

The Antifungal Protein AFP of *Aspergillus giganteus* Is an Oligonucleotide/Oligosaccharide Binding (OB) Fold-containing Protein That Produces Condensation of DNA*

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The antifungal protein AFP is a small polypeptide of 51 amino acid residues secreted by *Aspergillus giganteus*. Its potent activity against phytopathogenic fungi converts AFP in a promising tool in plant protection. However, no data have been reported regarding the mode of action of AFP. The three-dimensional structure of this protein, a small and compact β barrel composed of five highly twisted antiparallel β strands, displays the characteristic features of the oligonucleotide/oligosaccharide binding (OB fold) structural motif. A comparison of the structures of AFP and OB fold-containing proteins shows this structural similarity despite the absence of any significant sequence similarity. AFP, like most OB fold-containing proteins, binds nucleic acids. The protein promotes charge neutralization and condensation of DNA as demonstrated by electrophoretic mobility shift and ethidium bromide displacement assays. Nucleic acid produces quenching of the protein fluorescence emission. This nucleic acid interacting ability of AFP may be related to the antifungal activity of this small polypeptide.

The mold *Aspergillus giganteus* produces two major extracellular proteins, α -sarcin, a potent cytotoxic ribonuclease, and the so-called antifungal protein (AFP)¹ (1–3). AFP is a small polypeptide of 51 amino acid residues that is secreted under the form of a rather inactive larger precursor containing 6 extra amino acid residues at the NH₂-terminal end, which is processed in the extracellular medium (4). The amino acid and cDNA sequences of AFP have been reported, revealing a high content of disulfide bridges (four bonds) and tyrosines and lysines (6 and 12 residues, respectively) (5–7). AFP shows a significant degree of sequence similarity only with both the abundantly secreted antifungal PAF protein (55 amino acid residues) from *Penicillium chrysogenum* (47% sequence iden-

tity) (8) and the antifungal peptide Anafp (56 amino acid residues) secreted by *Aspergillus niger* (31% sequence identity) (9). AFP has been tested, at concentrations as large as 0.2 mM, against a wide variety of microorganisms, including prokaryotes and eukaryotes (3). This protein inhibits the growth of some filamentous fungi, the minimal protein concentration for total inhibition being in the range of 6–25 μ M (3), but it does not promote any effect on the producing mold or on bacteria or yeast. AFP is not active against *P. chrysogenum* or *A. niger* (3), the organisms producing PAF and Anafp proteins, respectively. Although these three proteins have been described as antifungal molecules, no data regarding their mode of action have been so far reported. We herein report the interaction of AFP with DNA.

EXPERIMENTAL PROCEDURES

Unless otherwise stated, all materials and reagents were molecular biology grade. Calf thymus double-stranded DNA (dsDNA) was purified as described previously (10, 11). The DNA fraction selected for the experiments described in this work (kept at –20 °C until used) was that containing 200 bp dsDNA on average according to their electrophoretic mobility on 0.7% agarose gels (see below). F1 single-stranded DNA (ssDNA) was prepared as described previously (12, 13) from *Escherichia coli* DH5 α F' {[F'] *endA1 hsdR17* ($r_K m_K$) *supE44 thi-1 recA1 gyrA* (NaI^R) *relA1* Δ (*lacZYA-argF*) U169 *deoR* [ϕ 80 *dlac* Δ (*lacZ*) M15]} cells after infection with a phage F1 preparation containing 6×10^{11} phage particles/ml. The phage suspension was digested for 20 min at room temperature with RNase A (1 mg/ml), and its ssDNA was extracted and kept at –20 °C until used. The concentration of DNA was determined spectrophotometrically based on A_{260} (1-cm optical path) = 1.0 for a 50 μ g/ml solution (0.154 mM nucleotide concentration using the average nucleotide molecular weight of 325).

