Biochemistry

 Volume 29, Number 48 December 4, 1990

Accelerated Publications

Studies of the Strand-Annealing Activity of Mammalian hnRNP Complex Protein A1

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Received July 24, 1990; Revised Manuscript Received September 18, 1990

ABSTRACT: A1 is a major core protein of the mammalian hnRNP complex, and as a purified protein of ~34 kDa, A1 is a strong single-stranded nucleic acid binding protein. Several lines of evidence suggest that the protein is organized in discrete domains consisting of an N-terminal segment of ~22 kDa and a C-terminal segment of ~12 kDa. Each of these domains as a purified fragment is capable of binding to both ssDNA and RNA. We report here that A1 and its C-terminal domain fragment are capable of potent strand-annealing activity for base-pair complementary single-stranded polynucleotides of both RNA and DNA. This effect is not stimulated by ATP. Compared with A1 and the C-terminal fragment, the N-terminal domain fragment has negligible annealing activity. These results indicate that A1 has biochemical activity consistent with a strand-annealing role in relevant reactions, such as pre-mRNA splicing.

A1 is one of the major core proteins of the mammalian hnRNP particle, and the purified protein is able to bind stoichiometrically and selectively to single-stranded conformations of RNA and DNA (Kumar et al., 1986; Cobianchi et al., 1988; Merrill et al., 1988). A1 is a basic protein of ~34 kDa with a blocked N-terminus. Its amino acid composition is characterized by the presence of the unusual amino acid dimethylarginine and high glycine content. The sequence of the A1 protein is highly conserved from rat, bovine, and human sources and is structurally related to other core hnRNP proteins (Cobianchi et al., 1986; Kumar et al., 1986; Riva et al., 1986). A1 is organized in two well-defined domains, and this property has been probed with recombinant rat A1 by controlled proteolysis (Kumar et al., 1990). The protease-resistant N-terminal domain of 195 amino acids has an internal repeat corresponding to residues 1–92 and 93–184, respectively (Williams et al., 1985); each repeat contains two short peptide sequences common in many RNA binding proteins (Bandziulis et al., 1989). The C-terminal domain of ~124 amino acids is composed of 12 imperfect repeated units with the consensus sequence of $GN^{F}_{Y}GG^{S}_{G}RG$ (Cobianchi et al., 1986, 1988).

Intact A1 binds far more tightly to single-stranded nucleic acids than the purified N-terminal fragment. Binding by A1 is cooperative, whereas the N-terminal fragment binds noncooperatively. Yet, the difference in binding affinity is not fully accounted for by this cooperativity (Cobianchi et al., 1988; Kumar et al., 1990). Further, the N-terminal fragment is a helix-destabilizing protein for double-stranded DNA (Herrick & Alberts, 1976; Herrick et al., 1976; Planck & Wilson, 1980) whereas A1 has no T_m depression activity, except under specialized conditions (Kumar et al., 1986, 1987; Nadler et al., 1990). Although the mechanism of interdomain interactions is unknown, these results have suggested that the C-terminal domain contributes to the binding properties of intact A1 by providing cooperativity and a portion of the intrinsic binding free energy (Cobianchi et al., 1988). This idea was recently supported by studies of a purified C-terminal domain fragment. The fragment was found to bind strongly to the single-stranded nucleic acid with positive cooperativity, indicating that the intact A1 protein has at least two functional nucleic acid binding domains that could potentially act in a modular fashion (Kumar et al., 1990).

The picture of more than one binding pocket in a single-stranded nucleic acid binding protein is reminescent of the *Escherichia coli* recA protein among others. recA is a multifunctional DNA binding protein of 38 kDa with a nucleotidyl transferase activity (ATPase) and several types of experimentally defined nucleic acid binding activities, such as single-stranded and double-stranded DNA lattice binding capacity (Sancar et al., 1980; Craig & Roberts, 1981). The protein can simultaneously bind more than one nucleic acid strand and also can promote strand annealing for homologous

single-stranded DNA lattices (Radding, 1982; Cox & Lehman, 1981; Soltis & Lehman, 1983).

We recently learned that the A1 protein is able to facilitate interstrand reannealing of DNA,[1] and in this paper we report a further examination of this activity with emphasis on the question of functional roles of the two A1 domains. This activity appears to reside in the C-terminal domain of A1, since strand annealing is seen with the purified C-terminal fragment, whereas the N-terminal fragment does not demonstrate this property. The A1 strand-annealing activity is different from that of recA, since A1 carries out RNA·RNA strand annealing in contrast to recA. The relevance of this strand-annealing activity for A1 is discussed.

