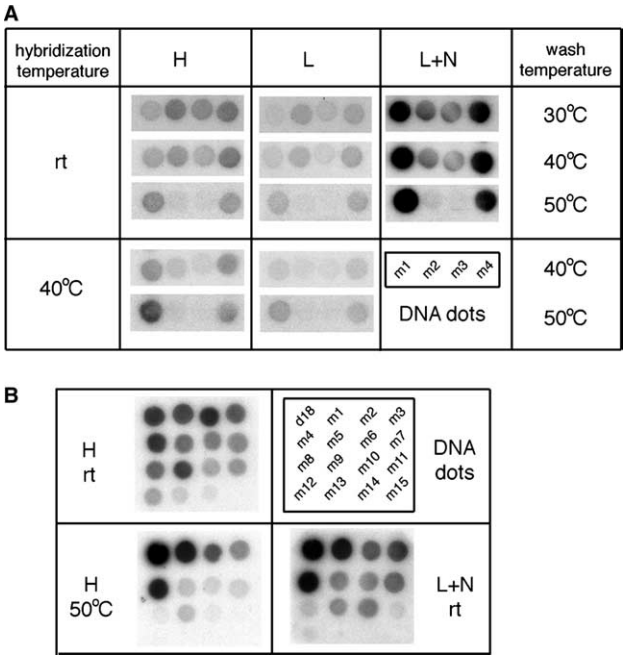

Fig. 2. The effect of NdAg on dot blot hybridization. (A) The blot containing 22.5 pmol of each d18 single mutant was probed with 1.3 pmole of probe (cd18) in 1 mL of the high ionic strength buffer (H), the low ionic strength buffer (L), or the low ionic strength buffer containing 5 μM NdAg (L + N) at room temperature (rt) or 40 °C for 1 h. The blot was then washed at different temperatures. (B) Sixteen DNA dots, 2.5 pmol of each, were arranged on the blot as illustrated. The blot was probed with 2 pmole of probe (cd18) in 1.5 mL of the high ionic strength buffer (H) at temperature (rt) or 50 °C for 1 h; or in 1.5 mL of the low ionic strength buffer containing 2 μM NdAg (L + N) or not (L) at room temperature (rt) for 1 h. Each blot was washed at 30 °C.

We prepared another blot that contained d18 and d18m1–d18m15, 2.5 pmol of each. d18, d18m1, d18m2, d18m4, and d18m9 gave stronger hybridization signals when hybridized in high ionic strength buffer at room temperature with labeled cd18 as the probe (1.3 μM, 2 pmol in 1.5 mL) (Fig. 2B). However, the hybridization signals of d18m2 and d18m9 were much weaker but d18, d18m1, and d18m4 gave similar signal intensities as before when the hybridization temperature was raised to 50 °C (Fig. 2B), indicating that d18, d18m1, and d18m4 formed more stable cd18 hybrids. Only d18, d18m1, and d18m4 hybridized strongly to the probe when the hybridization reaction was performed in the presence of 2 μM NdAg in low ionic strength buffer at room temperature (Fig. 2B). These results confirmed that in the presence of NdAg, the formation of the more stable cd18 hybrids was promoted over that of relatively weaker cd18 hybrids even under conditions of low stringency. Thus, promoting more stable duplex formation among competing sequences of NdAg can increase the specificity and the efficiency of dot blot hybridization.

3.3. NdAg stabilized the more stable duplex

To assess how NdAg promotes more stable duplex formation between cd18 and cd18 complementary sequences, we first investigated the effect of NdAg on the dissociation of a preformed cd18 duplex through the action of a competing DNA oligo. We found that in the absence of NdAg, the pre-annealed labeled d18/cd18 and labeled d18m3/cd18 duplexes dissociated extensively during the 30 min incubation with an excess amount of d18 or d18m3 that prevents the re-annealing of the dissociated labeled DNA oligo (Fig. 3, lanes 1–5, 11–15 and data not shown). However, in the presence of NdAg, the labeled d18/cd18 duplex became stable even if the reaction contained an excess amount of d18m3 (Fig. 3, lanes 6–10 versus 1–5). In contrast, the labeled d18m3/cd18 duplex dissociated at an elevated rate when the reaction contained an excess amount of d18 in the presence of NdAg (Fig. 3, lanes 16–20 versus 11–15). These results showed that both the perfectly matched duplex and the mismatched duplex are quite unstable in the absence of NdAg, and that NdAg selectively stabilized the pre-annealed duplex if the competing DNA oligo can only form a less stable duplex with cd18 than the pre-annealed duplex.