Two different 27-mer deoxyoligonucleotides were also used. They were synthesized at the DNA sequence facility of the Universidad Complutense (Madrid, Spain). The sequence of the one used as the single-stranded 27-mer (ss27-mer) was 5'-TTGAACGACCAGCTGAAC-CCCAAGACC-3'. This sequence was not chosen for any particular reason, but it was just one of the deoxyoligonucleotides available in our laboratory pool of oligos. The other one contained the complementary sequence and was used to obtain the double-stranded 27-mer deoxyoligomer (ds27-mer) by means of boiling an equimolar mixture of both oligos and then cooling down slowly to room temperature.

The AFP was purified to homogeneity from *A. giganteus* cultures as described previously (1–4). The protein concentration was determined from absorbance measurements based on an $E^{0.1\%}$ (1-cm optical path) at 278 nm of 1.76 (3). All the absorbance measurements were performed on a Uvikon 930 spectrophotometer.

Agarose gel electrophoresis shift assays were performed as described previously (11, 14). The protein and nucleic acid mixtures were incubated for 10 min at room temperature in 40 mM Tris acetate buffer, pH 7.0, containing 1 mM EDTA (TAE buffer) (11) and then loaded on 0.7% agarose gels equilibrated in the same buffer. The electrophoresis was performed at 5 V/cm for different lengths of time. The gels were stained with 0.5 μ g/ml ethidium bromide, and the nucleic acids were visualized on a UV transilluminator equipped with a 312 nm wavelength emitting

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¹ The abbreviations used are: AFP, antifungal protein; OB fold, oligonucleotide/oligosaccharide binding; ds, double-stranded; ss, single-stranded; CSD, cold shock domain; r.m.s., root mean square.

lamp. The intensity of the bands obtained was used to quantify the amount of free and bound nucleic acid. The volumogram (density or quantity of a spot calculated from its volume made of the sum of all pixel intensities composing the spot) of each nucleic acid band was obtained on a Photodocumentation System (UVItec). A linear relationship between the amount loaded on the gel and the value of the corresponding volumogram was previously verified with control samples.

Fluorescence emission spectra of AFP were obtained at 25 °C using an SLM Aminco 8000 spectrofluorimeter as described previously (3). The excitation wavelength used was 275 nm (the wavelength of the absorption maximum of tyrosine) since this protein does not contain Trp residues (5). Excitation and emission effective optical paths were 0.1 and 0.5 cm, respectively. A single emission band centered around 305 nm, corresponding to AFP tyrosine emission (3), was obtained. Cross (90°/0°) polarizers were used for the excitation and emission beams, respectively, to avoid interferences due to the turbidity of some DNA-protein samples. The fluorescence intensities were corrected for the inner filter effect, contribution of the water Raman signal, and dilution during titrations. Fluorescence quenching promoted by either DNA or oligonucleotides was calculated as $1 - F/F_0$, where F_0 is the fluorescence intensity in the absence of nucleic acid.

Ethidium bromide (EtBr) displacement was assayed following the decrease of the fluorescence emission at 595 nm (for excitation at 510 nm) after addition of AFP to a solution of DNA prelabeled with the probe (15). Displacement was calculated as $(F - F_p)/(F_0 - F_p)$, where F_0 and F_p are the fluorescence intensities of EtBr in the absence and presence of DNA, respectively.

Circular dichroism (CD) spectra were recorded at 25 °C in the 350–200 nm wavelength range in 0.01-cm optical path cuvettes. The instrument used was a Jasco 715 spectropolarimeter. Spectra were obtained at 0.2-nm/s scanning speed with a response time of 2 s. The results shown are the accumulation of at least four spectra in each case. Base lines were conveniently subtracted. Mean residue ellipticity is expressed in units of degree \times cm² \times dmol⁻¹ referred to the nucleotide molar concentration. The contribution of AFP to the CD spectra of polydeoxynucleotides is negligible at wavelengths above 250 nm since this protein shows a CD spectrum that is rather unique, with a major positive contribution centered around 230 nm, due to its high content of aromatic residues (3, 4). Images and superposition of protein structures were generated by the MOLMOL program (16).