Experimental Procedures

Materials. ϕX174 plus strand DNA and the 16-residue synthetic oligodeoxynucleotide were as previously described (Abbotts et al., 1988). T4 polynucleotide kinase and the NICK column were obtained from Pharmacia. Restriction enzymes and T7 RNA polymerase were obtained from Boehringer Mannheim. [γ-^{32}P]ATP (7000 Ci/mmol) and [α-^{32}P]UTP (3000 Ci/mmol) were obtained from Amersham. Electrophoresis grade acrylamide and bis(acrylamide) were obtained from Bio-Rad Laboratories. Recombinant rat A1 was purified to homogeneity from *E. coli* RRI (pRK248 cIts, pEX11) as described by Cobianchi et al. (1988). The protein was digested with V8 protease, and the N- and C-terminal fragments were isolated as described by Kumar et al. (1990). DNA and RNA concentrations are expressed in molecular units rather than as nucleotides. Concentrations were measured spectrophotometrically.

Assay of Annealing Activity Using Homologous ssDNA. The ~180-bp promoter fragment (−114 to +62) of the human β-polymerase gene (Widen et al., 1988) was 5′ end labeled with ^{32}P by the procedure described by Maxam and Gilbert (1980). Free [γ-^{32}P]ATP was removed by passing the kinase reaction mixture over a NICK column (Pharmacia) following the manufacturer's suggested protocol. The DNA was boiled for 15 min and cooled immediately with vigorous shaking in an ice bath. The reaction mixture in a final volume of 20 μL contained 20 mM Tris-HCl, pH 7.5, 40 mM NaCl, 50 μg/mL BSA, 5% glycerol, 2 mM EDTA, ^{32}P-labeled ssDNA (0.8×10^{-9} M), and A1 or N- or C-terminal fragments (22.5×10^{-9}, 500×10^{-9}, or 37.5×10^{-9} M, respectively). The mixture was incubated for 5 min at 25 °C, and the samples were run immediately on a 6% nondenaturing polyacrylamide gel in 0.5× TBE buffer at 14 mA for 2 h. After electrophoresis, the gel was transferred to Whatman 3 MM paper and covered with Saran wrap. Products were visualized by autoradiography with Kodak XAR-5 film at −70 °C.

Assay of Annealing Activity Using ϕX174 Plus Strand DNA and Homologous Oligodeoxynucleotide. ϕX174 plus strand DNA and a 16-residue synthetic oligodeoxynucleotide complementary to position 618–633 as shown here were tested for annealing activity: -(610)CTGTTGAGTTTATTGC-TGCCGTCATTGCTTA(640)- and AAATA-ACGACGGCAGT. This 16-mer oligodeoxynucleotide was 5′ end labeled with ^{32}P (Maxam & Gilbert, 1980). A 10-μL reaction mixture contained 0.15×10^{-9} M ϕX174 plus strand DNA, 0.17×10^{-9} M ^{32}P-labeled oligodeoxynucleotide, 20 mM Tris-HCl, pH 7.5, 40 mM NaCl, 50 μg/mL BSA, 5% glycerol, 2 mM EDTA, and 0.3×10^{-6}, 0.4×10^{-6}, or 0.4×10^{-6} M A1 or N- or C-terminal fragments, respectively. The mixture was incubated 5 min at 25 °C, and the samples were run immediately on a 0.8% agarose gel in TBE buffer at 60 V for 2 h. After electrophoresis, the gel was dried on DE-81 paper (Whatman) and exposed to Kodak XAR-5 film. In some experiments, the amount of protein added to the reaction mixture was altered as indicated. Annealing of the 16-residue ^{32}P-labeled oligodeoxynucleotide to the ϕX174 DNA did not alter the mobility in the gel of the ϕX174 DNA, as revealed by ethidium bromide staining.

Assay of Annealing Activity Using ϕX174 Plus Strand DNA and Nonhomologous Oligodeoxynucleotide. A 66-residue oligodeoxynucleotide was 5′ end labeled with ^{32}P; this oligonucleotide is not homologous to the ϕX174 plus strand DNA. A reaction mixture in final volume of 10 μL contained 3×10^{-9} M ϕX174 plus strand DNA and 3×10^{-9} M ^{32}P-labeled 66-residue oligodeoxynucleotide; the other components and the procedure were as described above for annealing of ϕX174 plus strand DNA and the homologous oligodeoxynucleotide.