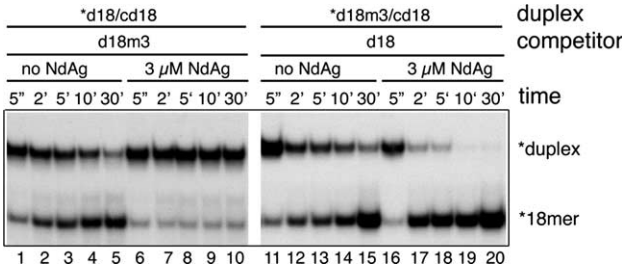

Fig. 3. NdAg stabilized the more stable duplex. A pre-annealed labeled duplex (d18/cd18 or d18m3/cd18) that was composed of 0.25 nM labeled top strand and 2.5 nM of cd18 was mixed with a competing DNA oligo (75 nM of d18 or d18m3) in the absence or presence of 3 μM NdAg at 37 °C for various periods of time. *18mer is the dissociated labeled top strand and *duplex is the remaining labeled duplex.

3.4. NdAg stimulated duplex conversion

We then set up an assay to simultaneously investigate the effect of NdAg on duplex dissociation and strand annealing. The assay included six DNA oligos with labeled cd18 as the bottom strand (2.5 nM) and five competing oligos that were either completely complementary to cd18 or contained a substitution, that causes a mismatched base pair in cd18 duplex, and had a dT tail of different lengths at the 3′ terminus or both termini (d18m2, d18m2T_5, d18T_{10}, d18T_{15}, and T_{10} d18 T_{10}, and T_n represents the length of dT tail) as the top strands (10 nM of each). Almost all cd18 participated in duplex formation in the absence of NdAg probably due to the relatively high DNA concentration, while two shorter DNA oligos (the ones with substitution) hybridized to cd18 more efficiently than three longer DNA oligos (the ones without substitution) with no obvious duplex conversion during the 60 min incubation period (Fig. 4A, lanes 1–4). The labeled cd18 distributed almost equally to different duplexes when the concentration of cd18 and cd18 complementary DNA oligos was elevated by a factor of 50 (Fig. 4B). These results showed that the preferential formation of the perfectly matched cd18 duplexes did not occur spontaneously at a wide range of DNA concentrations although high DNA oligo concentration would shift the equilibrium of the single-strand ↔ double strand transition.

When 5 μM NdAg was added to the reaction with 2.5 nM of cd18 and 10 nM of each top strand, two mismatched duplexes