RESULTS AND DISCUSSION

The folding pattern of AFP consists of five antiparallel highly twisted β strands, defining a small and compact β barrel stabilized by four disulfide bridges, the eight cysteine residues being deeply buried in the protein core (Fig. 1A) (17). A five-stranded β barrel capped by an α -helix was first noted as a structural motif in some proteins that bind single-stranded nucleic acids or oligosaccharides, and it was termed the oligonucleotide/oligosaccharide binding motif or OB fold (18). The number of known protein structures containing the OB fold has been steadily increasing, and there are now 10 families for which this common structural motif has been identified (19–21). Some of the features originally considered as characteristics of the OB fold appear not to be conserved for all these proteins. This is the case of the α -helix capping the barrel, which can be reduced to a single turn or even an irregular loop. The three-dimensional structure of AFP closely resembled the OB fold. This similar folding pattern became more evident when the backbone atoms of the five β strands of AFP were superimposed on those equivalents in known OB fold-containing proteins (Fig. 1B), although no significant sequence similarity was observed when AFP and OB fold-containing proteins were compared (Fig. 1C; only about 10% identity is observed upon sequence alignment based on the structure fitting). The AFP folding most closely resembled the cold shock domain (CSD), which defines a superfamily of proteins, the bacterial cold shock proteins being the first identified members (22). Among the structural determinants of the OB fold, and in particular of the CSD, the presence of a long and strongly coiled β -1 strand is highly remarkable. This coiling is promoted by a small residue (usually glycine). In addition, a β bulge in strand 1 and usually a glycine residue at the beginning of strand 4,

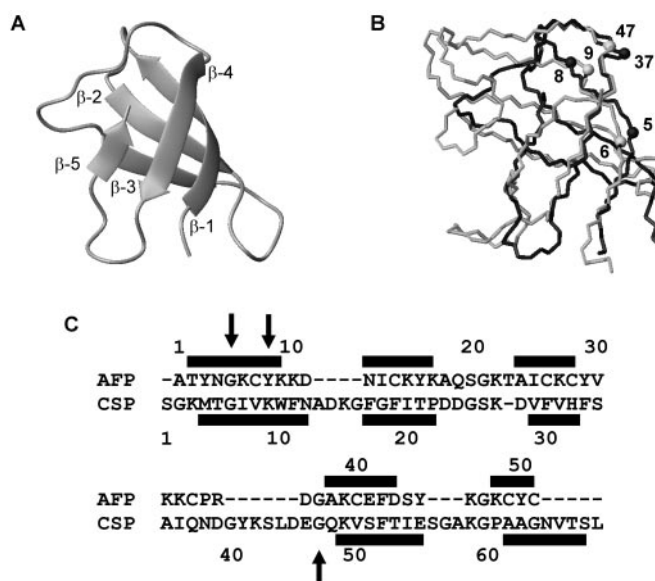


FIG. 1. Three-dimensional structure of AFP. A, diagram corresponding to the three-dimensional structure of AFP, constructed from the atomic coordinates deposited in the Protein Data Bank (reference 1afp), indicating the five strands (1–5 from the NH₂-terminal end of the protein). B, three-dimensional structures of AFP (black line) and major cold shock protein (CSP) from *E. coli* (light line). Only the backbone atoms are considered. The image was constructed from the atomic coordinates (cold shock protein: Protein Data Bank reference 1mjc). Both structures were fitted by considering the backbone atoms of residues corresponding to the five strands of both proteins (r.m.s. = 2.89; the r.m.s. values for all the backbone atoms of the 20 conformers of each of the three families of structures of AFP analyzed by NMR are 1.01, 0.89, and 1.01; Ref. 17). Residues Gly-5, Tyr-8, and Gly-37 of AFP (black) and Gly-6, Lys-9, and Gly-47 of cold shock protein (light) (corresponding to the coiling in strand 1, β bulge in strand 1, and beginning of strand 4, the three structural determinants of the CSD) are shown. Similar results have been obtained for other OB fold-containing proteins: human translation initiation factor Eif1a (r.m.s., 2.92), gene V protein from bacteriophage F1 (r.m.s., 3.19), verotoxin-1 (Shiga-like toxin-1) from *E. coli* (r.m.s., 3.40), and lysyl-tRNA synthetase from *E. coli* (r.m.s., 4.06). Images, corresponding to identical views of AFP, were generated by the MOLMOL program (16). C, sequence alignment of AFP and cold shock protein based on the structure fitting. Bars indicate β strand elements. Arrows mark the residues labeled in B.

