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HMG Box A in HMG2 Protein Functions as a Mediator of DNA Structural Alteration Together with Box B[1]

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Nonhistone protein HMG2, like HMG1, binds with B-DNA in a sequence-nonspecific manner and causes structural alterations in DNA such as bending, kinking and unwinding. Here, we studied the functions of HMG2 domains in the DNA structural alteration and modulation by using various HMG2 peptides, and we demonstrated several new findings. The HMG box itself as a DNA-binding motif may have the basic function of inducing curvature, resulting in the apparent DNA bending in the DNA cyclization assay, but not of abruptly kinking DNA. The DNA-binding activity of HMG box B, which is enhanced by the presence of box A, together with the flanking regions of box B, causes DNA bending accompanying the kinking of the DNA main chain. The DNA unwinding accompanied by DNA kinking diminishes cruciform structures in supercoiled DNA. Analysis using mutant peptides for box A confirmed that box A in HMG2 functions as a mediator of DNA structural alteration together with box B. The present studies on the functional properties of the respective regions of HMG2 may help to elucidate the protein function.

Key words: DNA bending, DNA kinking, DNA unwinding, HMG box, HMG2 protein.

HMG1 and 2 proteins are the most abundant, ubiquitously distributed nonhistone chromosomal proteins in nuclei of higher eukaryotic cells. They have two DNA-binding domains with nonidentical amino acid sequences called HMG boxes A and B (*1*, *2*). These boxes are connected by a short linker (l)-region. In addition, a unique carboxyl terminal region (C-tail) consisting of a continuous run of 23 acidic amino acids is connected to box B through a joiner (j)-region, as schematically represented in Fig. 1. These proteins play various important roles in organisms as transcription activators (*3–7*), DNA binding regulatory components of DNA-dependent protein kinase (*8*), novel antigens of perinuclear anti-neutrophil cytoplasmic antibodies (P-ANCAs) (*9–12*), and in other essential biological reactions (*13–18*, *19*). In addition, HMG1 functions as a quasi-transcriptional activator in cultured cells (*20*, *21*), and HMG2 may participate in cell proliferation (*22*).

HMG box proteins such as SRY and UBF may entail DNA structural changes in expressing their functions (*23–26*). Human SRY containing an HMG box binds with target DNA in a sequence-specific manner and induces a kink that unwinds the DNA (*27*). The DNA kink is a sharp DNA bend induced by DNA intercalation of a specific amino acid between base stacks. The DNA intercalation increases the distance between DNA base stacks, and subsequently the DNA strand unwinds. The HMG box of a mutant SRY obtained from human XY-female neither binds to DNA nor alters the DNA structure (*28*). Thus DNA alteration by the HMG box in SRY may be important for normal sex-determination (*29*, *30*). *Xenopus* UBF has five HMG boxes and a dimerization domain. The dimerized UBF molecules bind to DNA with high sequence-tolerance (*31*, *32*) and bend and unwind DNA cooperatively (*24–26*). UBF and SRY activate transcription accompanied by changes in DNA structure (*24*, *25*). DNA structural alteration by HMG1 and 2 may therefore play important roles in the expression of their functions.

HMG1 and 2, which bind to DNA in a sequence-nonspecific manner (*33*, *34*), show preferential interaction with supercoiled (*35–37*) and non-B DNA such as four-way junction (*38–41*), cruciform DNA (*35*, *36*, *42*), and B-Z junction (*43*). These proteins bend (*44–47*) and unwind DNA (*43*, *48*). These DNA structural alterations mediated by HMG1 and 2 may be induced by the HMG boxes (*6*, *7*, *37*, *41*, *47*, *49–52*). Box B in particular is a major mediator of DNA structural alteration, because it has an amino acid (Phe 102) intercalatable into the DNA backbone (*47*, *53*). The flanking basic regions of box B (*47*, *51*) and acidic C-tails (*52*, *54*) may modulate the binding force of HMG1 and 2. The stabilized array of two HMG boxes endowed by the linker sequence between them is requisite for the effective binding of HMG1 and 2 with DNA (*37*, *47*, *51*). However, the real participation of box A in the mediation of the DNA structural alteration by HMG proteins is not clear, although structure-specific binding of HMG1 to four-way junction is mediated by box A (*40*). The present study examined the HMG2 domains that may function in DNA structural alteration and modulation. We found that HMG

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Abbreviations: HMG, high mobility group; SDS, sodium dodecylsulfate; bp, base pair(s).

box A functions as a mediator of DNA structural alteration together with box B.

MATERIALS AND METHODS

Preparation of HMG Proteins and the Peptides—HMG1 and 2 were prepared from pig thymus as described previously (*55*). HMG2 peptides 2A (amino acid residues 1–76), 2Al (1–87), 2B (88–164), 2Bj (84–181), 2AlB (1–164), and 2AlBj (1–181), schematically represented in Fig. 1, were overexpressed in *Escherichia coli* BL21 (DE3) cells carrying the corresponding cDNA sequences downstream from the T7 promoter. HMG2 peptides were purified to homogeneity on SDS–polyacrylamide electrophoretic gel (Fig. 1b, *37*).