Assay of Annealing Activity Using Homologous RNA. (*a*) *Preparation of RNA.* RNAs were transcribed from cloning vectors pTZ18R and pTZ19R obtained from U.S. Biochemical Corp. The plasmid pTZ18R was linearized with *Pvu*I, while linearization of pTZ19R was achieved with *Eco*RI or *Sal*I. Transcription using plasmid pTZ18R was carried out in the presence of unlabeled nucleotides, while plasmid pTZ19R was transcribed in the presence of [α-^{32}P]UTP according to the procedure described by Melton et al. (1984). The RNAs were purified by NICK columns following the manufacturer's suggested protocol. RNA transcribed from plasmid pTZ18R is 175 residues long and is complementary to the labeled 55-residue- or 22-residue-long RNA transcribed from plasmid pTZ19R linearized with *Eco*RI or *Sal*I, respectively.

(*b*) *Annealing Assay.* A typical 10-μL reaction mixture contained 5×10^{-9} M 175-residue RNA and 7×10^{-9} M [^{32}P]UMP-labeled 55-residue or 22-residue RNA; the other components and the procedure were as described for annealing of ϕX174 plus strand DNA and the homologous oligodeoxynucleotide. The mixture was incubated for 5 min at 25 °C and the samples were run on a 6% nondenaturing polyacrylamide gel in 0.5× TBE buffer at 14 mA for 2 h.

Results

A1 Promotes Reassociation of Complementary ssDNA. E. coli RRI (pRK248 CIts, pEX11) overexpresses rat hnRNP complex protein A1 and is a source for A1 purified under nondenaturing conditions to homogeneity (Cobianchi et al., 1988). By use of controlled proteolysis with V8 protease, two domain fragments were prepared (Kumar et al., 1990). These are an ~22-kDa N-terminal fragment (residues 1–185) and an ~12-kDa C-terminal fragment corresponding to residues 186–319. These purified protein preparations were analyzed by SDS–polyacrylamide gel electrophoresis, as shown in Figure 1a, and were free of contaminating proteins of M_r greater than A1 and in particular of recA protein at M_r = ~38 000. The proteins also were examined for annealing activity in a preliminary experiment with a short DNA restriction fragment. When this ~180-bp duplex DNA was denatured by heating at 95 °C and allowed to cool rapidly on ice, the DNA remained single stranded. The single- and double-stranded forms migrate differently in our gel system (Figure 1b, lanes 1 and 2). Using this gel assay to distinguish dsDNA and ssDNA, we evaluated whether A1 and its fragments could promote annealing of the complementary ssDNA strands. It is evident from Figure 1b, lane 3, that when A1 was incubated with this ssDNA, dsDNA was formed. The N-terminal and C-terminal fragments of A1 also were examined in this assay; the C-

[1] Brian Pontius and Paul Berg, personal communication.

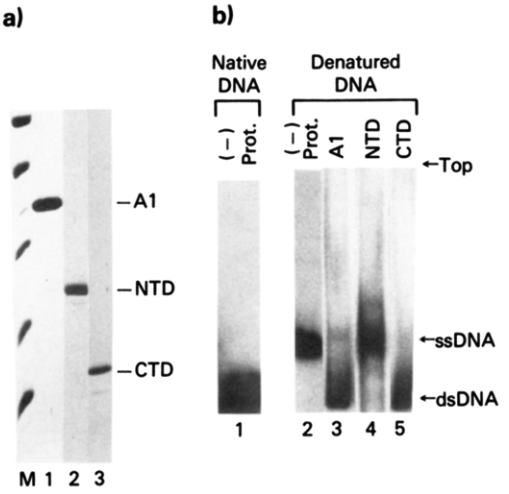

FIGURE 1: Analysis of A1 and the N-terminal and C-terminal fragments. (Panel a) Photograph of the Coomassie blue stained 12.5% SDS–polyacrylamide gel analysis of A1 and its fragments. A1 and the N-terminal (NTD) and C-terminal (CTD) fragments were purified and analyzed as described by Kumar et al. (1990), and 5 μg of protein was used for lanes 1–3. Marker proteins and molecular weights were bovine serum albumin (66.2), ovalbumin (42.7), carbonic anhydrase (31), soybean trypsin inhibitor (21.5), and lysozyme (14.4). (Panel b) Strand-annealing activity using base-pair complementary single-stranded DNA. A photograph of an autoradiogram is shown. The denatured ~180-bp restriction fragment was incubated in the absence or presence of proteins, as indicated at the top and as described under Experimental Procedures. The samples were applied to a 6% nondenaturing polyacrylamide gel as described under Experimental Procedures. The left-hand lane contained duplex DNA incubated in the absence of protein.