opposite the β bulge, are also characteristic. These features are not invariant, but at least one of them is always present in the known OB folds (19). These structural determinants are present in AFP (Fig. 1B) and correspond to Gly-5 and Tyr-8 in strand 1 and Gly-37 at the beginning of strand 4. Thus, AFP could be considered as a member of the CSD-containing family of proteins based on these structural criteria.

The proteins of the OB fold superfamily have unrelated functions with many of them having an unknown functionality. As indicated above, many OB fold-containing proteins bind nucleic acids. In particular, CSD-containing proteins have been shown to bind both dsDNA and ssDNA *in vitro* (19). Therefore, we studied this possibility, and we in fact observed that AFP bound polydeoxynucleotides. Considering that AFP is a cationic polypeptide (5), we first tested the effect of the protein on the electrophoretic behavior of the nucleic acid. Fig. 2 summarizes the results of gel shift electrophoresis assays under non-denaturing conditions for double-stranded calf thymus DNA. This assay allows separation of free nucleic acid from protein-bound nucleic acid by retardation of the electrophoretic mobility. This study was also performed for phage F1 circular single-stranded DNA with similar results (data not shown). Complete retardation of DNA was produced with no bands of intermediate mobility observed, indicating charge neutralization of the nucleic acid by the protein. The fraction of free nucleic acid

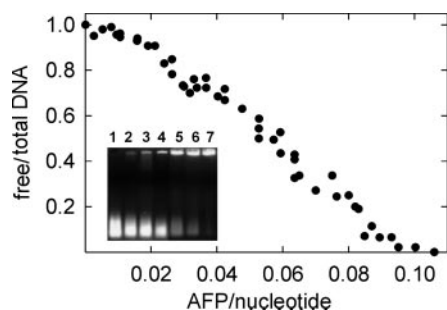


FIG. 2. Electrophoretic mobility shift assay on agarose gel corresponding to the AFP binding to calf thymus DNA. Data are expressed in terms of free/total DNA ratio versus AFP/total nucleotide molar ratio. Free to total nucleic acid ratios were calculated from the image analysis of the corresponding electrophoresis gels (see "Experimental Procedures"). Inset, agarose gel (ethidium bromide staining) of AFP-calf thymus DNA (370 μM nucleotide concentration) at different protein/nucleotide molar ratios (lanes 1–7): 0, 0.02, 0.03, 0.04, 0.07, 0.08, and 0.09, respectively. DNA is retained in the well upon charge neutralization complex formation with AFP.

decreased as the amount of AFP increased, and no free nucleic acid was observed at a minimum protein/nucleotide molar ratio of about 0.1. AFP contains 1 Arg and 12 Lys residues and 1 Glu and 3 Asp residues; its net charge is about +9 at neutral pH. Thus, no free nucleic acid was observed when a protein (+)/nucleotide (–) charge molar ratio of ~ 0.9 was reached in the assayed mixture (Fig. 2). The cationic character of AFP and the observed charge neutralization strongly suggested that DNA condensation occurred. In fact, 89–90% of the DNA charge (as observed in this case) must be neutralized for condensation to occur (23). AFP-DNA mixtures were also analyzed by measuring the intensity of the 90° scattered light, and they were also centrifuged at high speed in Eppendorf tubes to evaluate the nucleic acid in the supernatant. The results obtained are shown in Fig. 3. At about a 0.1 protein/nucleotide molar ratio, virtually all the DNA was pelleted, and a maximum of scattered light was also observed at such a ratio in agreement with the results obtained from the electrophoretic study. In addition, these assays demonstrated a high affinity of the protein for the deoxyoligonucleotides since virtually all added protein is bound to the polynucleotide. Moreover, the DNA-protein interaction did not require any specific nucleotide sequence because 90% charge neutralization was achieved. However, the results of this assay did not allow the estimation of a binding stoichiometry. The nucleic acid could bind more protein molecules than those producing charge neutralization.