Preparation of Mutant 2AlB Peptides—The DNAs were amplified from the cDNA encoding the 2AlB peptide using primers containing the mutation for box A. These PCR products were inserted into the pGEM plasmid and transformed into *Escherichia coli* BL21 (DE3). The expression and purification of the mutant 2AlB peptides were conducted as described above. Box A mutants in 2AlB peptide are 2A-FA (Y15F), -FF (Y15F and A16F), -FS (Y15F and A16S), -SA (Y15S), -SF (Y15S and A16F), and -YF (A16F).

DNA Topoisomerase I—DNA topoisomerase I was prepared from pig thymus nuclei as described for calf thymus (*56*). The partially purified fraction eluted from the second hydroxyapatite column was used.

Preparation of DNA Probes—A zigzag DNA probe of 75-bp, GATCCGCGG[GCCGGAAAAAAGGCG]$_4$GCCCCG, has four consecutive 15-bp bending sequences containing the $(dA)_6$ tract at the center and sticky *Bam*HI ends. The shape of the DNA probe may appear to be straight as a whole, but may contain less apparent zigzag in the minute structure (*57*). The probe was labeled at the 5′ end with [γ-^{32}P]ATP (111 Bq/mmol) with T4 polynucleotide kinase. Form Ir (relaxed closed-circular) pBR322 DNA was prepared from the form I (negatively supercoiled) DNA by incubation with DNA topoisomerase I.

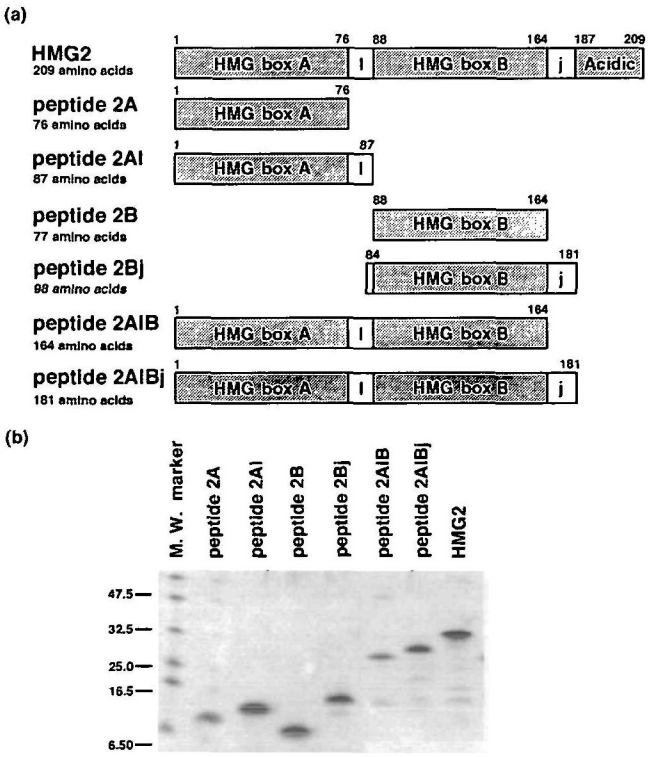

Fig. 1. **(a) Schematic representation of HMG2 protein and the peptides containing one or two HMG boxes.** The intact HMG2 protein is composed of HMG box A, l-region, HMG box B, j-region and an acidic carboxyl tail. **(b) SDS–polyacrylamide gel electrophoretic profile of the HMG2 peptides stained with Coomassie Brilliant Blue R-250.**

Gel Retardation Assay—An aliquot of HMG peptide solution was mixed with 2.5 ng of DNA in a reaction buffer (10 mM Tris-HCl, pH 7.8, 50 mM NaCl, 1 mM EDTA, 10 mM $MgCl_2$, 10 mM dithiothreitol, 66 μM ATP, 10% glycerol, and 0.1 μg/μl bovine serum albumin) used for DNA cyclization assay. The solution in a total volume of 10 μl was kept on ice for 30 min, then electrophoresed on a 5% polyacrylamide gel in 0.25× TBE buffer (1× TBE buffer contained 89 mM Tris-borate and 2 mM EDTA, pH 8.0). After electrophoresis, the gel was dried and exposed to an X-ray film.

DNA Cyclization Assay—The DNA-HMG mixture prepared as described above was incubated with 0.5 unit of T4 DNA ligase (Takara) at 12°C for 16 h. The resulting DNA was purified by phenol–chloroform extraction, precipitated with ethanol, and subjected to polyacrylamide gel electrophoresis with 0.5× TBE buffer as described above. After electrophoresis, the gel was dried and processed for autoradiography.

DNA Unwinding Assay—An aliquot of HMG peptide was mixed with 0.4 μg of form I DNA in a reaction buffer containing 10 mM Tris-HCl, pH 7.8, 100 mM NaCl, 1 mM EDTA, 10 mM $MgCl_2$, 1 mM dithiothreitol, 10% glycerol, and 1 μg/μl bovine serum albumin in a total volume of 20 μl. The solution was kept at 25°C for 60 min, then 2.5 μl of topoisomerase I was added. After incubation at 37°C for 60 min, DNA was purified by phenol–chloroform extraction followed by ethanol precipitation. The DNA samples were electrophoresed on a 1.2% agarose gel in TAE buffer (40 mM Tris-acetate, pH 8.0, containing 1 mM EDTA) at 4°C. After electrophoresis, the gel was stained with ethidium bromide.