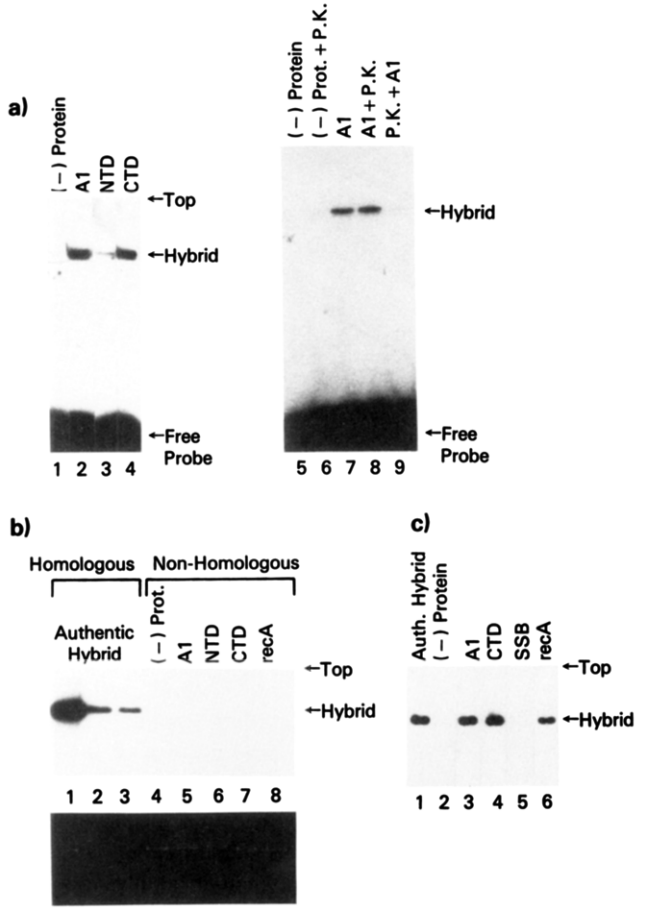

FIGURE 2: Composite showing annealing with homologous or nonhomologous oligodeoxynucleotides and ssDNA. Photographs of autoradiograms are shown. (Panel a) Annealing of φX174 plus strand DNA and the complementary ^{32}P-labeled 16-residue oligodeoxynucleotide in the absence or presence of proteins as indicated at the top (lanes 1–4). Incubation conditions were 0.3×10^{-6} M A1, 0.4×10^{-6} M NTD, or 0.4×10^{-6} M CTD as described under Experimental Procedures. Lane 5 represents an annealing reaction in the absence of protein. In lanes 6, 8, and 9, samples were treated with proteinase K (P.K.). In lanes 6 and 8, after the annealing reaction, samples were incubated with 0.2 μg/mL proteinase K at 25 °C for 15 min. Lane 7 indicates an annealing reaction in the presence of A1. In lane 9, A1 was digested first with proteinase K as above and then added to the annealing reaction mixture. (Panel b) Analysis of the nonhomologous ^{32}P-labeled 66-residue oligodeoxynucleotide and φX174 plus strand DNA (Lanes 4–8). As a control for the experiment, in lanes 1–3, the homologous 16-residue oligodeoxynucleotide was used. Lane 1, 3×10^{-9} M 16-mer and φX174 DNA boiled at 90 °C for 2 min and cooled slowly at 25 °C; lanes 2 and 3, 0.25×10^{-9} M 16-mer and φX174 DNA incubated at 25 °C for 5 min; lanes 4–8, 3×10^{-9} M nonhomologous oligodeoxynucleotide and φX174 in the presence or absence of proteins as indicated at the top. In the lower panel, a photograph of the ethidium bromide stained gel is shown; the band corresponds to φX174 DNA. (Panel c) Analysis of *E. coli* proteins using homologous ^{32}P-labeled 16-mer and φX174 DNA. Oligodeoxynucleotide and φX174 DNA were incubated in the absence or presence of proteins as indicated at the top of the gel (lanes 2–6). Lane 1 is same as described in panel b, lane 2 or 3. The amounts of protein used in the incubations for panels b and c were as follows: A1, 0.3×10^{-6} M; N-terminal fragment, 0.4×10^{-6} M; C-terminal fragment, 0.4×10^{-6} M; SSB 0.3×10^{-6} M; recA, 0.3×10^{-6} M. Unless otherwise indicated, incubations for all the lanes in this figure had 0.17×10^{-9} M homologous oligodeoxynucleotide and 0.15×10^{-9} M φX174 DNA.

terminal fragment was able to promote annealing of the ssDNA (Figure 1b, lane 5), whereas the N-terminal fragment was not (Figure 1b, lane 4).