The simplest DNA-condensing agents are multivalent cations with valence ≥ 3 as demonstrated for the trivalent cation spermidine (24). But, in addition to the nonspecific interaction of charge neutralization, specific interactions depending on the condensing agent have important impacts on DNA condensation (25). Three types of interactions account for binding of proteins by DNA involving different amino acid side chains: hydrogen bonding (hydrophilic side chains), stacking (aromatic side chains), and ionic (positively charged side chains). The results from the above assays indicated the involvement of ionic interactions in AFP-DNA binding. Aromatic residues on the twisted surface of a β sheet are a means to bind bases of nucleic acids by stacking interactions (20). AFP contains 1 Phe and 6 Tyr residues, three of them displaying a high solvent accessibility (Tyr-29, Tyr-45, and Tyr-50) (17). The fluorescence emission of AFP is related to these three exposed tyrosines since the characteristic protein fluorescence was abolished when three Tyr were titrated to tyrosinate ion at around pH 10 (3). Thus, the interaction between AFP and nucleic acids was also studied by measuring the intrinsic fluorescence emission

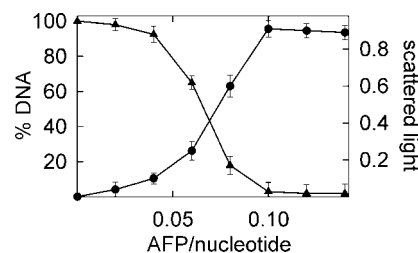


FIG. 3. Condensation of DNA promoted by AFP. Variation of percentage of DNA (%DNA) remaining in the supernatant after centrifugation of AFP-calf thymus DNA samples (triangles) and 90° scattered light of these samples (circles) at different protein/nucleotide molar ratios (average values \pm S.D. of three different experiments).

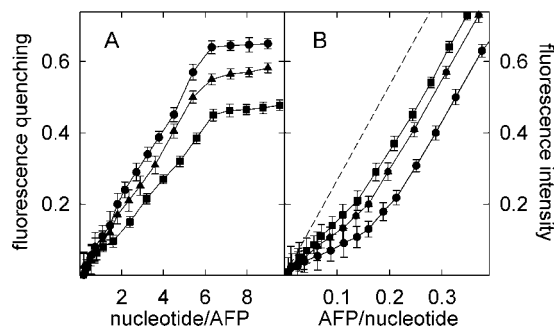


FIG. 4. Quenching of the tyrosine fluorescence emission of AFP upon binding to DNA. A, quenching of the fluorescence intensity as a function of the DNA concentration expressed in terms of nucleotide/AFP molar ratio. Experiments were performed at a constant AFP concentration of 11.58 μM . Quenching values were calculated as $1 - F/F_0$, where F_0 is the fluorescence emission at 305 nm (for excitation at 275 nm) of free AFP, and F is the fluorescence in the presence of the corresponding DNA concentration. Squares, calf thymus DNA; triangles, double-stranded 27-mer oligonucleotide; circles, single-stranded 27-mer oligonucleotide. B, variation of the fluorescence at 305 nm emission for the titration of dsDNA and ssDNA (same symbols as in A) with AFP (protein concentration expressed in terms of AFP/nucleotide molar ratio; experiments were performed at a constant nucleotide concentration of 55.4 μM). The fluorescence emission corresponding to AFP in the absence of nucleic acid is given by the dashed line. Average values \pm S.D. of three different experiments are shown.