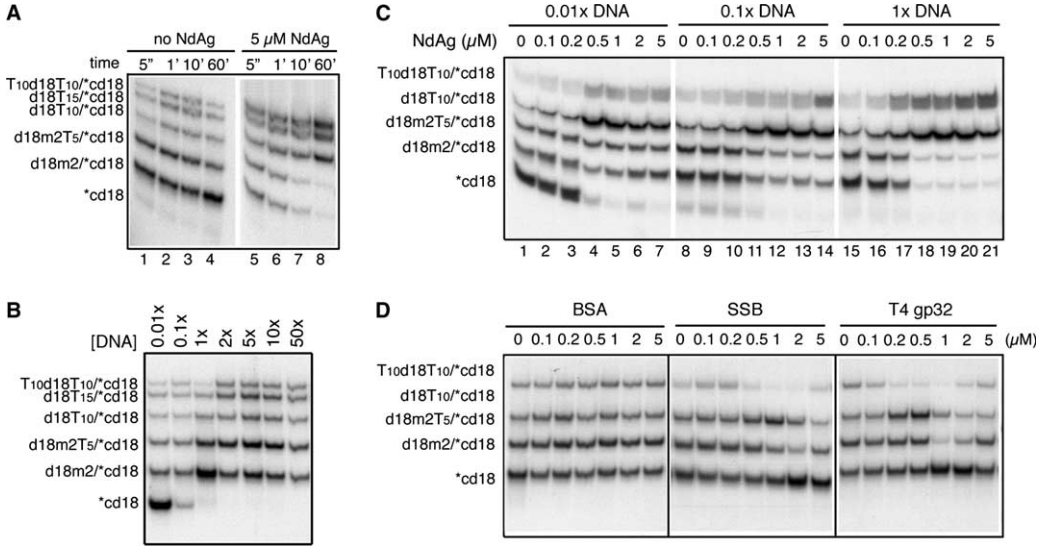

Fig. 4. NdAg promotes DNA duplex conversion. (A) Labeled cd18 (2.5 nM) was incubated with a mixture of five DNA oligos (10 nM of each) in the absence or presence of 5 μM NdAg at 37 °C for various periods of time. (B) DNA duplex formation in the absence of NdAg at 37 °C for 60 min at various DNA concentrations. DNA concentration of the "1×" condition was the same as that of (A) and DNA concentration was varied by a factor of 0.01-fold to 50-fold. (C) Labeled cd18 (the top strand) was incubated with a mixture of four DNA oligos (the bottom strands) in the presence of the indicated concentration of NdAg at 37 °C for 60 min. The DNA oligo concentrations were: 0.025 nM bottom strand and 0.1 nM each top strand (~0.011 μM nucleotide in total) for lanes 1–7, 0.25 nM bottom strand and 1 nM each top strand (~0.11 μM nucleotide in total) for lanes 8–14, and 2.5 nM bottom strand and 10 nM each top strand (~1.1 μM nucleotide in total) for lanes 15–21. (D) Labeled cd18 (2.5 nM) was incubated with a mixture of four DNA oligos (10 nM of each) in the presence of the indicated concentration of BSA, T4 gp32, or *E. coli* SSB at 37 °C for 60 min.

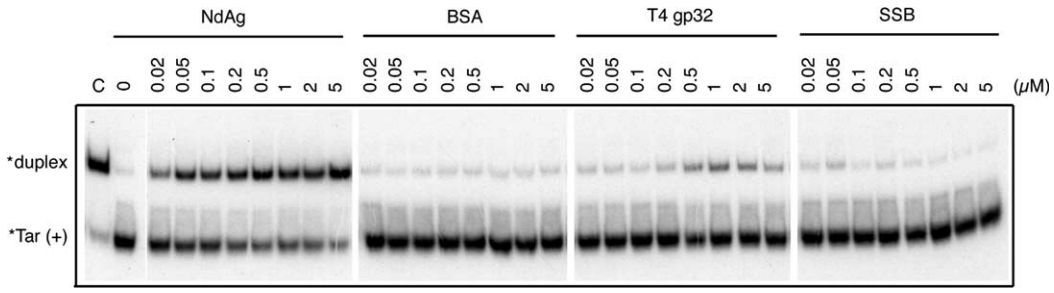

Fig. 5. NdAg promotes strand annealing. Labeled Tar(+) DNA (1 nM) and Tar(−) DNA (1 nM) were incubated with various concentrations of NdAg, T4 gp32, or *E. coli* SSB at 37 °C for 10 min. The control hybridization (C) was performed at 65 °C for 30 min in the absence of any protein. *Tar DNA is the free Tar(+) DNA and *duplex is the Tar(−)/Tar(+) hybrid.

(d18m2/cd18 and d18m2T_5/cd18) progressively converted to three perfectly matched duplexes (d18T_{10}/cd18, d18T_{15}/cd18, and T_{10} d18T_{10}/cd18), and the labeled cd18 hybridized predominantly to completely complementary DNA oligos after 60 min incubation with NdAg regardless of the location and the length of dT tail (Fig. 4A, lanes 5–8). Thus, NdAg could promote more stable duplex formation through catalyzing duplex conversion. The selective duplex conversion activity of NdAg is determined by the stability of the potential base-paired region only and is not altered when the duplex has single-stranded overhang(s) even though NdAg has a higher affinity to longer nucleic acids due to the cooperative interaction of NdAg molecules [24].