Annealing of Complementary Oligodeoxynucleotide and ssDNA. Hybridization of complementary ssDNA was further examined by using a single-stranded circular phage DNA (φX174) and several synthetic oligodeoxynucleotides. Gel electrophoresis was employed to measure annealing activity by the transfer of signal from the oligonucleotide position in the gel to the position of the much larger phage DNA. The migration position of the annealed labeled oligonucleotide was coincident with the position of φX174 DNA, as revealed by autoradiography and staining of the gel with ethidium bromide (e.g., see Figure 2b, lane 1). Spontaneous hybridization was seen at the concentration of 3×10^{-9} M each φX174 plus strand DNA and the complementary 16-residue oligodeoxynucleotide, with boiling at 90 °C for 2 min followed by slow cooling (Figure 2b, lane 1), and spontaneous hybridization also was seen following simple addition of 0.25×10^{-9} M each DNA and oligodeoxynucleotide to the usual incubation mixture at 25 °C (Figure 2b, lane 2 or 3). In experiments not shown, the incubation time and concentration requirements for spontaneous annealing appeared to follow approximate second-order kinetics, as expected. When the DNA concentrations were lowered, the hybridization signal was significantly reduced. At concentrations of 0.15×10^{-9}, 0.06×10^{-9}, and 0.045×10^{-9} M for φX174 DNA and oligodeoxynucleotide, no spontaneous hybridization signal was detected (data not shown). All the experiments described below were carried out with 0.15×10^{-9} M φX174 plus strand DNA and 0.17×10^{-9} M complementary oligodeoxynucleotide.

After finding conditions where no hybridization signal is detected in the absence of protein, we examined whether A1 and the two fragments could promote annealing of the φX174 plus strand DNA and the base-pair complementary 16-residue oligodeoxynucleotide. Results in Figure 2a demonstrate that annealing activity was observed with A1 and the C-terminal fragment, whereas the N-terminal fragment had no such activity under the conditions used. Annealing activity or transfer

of label to the hybrid position in the gel was not observed when ϕX174 DNA was omitted from the incubation; addition of A1 to the preannealed hybrid did not alter its migration position in the gel (data not shown). The migration position of the hybrid detected in the presence of A1 did not change when the incubation was digested with proteinase K (Figure 2a, lanes 5–9), indicating that the putative hybrid signal once formed did not depend upon the presence of A1. Therefore, we conclude from these experiments that A1 and the C-terminal fragment have the ability to facilitate annealing of complementary DNA strands. The very slight increase in hybrid formation seen with the N-terminal domain fragment (Figure 2a, lane 3) could mean that the fragment has low, marginal activity compared with A1 and the C-terminal fragment under our conditions. Alternatively, the N-terminal fragment could have been contaminated with a slight amount of A1 or C-terminal fragment. We have no further information at the moment on these possibilities.

Annealing of Nonhomologous Oligodeoxynucleotide and ssDNA. ϕX174 plus strand DNA and a nonhomologous 66 residue long oligodeoxynucleotide were incubated with A1 or N-terminal or C-terminal fragments, and the results are depicted in Figure 2b, lanes 5–7, respectively. Under these conditions in each case, no hybridization signal was detected by autoradiography. Ethidium bromide staining of this gel demonstrated that the ϕX174 DNA migrated to the same position with or without annealed oligonucleotide and that ϕX174 DNA migrated to about the same position in the presence and absence of the proteins (Figure 2b, lower panel). These results indicate that strand-annealing activity is specific for base-pair complementary DNA, as expected.

Annealing Activity Using E. coli Proteins. We also examined the two reference DNA binding proteins, *E. coli* ssDNA binding protein (SSB) (Sancar et al., 1981; Chase & Williams, 1986) and *E. coli* recA protein (Sancar et al., 1980; Radding, 1982). As shown in the Figure 2c, A1 and its C-terminal fragment and recA all have the ability to anneal a base-pair complementary oligodeoxynucleotide to ϕX174 plus strand DNA. This experiment was conducted at a similar concentration for each protein, and ATP was not included in the binding mixture. However, in case of SSB, no hybridization signal was observed. These results indicate that the annealing activity of A1 in some respects is similar to that of recA, which is a well-studied DNA strand-annealing protein. We did not observe any effect of including 1 mM ATP on the annealing activities of A1 or the C-terminal fragment reported here (data not shown).