of the protein. The interaction of AFP with DNA resulted in quenching of the tyrosine fluorescence (Fig. 4). Calf thymus DNA promoted a maximum quenching of about 45%, and the saturation was observed at a nucleotide/protein molar ratio of ~ 6 (Fig. 4A). These results were corroborated by titrating a DNA solution with AFP. This plot should display two portions, the first one corresponding to the bound protein and the second one to the free unbound protein after saturation of the nucleic acid. Addition of the protein produced an increase of the fluorescence emission up to an AFP/nucleotide molar ratio of about 0.15 (~ 6 nucleotides/AFP) but lower than that corresponding to the free protein at identical concentration (Fig. 4B). Upon further addition of AFP, a linear increase was observed with a slope corresponding to that of the free protein (Fig. 4B). This indicated that all the excess protein above such a saturating ratio remained unbound. The ratio of the slopes of the two portions of the plot would give an estimation of the proportion of unquenched fluorescence. This ratio was about 0.6 (40% quenching) in fair agreement with the maximum quenching observed upon titration of the protein. Similar results were obtained when F1 DNA was used (data not shown) and also when these assays were performed for both the single- and double-stranded 27-mer oligonucleotide (see "Experimental Procedures") (Fig. 4). The saturating protein/nucleotide molar ratios were identical, but the maximum quenching was 55 and 65% for double- and single-stranded oligonucleotide, respectively.

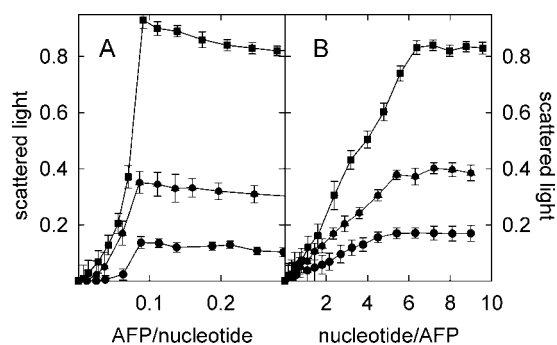


FIG. 5. **Light scattering of AFP-nucleic acid samples.** Variation of the intensity of the scattered light (in arbitrary units) versus AFP/nucleotide (A) and nucleotide/AFP (B) molar ratios in nucleic acid titration by addition of AFP and protein titration by addition of polydeoxynucleotide, respectively. Squares, calf thymus DNA; triangles, double-stranded 27-mer oligonucleotide; circles, single-stranded 27-mer oligonucleotide. Average values \pm S.D. of three different experiments are shown.

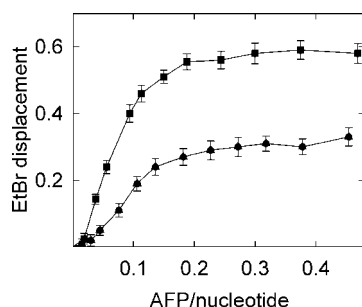


FIG. 6. **Ethidium bromide displacement assay in nucleic acid titration by addition of AFP.** Squares, calf thymus DNA; triangles, double-stranded 27-mer oligonucleotide (average values \pm S.D. of three different experiments). Displacement was calculated as $(F - F_p)/(F_b - F_p)$, where F_f and F_b are the fluorescence intensities at 600 nm of EtBr in the absence and presence of DNA, respectively. Experiments were performed at a constant nucleotide concentration of 37.0 μ M.

These results would indicate that polynucleotide chain length is not decisive for the interaction, and this would not require a double-stranded structure. Considering that the fluorescence emission of AFP arises from three tyrosines, the obtained results may suggest that 1–2 Tyr residues were involved in the interaction with the nucleic acids if it is assumed that each of these 3 residues have a similar contribution to the protein fluorescence.

Protein fluorescence quenching upon nucleic acid binding has also been observed for other OB fold-containing proteins. Thus, the fluorescence emission of the gene V protein encoded by bacteriophage M13, which binds to single-stranded DNA, is quenched upon binding to different oligodeoxynucleotides. The extent of maximum quenching is in the range of 17–45% depending on the length of the oligonucleotide (26). The major cold shock protein of *Bacillus subtilis*, which also binds to single-stranded deoxynucleic acids, also shows fluorescence quenching upon nucleic acid binding (27), the quenching ranging from 25–90% depending on the sequence of the considered oligonucleotide since this protein can preferentially bind polypyrimidine templates.