Nuclease S1 Protection Assay—One microgram of form I plasmid DNA was preincubated at 37°C for 60 min with an aliquot of HMG peptide solution in 50 μl of a reaction buffer containing 10 mM Tris-HCl, pH 8.0, 100 mM NaCl, 0.2 mM EDTA, and 0.2 mM phenylmethanesulfonyl fluoride. The solution was mixed with 4 units of nuclease S1 (Boehringer Mannheim) and $ZnCl_2$ at a final concentration of 2 mM, and incubated for an additional 60 min. The reaction was stopped by addition of 20 mM EDTA and 1% SDS in final concentrations. DNA was purified by phenol–chloroform–isoamylalcohol (25:24:1, v/v/v) extraction followed by ethanol precipitation. These DNA samples were analyzed by a similar method to the gel retardation assay described above.

RESULTS

DNA-Bending Activity of HMG2—DNA-bending activity was analyzed with a 75-bp rigid DNA probe, GATCCGCGG[GCCGGAAAAAAGGCG]$_4$GCCCCG, with zigzag structure containing four 15-bp bendable sequences to which HMG2 may bind preferentially (*58*). Electrophoresis after DNA ligation demonstrated many closed-circular and lin-

ear bands resulting from intra-, inter-, and inter-intra-ligations of the probe with sticky *Bam*HI ends, as shown on the left panel of Fig. 2a. The linear molecules were distinguished from closed-circular ones by exonuclease III digestion (Fig. 2a, right). The numbers of probes in linear molecules were determined with molecular standards, and in closed-circular molecules by partial *Bam*HI digestion of the respective bands (data not shown). In the absence of HMG2, closed-circular molecules composed of three (3c) and four (4c) probes were observed. The closed-circular molecules composed of one (1c) or two (2c) probes were observed with increasing amounts of HMG2. These results showed that HMG2 bent the DNA. Similar results were obtained for HMG1 (Fig. 2a left panel). No apparent difference was observed between the above 75-bp zigzag DNA and 75-bp random sequence DNA of GATCCCCGGGTACCGGGCCCCCCCTCGAGGTCGAGGTATCGATAAGCTTGATATCGAATTCCTGCAGCCCGGGG for DNA bending by HMG2 (data not shown).

DNA-Bending Activity of HMG2 Peptides—To determine which HMG2 region participates in the DNA bending, cyclization activities for various HMG2 peptides were assayed. The activity of peptide 2A was too weak to form a monomer circular product (Fig. 2b), although 2c band was observed at higher peptide/DNA ratios (P/D), indicating the weak bending activity. The activity of peptide 2B was similar to that of peptide 2A. The peptides 2Al and 2Bj activities were three and five times stronger than those of peptides 2A and 2B, respectively. These results indicated that the DNA-bending activity of single DNA-binding domains is relatively weak and that the flanking regions, especially the j-region for box B, enhance the activity. The peptides 2AlB and 2AlBj, which contain two HMG boxes, showed stronger activity than peptide 2Al. At higher P/D, the amount of monomer circular DNA was decreased by probable over-bending. Thus, the tandem array of two HMG boxes linked by l-region enhanced DNA-bending activity. Noticeable changes at higher P/D than 66 were the in-