Annealing Activity as a Function of Protein Concentration and Time. To evaluate the capacity of A1 proteins to enhance annealing activity in a concentration-dependent fashion, different levels of A1 and the C-terminal fragment were used in the reaction mixture (Figure 3a). At 20×10^{-9}, 30×10^{-9}, and 60×10^{-9} M A1, annealing activity increases in a concentration-dependent manner, where the protein:nucleotide ratio was 1:40, 1:27, and 1:13, respectively. Similar results were obtained when the C-terminal fragment was added in increasing amounts to the annealing mixture (Figure 3a); for the C-terminal fragment, more protein is required in comparison to A1, and the protein:nucleic acid ratios are 1:11, 1:5.5, and 1:2.6, respectively, at the C-terminal fragment concentrations used in Figure 3a. These results indicate that A1 and the C-terminal fragment carry out strand annealing in a protein-concentration-dependent manner and intact A1 appears to be more active than the C-terminal fragment on a molar basis. The time course of annealing is shown in Figure 3b. A1 increases the rate of annealing by at least 200-fold, since the respective $t_{1/2}$ values are approximately 5400 and $\leq$ 30 s.

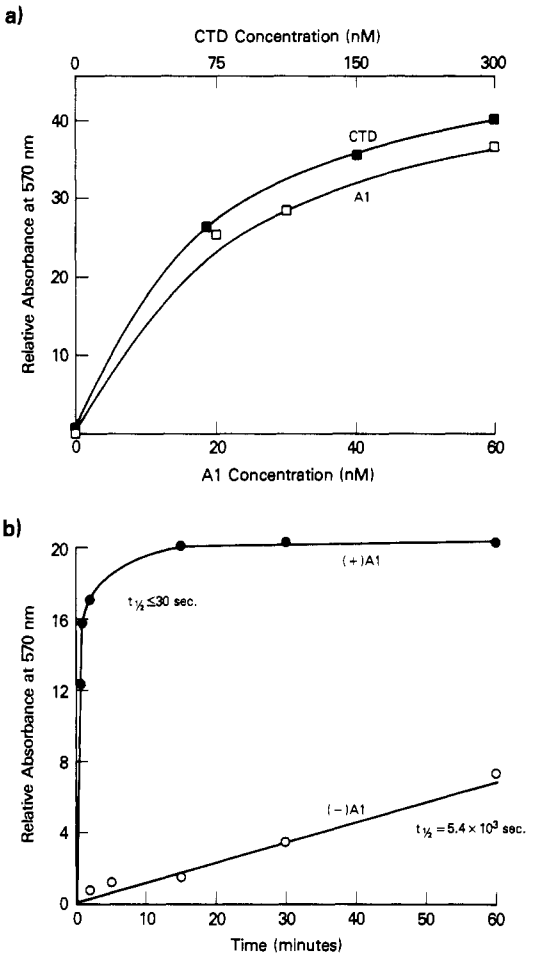

FIGURE 3: (Panel a) Annealing activity as a function of protein concentration of A1 and the C-terminal fragment (CTD). Increasing concentrations of A1 (□) or CTD (■) were incubated with the homologous ^{32}P-labeled 16-residue oligonucleotide (0.17×10^{-9} M) and ϕX174 DNA (0.15×10^{-9} M), and samples were processed as described under Experimental Procedures. (Panel b) Time course of annealing of the base-pair complementary oligodeoxynucleotide and ssDNA. ϕX174 DNA (0.15×10^{-9} M) was incubated with the ^{32}P-labeled 16-residue oligodeoxynucleotide (0.17×10^{-9} M) in the absence (○) or presence (●) of A1 (0.3×10^{-6} M). Samples were taken at different times and displayed on an 0.8% agarose gel as described under Experimental Procedures. Autoradiograms were evaluated for band intensity with a Zeineh soft-laser scanning densitometer (Biomad Instruments). Areas under peaks were determined by cutting and weighing.