DNA-AFP binding produced turbidity, characteristic of a DNA condensation process. Thus, the course of the binding was also analyzed by measurement of the scattered light at a right angle. The results obtained are given in Fig. 5. Titration of the polynucleotides by addition of AFP produced a variation of the scattered light that showed a maximum at an AFP/nucleotide molar ratio of \sim 0.1, the scattered intensity decreasing in the order DNA > ds27-mer > ss27-mer (Fig. 5A), which would be

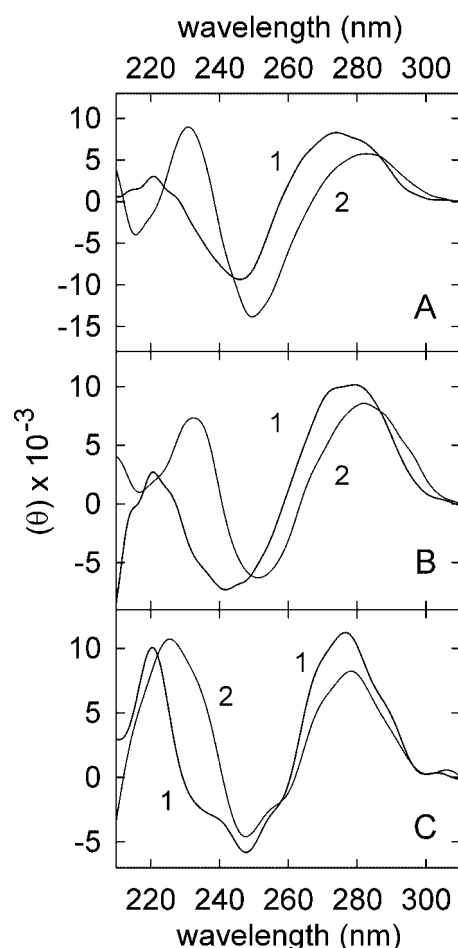


FIG. 7. **Circular dichroism of AFP-nucleic acid complexes.** Shown are the CD spectra of calf thymus DNA (A), ds27-mer (B), and ss27-mer (C) (spectrum 1) and in the presence of AFP at 0.05 protein/nucleotide molar ratio (spectrum 2). Ellipticity values are expressed in units of degree \times cm² \times dmol⁻¹ of nucleotide.

correlated with the decrease in size of the corresponding aggregates. This ratio is that at which charge neutralization of DNA was observed from the retardation electrophoretic mobility assays (Fig. 2). It is well known that addition of cationic peptides to DNA promotes charge neutralization and a collapse of the DNA structure resulting in compact condensed structures (25, 28). Thus, this result was consistent with a protein binding by strong electrostatic interactions followed by aggregation as charge neutralization occurs (29). However, more protein molecules than those required for charge neutralization can be bound to the nucleic acid as deduced from the fluorescence titrations and also from the measurement of the scattering in titration of AFP by addition of polydeoxynucleotides (Fig. 5B). A continuous increase of the light scattering up to a saturating value of \sim 6 nucleotide/AFP molar ratio was observed in agreement with the results from the protein fluorescence quenching measurements (Fig. 4), the maximum value also decreasing in the order DNA > ds27-mer > ss27-mer.

EtBr, a widely used fluorescent probe for DNA, is bound by intercalation between the base pairs. EtBr fluorescence enhancement when the probe intercalates DNA and the subsequent fluorescence decrease upon probe displacement by ligand binding to DNA are well known. The binding constant of this probe is dependent on the molecular flexibility of DNA, which is altered through cationic compaction (30). Thus, DNA condensation promotes exclusion of EtBr from the DNA (31). Therefore, DNA condensation induced by AFP was studied by

an EtBr displacement assay. The results obtained for AFP are summarized in Fig. 6. AFP promoted a decrease of the fluorescence emission of EtBr-DNA complex (about 60 and 30% for calf thymus DNA and ds27-mer, respectively). These values were roughly in the same proportion as those corresponding to the intensity of the scattered light in the equivalent experiments (Fig. 5A) as it would be expected if the probe displacement was a consequence of the extent of DNA condensation.