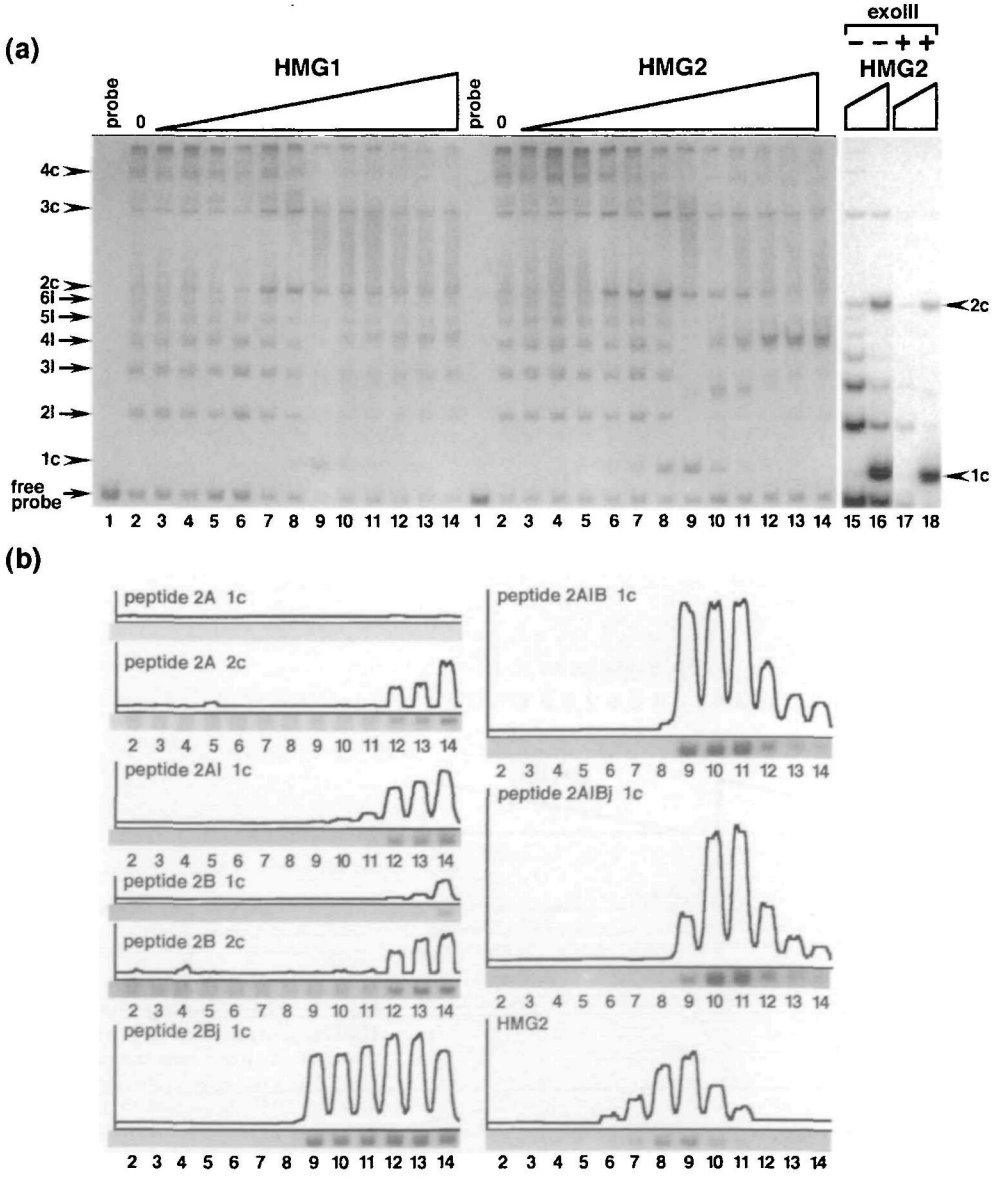

Fig. 2. **DNA-bending activities of HMG2 protein and the peptides.** (a) DNA cyclization assay for a 75-bp DNA probe at different HMG1 and HMG2 (left panel) to DNA molar ratios (P/D): 0 (lane 2), 0.66 (lane 3), 1.32 (lane 4), 2.00 (lane 5), 6.60 (lane 6), 13.2 (lane 7), 20.0 (lane 8), 66.0 (lane 9), 132 (lane 10), 200 (lane 11), 660 (lane 12), 1320 (lane 13), and 2000 (lane 14). Abbreviations c and l in the left column represent the circular and linear products, and the numbers preceding these abbreviations indicate the number of DNA probes in the ligation products. The circular products (1c and 2c) in HMG2 before (–exoIII, lanes 15 and 16) and after (+exoIII, lanes 17 and 18) exonuclease III digestion are shown at the right of panel (a). The P/D of lanes 15 and 17 are equal to that of lane 6, and those of lanes 16 and 18 are equal to that of lane 8. (b) The amount of cyclization products in the presence of peptides 2A, 2Al, 2B, 2Bj, 2AlB, and 2AlBj at different P/D described in (a). The autoradiogram of 1c (and 2c for peptides 2A and 2B) products and the densitometric tracing profiles are shown.

crease in the closed circular molecules and the decrease in the linear ones observed with peptides 2Bj, 2AlB, and 2AlBj (not shown) as well as HMG1 and HMG2 proteins (Fig. 2a).

DNA-Binding Activity of HMG2 Peptides—Since the j-region contains numerous basic amino acids, the effective cyclization by peptide 2Bj was considered to result from an increase in DNA-binding activity in the presence of j-region. Therefore, the DNA-binding activity of HMG2 peptides with 75-bp zigzag DNA was examined by gel retardation assay. As shown in Fig. 3, all of the HMG2 peptides as well as HMG2 protein bound with the DNA. The relative DNA-binding activity could be compared in spite of their smeared band shifts. Peptides 2A and 2B showed weaker DNA-binding activity than HMG2. The activity of peptides 2A and 2B was enhanced in the presence of the l- and j-regions, respectively, with peptide 2Bj in particular showing distinct shift bands. The P/D required to give more than four distinct shift bands was higher than 66, consistent with the ratio required to give monomer circular molecules (1c) in the DNA cyclization assay (Fig. 2b). Similarly, peptides 2AlB and 2AlBj presented distinct band shifts, although that caused by 2AlBj peptide was smaller than that by 2AlB peptide. These results suggest that the DNA-binding activity of the HMG boxes is enhanced by the respective flanking sequences and is correlated with their DNA-bending activity. The j-region that flanks the HMG box B may be most effective in these reactions. These activities