The duplex conversion assay was performed under a variety of conditions to determine the optimum conditions in which NdAg would stimulate more stable duplex formation. In reactions containing cd18 (2.5 nM), d18m2, d18m2T_5, d18T_{10}, and T_{10} d18T_{10} (10 nM of each), and 0.5–5 μM NdAg, the formation of perfectly matched duplexes was dominant over the formation of mismatched duplexes (Fig. 4C, lanes 18–21). However, predominant formation of perfectly matched duplexes was not so obvious when reactions were performed with the same concentrations of DNA oligos (the total nucleotide concentration of five DNA oligos in the reaction was ~1.1 μM) but the NdAg concentration was reduced to less than 0.5 μM (Fig. 4C, lanes 15–17). This was also true when NdAg was kept at greater than 0.5 μM but the DNA oligo concentration was reduced by one or two orders of magnitude (to ~0.11 or ~0.011 μM nucleotide in total, Fig. 4C, lanes 1–14). Furthermore, BSA or nucleic acid binding proteins, such as T4 gp32 and *E. coli* SSB, did not stimulate duplex conversion for the formation of perfectly matched duplexes under the same reaction conditions (Fig. 4D). These results showed that NdAg could promote more stable duplexes formation through catalyzing duplex conversion and the selective duplex conversion process occurred when the nucleotide and protein concentrations were both at M level and at a nucleotide-to-NdAg ratio of around 0.2 ~2 to 1.

3.5. NdAg possessed DNA annealing activity

To further assess the strand annealing activity of NdAg, the ability of the protein to stimulate the annealing of complementary DNA oligos that hardly hybridized spontaneously was investigated. To this end, complementary DNA oligos Tar(−) and Tar(+) that had intramolecular interactions and were utilized by other researchers for identifying the nucleic acid chaperone activity of a variety of nucleic acid binding proteins [12,13] were incubated with NdAg and Tar(−)/Tar(+) hybrid formation was analyzed after removing the protein by SDS. The results showed that NdAg promoted Tar(−)/Tar(+) hybrid formation, whereas BSA, T4 gp32, as well as *E. coli* SSB of the same concentrations were not able to stimulate Tar(−)/Tar(+) hybrid formation (Fig. 5). These results reveal that the strand annealing activity of NdAg is not simply due to molecular crowding or charge neutralization effect, and that NdAg possesses a bona fide chaperoning activity.

4. Conclusion

NdAg containing the N terminal domain of NdAg is a general nucleic acid binding protein [24] and NdAg helps a variety of RNA molecules to form more stable intra- or intermolecular structure by acting as an RNA chaperone [23]. In this study, we used synthetic DNA oligos to further investigate the nucleic acid chaperone activity of NdAg. We demonstrated that NdAg promoted the annealing of complementary DNA oligos whether they were highly structured or not, and NdAg directed more stable duplex formation among competing sequences by facilitating strand annealing selectively, by stabilizing the more stable duplex selectively, as well as by catalyzing duplex conversion selectively. NdAg stimulated the dissociation of the less stable duplex to form the more stable duplex, and the dissociation of the initial duplex and the formation of the final duplex appeared to occur simultaneously. The efficiency of the duplex conversion reaction in the presence of NdAg, as revealed by the competition annealing assay, was dependent upon the relative thermal stability of the competing duplexes, the bigger the difference the more efficient the duplex conversion reaction. Therefore, NdAg possessed a bona fide chaperone activity, while the molecular mechanism underlying the nucleic acid chaperone activity of NdAg remained to be explored.

The ability of NdAg to mediate more stable duplex formation among competing sequences and catalyze strand annealing in the presence of non-homologous nucleic acids [23] implied that this nucleic acid chaperone was useful for biotechnological applications. In this report, we showed that NdAg elevated the specificity and efficiency of DNA hybridization under low stringency conditions, i.e., in low ionic strength buffer and at low temperature. These findings disclosed that NdAg would be able to improve the accuracy of nucleic acid annealing for reverse transcription reactions, primer extension reactions, polymerase chain reactions, and other kinds of

hybridization reactions, such as Northern, Southern, and in situ hybridizations. However, the loss of the nucleic acid chaperone activity at high ionic strength of NdAg [24] and the narrow window of nucleotide-to-NdAg ratio required for promoting selective strand exchange would limit the potential application of this protein.

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