The interaction of AFP with polydeoxynucleotides (calf thymus DNA, ds27-mer, and ss27-mer) was also analyzed by circular dichroism measurements. These studies can only be reliably performed up to an AFP/nucleotide molar ratio of about 0.05 since at higher ratios the turbidity of the samples distorted the CD spectra. Short (0.01-cm) optical path cells were used to minimize the effect of turbidity. The obtained results are given in Fig. 7. A progressive decrease in the amplitude of the positive CD band at about 280 nm was observed for the three polydeoxynucleotides as the proportion of AFP increased, suggesting a loss of the stacking interactions. Thus, the interaction of AFP with DNA would be consistent with a protein binding by strong electrostatic interactions with alteration of the base stacking interactions, involving Tyr residues of AFP, followed by collapse of the nucleic acid as charge neutrality is approached.

There are many strategies found in protein-nucleic acid interaction. Exposed aromatic amino acids and extended loops, frequently poorly structured, are among them (20), and they are present in the AFP structure. In addition to AFP two other antifungal proteins from molds, PAF and Anafp, have been described (8, 9). AFP displays 45 and 31% sequence identity, respectively, with these two proteins. There are 14 positions conserved within the three proteins and four more exhibiting substitutions of conservative character. Eleven of the conserved positions are in the protein core according to the known structure of AFP. Six of the remaining conserved positions are highly exposed (Lys-17, Gln-19, Lys-22, Lys-32, Asp-36, and Tyr-45). Although PAF and Anafp have not been described to bind nucleic acids, the sequence similarity between these three proteins may suggest a relevant role for these exposed and conserved residues in the AFP-nucleic acid interaction. The three conserved positively charged residues deserve more significance because PAF and Anafp display a lower net positive charge (+5 and 0, respectively) than AFP (+9). The results obtained from the spectroscopical analysis of the AFP-nucleic acid interaction strongly suggest a particular role for the conserved Tyr-45 residue in the binding.

In summary, the antifungal protein AFP can be considered as an OB fold-containing protein based on the similarities observed at the level of three-dimensional structure between AFP and these proteins. In addition, AFP exhibits the characteristic ability of this group of proteins to bind nucleic acids. This *in vitro* interaction of AFP with nucleic acids resulting from its OB fold structure strongly suggests that this ability is related to its physiological activity. The CSD is regularly found in proteins involved in translation initiation and in RNA turnover (for a review, see Ref. 32). Thus, the major cold shock protein from *E. coli* is a chaperone for mRNA during the cold shock response (33). AFP and α -sarcin appear in the extracellular medium of *A. giganteus* under a "shock" circumstance once the corn starch, the glucose source, is consumed (2), and α -sarcin is related to the RNA structure (it is a highly specific cytotoxic ribonuclease degrading a single phosphodiester bond of the ribosomal RNA). In addition, it has been recently reported that the expression of the *afp* gene is up-regulated by heat shock upon growth in the presence of excess NaCl and ethanol and under conditions of carbon starvation (34).

AFP also interacts with phospholipid membranes (3). Other

nucleic acid-binding proteins also interact with acidic phospholipids. This is the case for DnaA protein, the initiator of DNA replication in *E. coli* that specifically binds to *oriC*, the unique sequence for the initiation of the chromosomal DNA replication, and recruits other replication proteins (35, 36). This protein interacts with acidic phospholipids, and its activity seems to be regulated by membrane binding in cells (37). Since AFP is an extracellular protein, the potential involvement of its two binding abilities, to membranes and DNA, in its antifungal action should be considered. In regard to this, it has been reported that AFP completely inhibits the growth of phytopathogenic fungi in rice with no toxicity toward plant protoplasts (38) and that heterologous expression of the *afp* gene enhanced fungal resistance in transgenic wheat (39), which opens the potential use of the *afp* gene to enhance crop protection against fungal pathogens in transgenic plants. Finally, the activity of AFP on promoting DNA condensation and interacting with lipid membranes may also be useful in designing non-viral gene delivery systems.

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