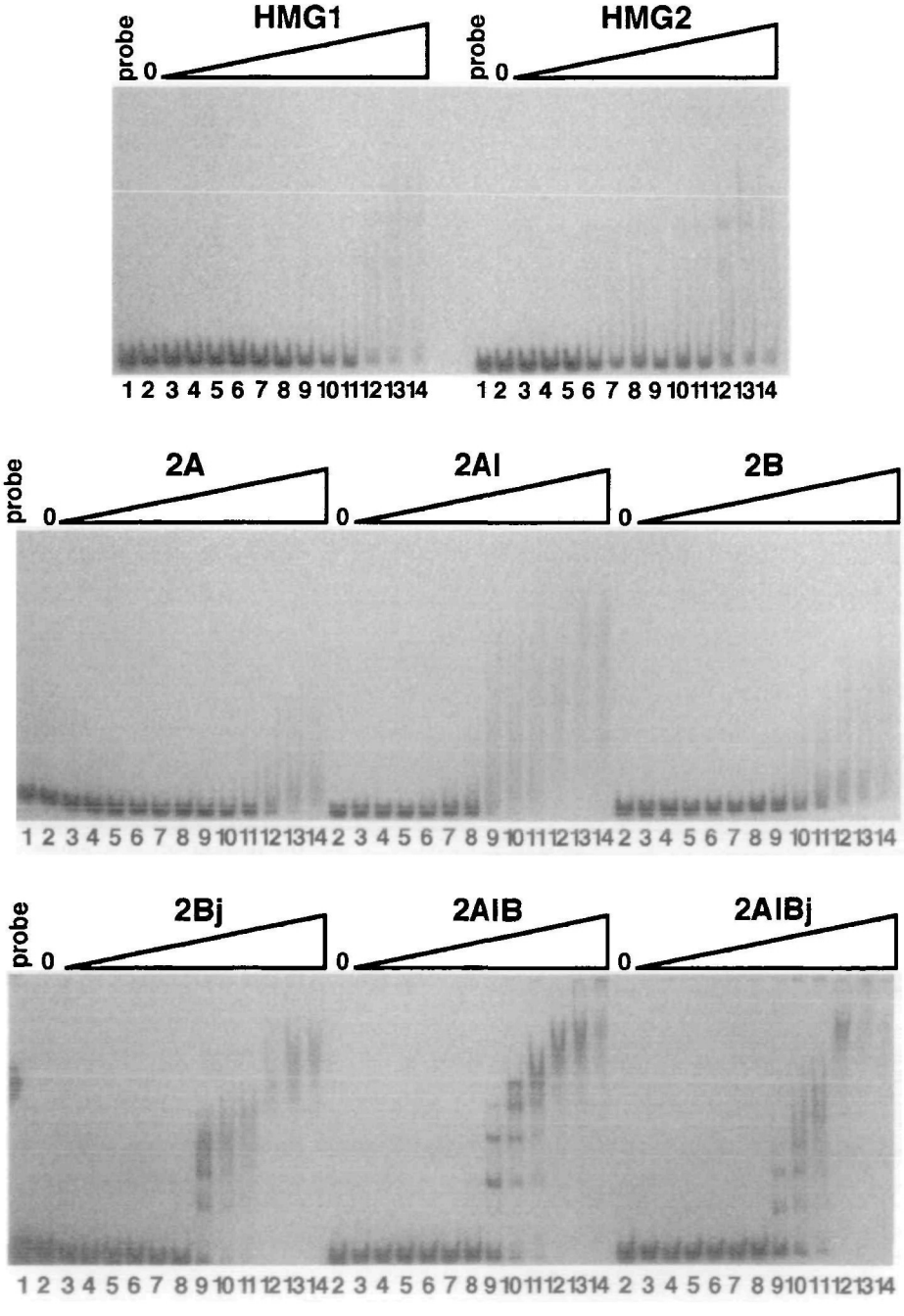

Fig. 3. **DNA-binding activity of HMG2 protein and the peptides.** A 75-bp DNA probe was complexed with HMG2 and the respective peptide at the different P/D described in Fig. 2, and subjected to polyacrylamide gel electrophoresis.

were also markedly enhanced in the presence of two HMG boxes with their flanking sequences.

DNA-Unwinding Activity of HMG2 Peptides—DNA bending is generally thought to be accompanied by the unwinding of DNA double helix (*59*, *60*). To confirm the DNA unwinding by HMG2 peptides, DNA unwinding assay which detects the presence of relaxed molecules by changes in DNA superhelicity was employed. In the presence of peptides 2Bj, 2AlB, 2AlBj, and HMG2, numerous topoisomers were observed with increases in P/D (Fig. 4a). These topoisomer DNAs were identified as negatively supercoiled on two-dimensional electrophoresis (data not shown), showing that the DNA has been unwound in complexes with the peptides. It should be noted that peptides 2A and 2B had no unwinding activity in this assay, and that peptide 2AlB seems to have higher unwinding activity than peptide 2AlBj.