Annealing of Complementary RNAs. A1 and its fragments bind natural ssDNA and RNA with roughly equal affinity. In view of this and the fact that A1 is an hnRNP component, we asked the question whether these proteins can stimulate RNA·RNA hybridization. Two RNA molecules of different lengths were used. A 55-residue-long ^{32}P-labeled RNA and a 175-residue-long RNA with a base-pair complementary segment were tested for the annealing activity. Typical results are depicted in Figure 4. No hybridization signal was seen in the absence of protein under the conditions used. A1 and the C-terminal fragment promoted annealing, whereas the N-terminal fragment did not. Four hybridization bands are visible in the autoradiogram, which are due to heterogeneity in the preparation of 175-residue RNA, as revealed by analysis of labeled transcripts (data not shown). When the 175-residue transcript was eluted from the gel and then used in an annealing reaction, a single hybridization band was observed

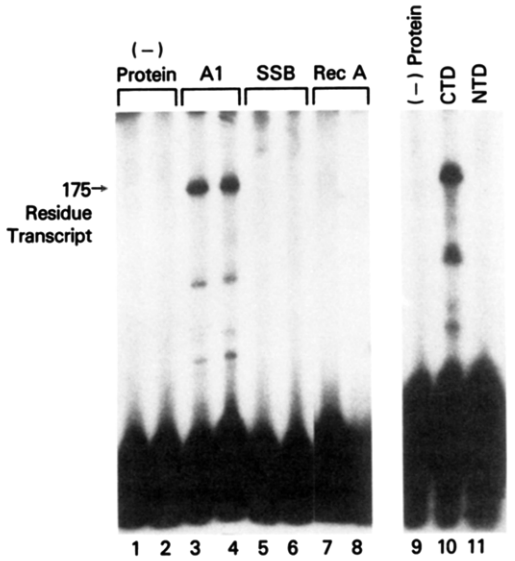

FIGURE 4: Strand annealing of base-pair complementary RNA molecules. Photographs of autoradiograms are shown. The 175-residue-long and ^{32}P-labeled 55-residue-long RNAs were prepared as described under Experimental Procedures. These RNAs were incubated in the absence or presence of proteins as indicated at top, and the hybridization products were examined by 6% nondenaturing polyacrylamide gel electrophoresis. The amounts of protein used in the incubations were as follows: A1, 0.3×10^{-6} M; N-terminal fragment, 0.4×10^{-6} M; C-terminal fragment, 0.4×10^{-6} M; SSB, 0.3×10^{-6} M; recA, 0.3×10^{-6} M. Annealing reactions were carried out in duplicate with no protein (lanes 1 and 2), A1 (lanes 3 and 4), SSB (lanes 5 and 6), and recA (lanes 7 and 8). The migration position of the 175-residue transcript is indicated in the margin.

corresponding to the slower migrating band in Figure 4 (not shown). SSB, recA, and the N-terminal fragment were unable to promote annealing; this was not surprising in the case of recA and SSB, as these proteins do not bind to RNA. Results similar to those shown in Figure 4 also were obtained when a 22-residue-long ^{32}P-labeled complementary RNA was used in the annealing mixture, whereas noncomplementary RNA of 55 or 22 residues failed to hybridize (data not shown). These results demonstrate RNA·RNA annealing by A1 and the C-terminal fragment and also that the annealing activities of A1 and recA are different, in that recA has no activity with RNA.

Discussion

We have shown that A1 has strand-annealing activity for complementary single-stranded DNA that is similar to the annealing activity of *E. coli* recA on a protein concentration basis. The A1 activity is not due to contaminating recA, because the sample contains no other polypeptides, at the detection level of at least 50:1, and in contrast to recA, A1 also has annealing activity for complementary single-stranded RNA molecules. With both RNA and DNA, A1 strand-annealing activity is seen at a ratio of A1 to polynucleotide occluded site (i.e., 12 nucleotide residues per site) of 1:1 or less and at a concentration ~10-fold lower than the K_d for binding, suggesting that the protein need not fully saturate the polynucleotide strands to promote annealing.

We used gel electrophoresis to assay for hybrid formation with three different examples of base-pair complementary nucleic acids. Evidence that an authentic hybrid structure was formed is indirect. In the case of ϕX174 DNA, A1 promotes transfer of the labeled oligodeoxynucleotide probe away from the gel front to a position coincident with that of the authentic hybrid. This is expected for a protein–ϕX174 DNA hybrid complex, since we know that the nanomolar concentration range and amount of A1 used are insufficient to saturate the ϕX174 DNA and produce a shift in gel migration. In addition, the complex is not seen with a nonhomologous probe or in the absence of ϕX174 DNA, and the complex once formed does not depend upon the presence of A1. Therefore, we conclude that this complex is indeed a base-pair complementary hybrid.