Nuclease S1 Protection Assay for HMG2 Peptides—Our previous studies (*36*) showed that nicking and subsequent linearization of form I plasmid DNA by nuclease S1 was prevented by incubation of the DNA in the presence of HMG1. These results suggested that HMG1 binds at or in the vicinity of a nuclease S1-sensitive site of the cruciform DNA to protect against nuclease S1 digestion. However, the present results suggest that the protection from nuclease S1 afforded by HMG1 may simply be due to nonspecific, random binding of HMG1 to double-stranded DNA such that the whole plasmid DNA conformation is altered, resulting in loss of the cruciform. In the former case, even a single HMG box (peptide 2A or 2B) bound with the DNA should be sufficient to protect it from nuclease S1 digestion. To analyze these possibilities, nuclease S1 protection assay was conducted for HMG2 and the peptides instead of HMG1 (*36*). As shown in Fig. 4b, peptides 2A, 2Al, and 2B were not able to protect the form I structure from nuclease S1 digestion, whereas HMG2 was. These results support the latter possibility that HMG2 alters the entire plasmid DNA conformation owing to its DNA-unwinding activity. Peptide 2Bj at a P/D of more than 80 protected against nuclease S1 digestion, while peptides 2AlB and 2AlBj at a ratio of 40 showed the activity. These results suggested that peptides 2AlB and 2AlBj had stronger nuclease S1 protection activity than peptide 2Bj and HMG2. In addition, the j-region flanking the HMG box B may also be important for this protection as well as the presence of the both boxes.

DNA Bending, Binding, and Unwinding Activities of 2AlB Mutants—The above results indicated that box A linked to box B by the l-region also participates in the bending, binding, unwinding and nuclease S1 protection activities. To confirm this, the activities of several 2AlB mutants for box A were examined. These mutants contain mutations at 15Y and 16A in box A, equivalent respectively to 101F, and 102F, which are concerned with the DNA intercalation of box B (*37*). The DNA-bending activities of the mutants were similar to that of wild peptide 2A-YA (Fig. 5a), although the binding activities to form I DNA

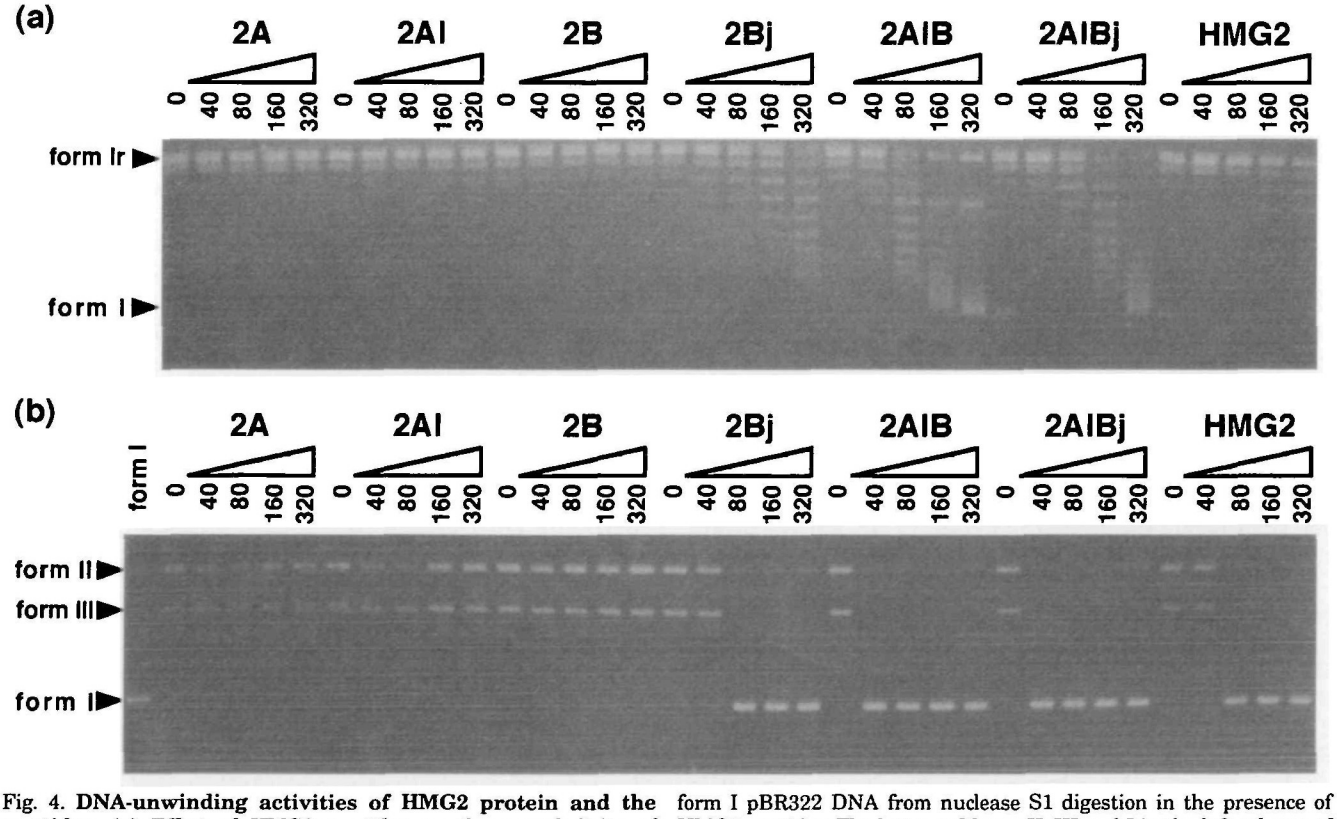

Fig. 4. **DNA-unwinding activities of HMG2 protein and the peptides.** (a) Effect of HMG2 peptides on the superhelicity of pBR322 DNA in the presence of topoisomerase I. The letters of forms Ir and I at the left column of the panel represent relaxed closed-circular and negatively supercoiled DNA, respectively. (b) Protection of form I pBR322 DNA from nuclease S1 digestion in the presence of HMG2 peptides. The letters of forms II, III and I in the left column of the panel represent nicked circular, linear and negatively supercoiled plasmid DNA, respectively.

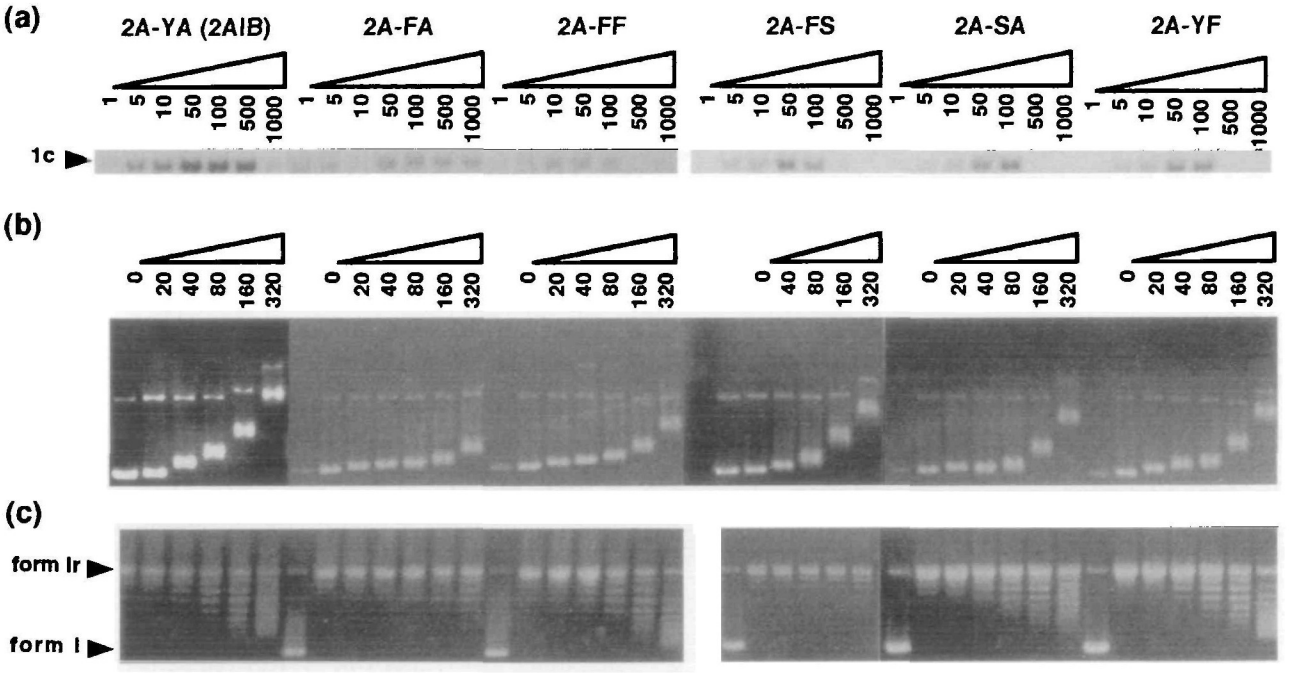

Fig. 5. **DNA bending, binding, and unwinding activities of peptide 2AlB mutants.** (a) DNA cyclization assay with use of a 75-bp DNA probe of wild peptide 2AlB (2A-YA) and five mutants at P/D ratios of 1, 5, 10, 50, 100, 500, and 1000. (b) DNA-binding assay with use of pBR322 plasmid DNA (form I) of the 2AlB mutants at various P/D described in Fig. 4. (c) DNA-unwinding assay conducted as described in (b). The letters of forms Ir and I in the left column of the panel represent relaxed closed-circular and negatively supercoiled DNA, respectively.

were decreased (Fig. 5b). The DNA-unwinding activities of mutants 2A-FF, 2A-SA, and 2A-YF were similar to that of wild peptide. On the other hand, the activity was decreased for mutants 2A-FA and 2A-FS (Fig. 5c). These mutational analyses confirmed that box A linked to box B by the l-region mediates the DNA structural alteration in combination with box B flanked by the j-region.

DISCUSSION

Circular permutation assays indicated that HMG box proteins such as LEF-1 and SRY, which bind with DNA in a sequence-specific manner, bend DNA (*61–66*). In the present studies, the DNA-bending activity of HMG2 and the peptides were analyzed by DNA cyclization assay because of their sequence-nonspecific binding properties. HMG2 as well as HMG1 bent DNA (Fig. 2a) in agreement with previous results (*37, 44, 51, 61, 67*). HMG2 seemed have a stronger DNA-bending activity than HMG1 (Fig. 2a), consistent with our previous results (*34*).