We did not attempt to assign a precise value for the strand-annealing rate enhancement conferred by A1, although the protein has roughly the same effect as increasing the concentration of complementary strands by ~30-fold. This corresponds to a 2 order of magnitude increase in the rate constant for annealing, assuming that the annealing reaction follows the standard second-order rate equation (Tinoco et al., 1978), and this idea is consistent with the time–course results obtained here; i.e., $t_{1/2}$ decreased from 5400 s (−A1) to ≤30 s (+A1). Although the N-terminal domain fragment of A1 has been studied extensively as a helix-destabilizing or melting protein [Herrick et al., 1976; for review see Wilson (1990)], intact A1 does not carry out T_m depression (melting) (Kumar et al., 1986, 1987), except under specialized conditions at low protein levels (Nadler et al., 1990). Evidently, the strand-annealing activity of the C-terminal domain can counterbalance the melting activity of the N-terminal domain in the intact protein.

The observation of interstrand-annealing activity for A1, in some respects, is not surprising. For example, in the cases of T4 gene 32 protein and *E. coli* SSB, it is well-known that the proteins can promote DNA reannealing under specialized conditions, and in general, T_m depressing proteins are viewed as enhancing rates of the reversible reaction, single-stranded DNA ⇄ double-stranded DNA, by virtue of single-strand binding and removal of kinetically unfavorable structures for reannealing. It is also well-known that the N-terminal domain fragment of calf A1, known as UP1, can accelerate intrastrand renaturation of a variety of tRNAs and 5S RNA from biologically inactive conformations [Karpel et al., 1974; for review, see Karpel et al. (1982)]. These effects of single-strand-specific binding proteins may be quantitatively different from the interstrand-annealing activities of recA and A1. For such intermolecular annealing, it is reasonable to imagine that the two strands must be brought physically into close proximity. There are two ways A1 and the C-terminal fragment could bring this about. First, we know that A1 has at least two nucleic acid binding pockets, in the N-terminal domain and in the C-terminal domain. These could independently bind complementary strands and create the local concentration necessary to facilitate annealing. The same reasoning could apply to the C-terminal domain fragment, although its capacity to bind more than one strand has not been demonstrated. Second, both A1 and the C-terminal fragment show strong protein–protein binding; this could be responsible for increasing local concentrations of strands bound on a 1:1 basis with each protein molecule. Interestingly, the lack of protein–protein binding by the N-terminal domain fragment correlates with its lack of interstrand-annealing activity.

It is obvious to speculate that the strand-annealing activity of A1 could play a role in such complex reactions as homologous DNA recombination and pre-mRNA splicing. This latter process may be particularly interesting, since A1 is a major component of the spliceosome complex, corresponding to approximately 30% of the protein mass in the HeLa hnRNP complex. There would appear to be a sufficient local concentration of A1 to promote annealing between the complementary region of U1 SnRNP and the 5′ splice site (Zhuang & Weiner, 1986) and U2 SnRNP to the branch site of pre-

mRNA (Nelson & Green, 1989). It is interesting to note that binding of A1 to pre-mRNA is dependent on U1 and U2 SnRNPs (Mayrand & Pederson, 1990). Experimental confirmation of this idea may be available through studies of in vitro splicing and antibodies against the C-terminal domain of A1 (Trauger et al., 1990). The question of whether A1 in the hnRNP particle is sufficiently exposed to participate in annealing of pre-mRNA with SnRNPs remains to be determined, but it is interesting to note that NaCl washing of the particle releases A1 under conditions where most of the other components remain particulate (LeStourgeon et al., 1990).

Finally, the annealing activity of A1 appears to be conferred by the glycine-rich C-terminal domain, and this region shows some degree of sequence similarity with the other abundant hnRNP core proteins A2, B1, and B2. On the basis of the results with A1, it should be possible to examine these proteins for annealing activity, since only small amounts of protein may be required and there is no apparent need to await recombinant expression. Nucleic acid annealing activities have been detected earlier in crude extracts of mammalian cells and in some cases with partially purified proteins. It is unclear whether any of these activities correspond to A1, but in one case the apparent M_r of the annealing protein was similar to that of A1 (~31 000) (Kawasaki et al., 1989).

ACKNOWLEDGMENTS

We thank Brian Pontius and Paul Berg for informing us about their results on A1 annealing activity prior to the start of the work described in this paper.

Registry No. AAATAACGACGGCAGT, 129964-71-2.

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