The various activities of HMG2 peptides observed in the present studies are summarized in Table I. High correlation was found between the activities of these peptides. The DNA bending by the peptides seemed to be correlated with their respective DNA-binding activities. The peptides 2Bj, 2AlB, and 2AlBj showed stronger DNA-bending activities. 68I in the HMG box in SRY intercalates the DNA base stack and kinks the structure (*27, 28, 68, 69*). Box B in HMG2 has 102F, which corresponds to 68I in SRY (*37*). The side chain of 102F in HMG box B tightly bound in the presence of the flanking regions of the box might intercalate into the base stack, resulting in kinking the DNA main chain, as our previous suggestion (*37*). Peptide 2Al showed a weaker DNA-bending activity in DNA cyclization assay (Fig. 2). The HMG box A has no amino acid residue which possesses an intercalatable side chain corresponding to those of 102F in HMG2 box B and 68I in HMG box of SRY. Thus, only when an intercalatable amino acid residue is present DNA may be abruptly kinked by the intercalation of the side chain. In other words, the HMG box itself as a DNA-binding motif may have the basic function of inducing curvature, resulting in the apparent DNA bending in the DNA cyclization assay, but not of abruptly kinking DNA. Our simulation results of the binding structure of the complex between DNA and HMG boxes by molecular dynamics support these suggestions (*53*).

HMG proteins have DNA-unwinding activity (*37, 49, 52, 54, 70*). The DNA unwinding is a DNA alteration caused by DNA kinking (*71*). The DNA unwinding and nuclease S1 resistance activities were observed with peptides 2Bj, 2AlB, and 2AlBj but not with peptides 2A, 2Al, and 2B (Fig. 4). These results may show that these activities are resulted from the DNA kinking by HMG box B in the presence of the flanking sequences. DNA unwinding accompanied by the DNA kinking may diminish the cruciform structures in form I pBR322 DNA (*36*) to confer resistance against nuclease S1 digestion. The DNA curvature by box A may be insufficient to cause such a DNA alteration.

Interestingly, the peptide 2AlB showed larger and more distinct shift bands than peptide 2AlBj in gel shift assay (Fig. 3). The peptide 2AlBj has similar DNA-binding ability to peptide 2AlB in SPR-binding measurement (*37*). In addition, peptide 2AlB more strongly unwound DNA than peptide 2AlBj (Fig. 4b). Our simulated model of HMG1-DNA

TABLE I. **Summary of the relative DNA bending, binding, unwinding, and nuclease S1 resistance activities of the various HMG2 peptides.** The DNA-bending activity was quantified based on the amount of 1c bands (Fig. 2b). The DNA-binding activity was quantified based on the amount of DNA in the lowest mobility band in gel retardation assay (Fig. 3). The DNA-unwinding activity was determined by the amount of DNA topoisomers (Fig. 4a). The nuclease S1 resistance activity was determined by the amount of form I DNA remaining (Fig. 4b).

Activities	Peptides						
	2A	2Al	2B	2Bj	2AlB	2AlBj	HMG2
DNA bending (cyclization)	−	++	+	+++	++++	+++	++++
DNA binding (gel retardation)	+	++	+	+++	++++	+++	++
DNA unwinding (topoisomerase I)	−	−	−	++	++++	+++	+
Nuclease S1 resistance	−	−	−	+++	++++	++++	+++

complex by molecular dynamics suggested that the j-region nears the l-region in the complex (*53*). Thus the l-region may compete with the j-region in a step of DNA binding. The competition may destabilize the 2AlBj binding with DNA, accompanying the structural alteration of the complex. The whole structures of HMG1 and 2 remain unclear, although the DNA-binding structure of HMG1 without the acidic C-tail was simulated (*72*). The acidic C-tail in the whole HMG2 protein weakens its DNA binding (Fig. 3, *20*, *34*, *37*, *44*, *52*, *54*) and DNA unwinding (Fig. 4a, *37*, *52*). These results suggest that the acidic C-tail may interact with DNA-binding domains or the flanking regions and/or compete with DNA in the interaction of the protein.

The presence of box A linked by the l-region strengthens the activities of DNA bending, binding, unwinding and nuclease S1-resistance. The mutations of box A in peptide 2AlB changed the DNA-binding and DNA-unwinding activities of the peptides (Fig. 5, b and c). These results may show that box A in HMG2 functions as a mediator of B-DNA structural alteration together with box B. Recently, Webb *et al.* showed that box A, but not box B, in peptide 2AlB played a major role in the preference of HMG2 for distorted DNA (four-way junction), and that the DNA-binding behavior of box B in peptide 2AlB was different from that of isolated box B (*40*).

Many HMG box proteins have two or more HMG boxes (*1*, *2*, *72*). The present studies presented several information for meaning of the presence of two DNA-binding regions in HMG2. (I) The HMG box itself as a DNA-binding motif may have a basic function of inducing curvature, giving rise to apparent DNA bending in the DNA cyclization assay, but not of abruptly kinking DNA. (II) The DNA binding activity of the HMG box B, which causes DNA bending accompanying the kinking of the DNA main chain, is enhanced by the presence of box A, as well as by the flanking regions of box B. (III) The DNA-unwinding and nuclease S1 resistance activities of HMG2 are resulted from the DNA kinking by the HMG box B. The DNA unwinding that accompanies DNA kinking diminishes cruciform structures in supercoiled DNA. (IV) Box A in HMG2 functions as a mediator of DNA structural alteration together with box B. The present studies to show the functional properties of the respective regions in HMG2 may help to elucidate the protein function.

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