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Dimer:dimer stacking Interactions are important for nucleic acid binding by the archaeal chromatin protein Alba

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short title: Dimer stacking by the archaeal chromatin protein Alba

Abbreviations used: dsDNA, double stranded DNA; ssDNA, single stranded DNA; SDSL, site-directed spin-label; MTSSL, (1-Oxyl-2,2,5,5-tetramethylpyrroline-3-methyl) methanethiosulfonate; HSQC, heteronuclear single quantum coherence; DEER, double electron-electron resonance.

Synopsis

Archaea use a variety of small basic proteins to package their DNA. One of the most widespread and highly conserved is the Alba (Sso10b) protein. Alba interacts with both DNA and RNA *in vitro*, and we show here that it binds more tightly to dsDNA than to either ssDNA or RNA. The Alba protein is dimeric in solution, and forms distinct ordered complexes with DNA that have been visualised by electron microscopy studies, these studies suggest that on binding dsDNA the protein forms extended helical protein fibres. An end-to-end association of consecutive Alba dimers is suggested by the presence of a dimer:dimer interface in crystal structures of Alba from several species, and by the strong conservation of the interface residues, centred on Arg-59 and Phe-60. Here we map perturbation of the polypeptide backbone of Alba upon binding to DNA and RNA by NMR, and demonstrate the central role of Phe-60 in forming the dimer:dimer interface. Site directed spin labelling and pulsed electron spin resonance are used to confirm that an end-to-end, dimer:dimer interaction forms in the presence of dsDNA.

Keywords: archaea, Alba, NMR, site-directed spin labelling, electron spin resonance

INTRODUCTION

In contrast to the eukaryotic histone, there is no universal archaeal chromatin protein. Instead, archaea tend to utilise two or more unrelated proteins to package their DNA. The archaeal histone is conserved in the euryarchaeal branch of the archaeal lineage and like its eukaryotic counterpart it forms a nucleosome that wraps dsDNA (reviewed in [1]). Histone proteins are also present in the marine archaeon *Cenarchaeum symbiosum* [2], but are absent from most crenarchaea and also from euryarchaeal thermoacidophiles such as *Thermoplasma acidophilum* [3]. The second widely distributed chromatin protein, Alba (also known as Sac10b or Sso10b) is present in one or two copies in the genomes of all archaea sequenced to date with the exception of the *Methanosarcinas* and Halophiles [4].

Alba is a dimeric, highly basic protein with a subunit size around 10 kDa. The Alba1 protein is highly abundant in the *Sulfolobus* species from which it was originally purified [5], representing approximately 4% of the total soluble protein [6]. The crystal structure of Alba1 from *Sulfolobus solfataricus* revealed a compact α/β structure similar to DNaseI, translation initiation factor IF3 and other bacterial proteins [7]. The structural similarity to RNA binding proteins, coupled with the observation that Alba can bind to RNA *in vivo* and *in vitro* [8], has prompted the suggestion that Alba may also function as an RNA binding protein *in vivo* [9]. A role in DNA binding *in vivo* has been confirmed by chromatin immunoprecipitation experiments, which show a wide distribution of *S. solfataricus* Alba1 at different gene loci [10]. In *S. solfataricus*, a second Alba paralogue, Alba2, is expressed at 5-10% of the Alba1 protein level. Alba2 forms heterodimers with Alba1, resulting in a reduction in DNA binding affinity, and it has been suggested that this may provide a mechanism for the control of chromatin packaging in this organism [11].

The Alba1 protein in *S. solfataricus* is reversibly acetylated on a single lysine residue, and this results in a reduction in the DNA binding affinity [12]. *In vitro*, acetylation of Alba1 reduces the repressive effects of the protein on transcription [12] and on strand separation by the replicative helicase MCM [13]. However, the relevance of these findings in other archaeal species is not yet clear [4]. DNA binding by Alba is a highly cooperative process, with a final stoichiometry of one Alba dimer for every 6 bp of dsDNA bound, suggesting a high binding density [5, 6]. Electron microscopy studies of DNA binding by Alba from *Sulfolobus acidocaldarius* revealed the formation of fibre-like structures that were thought to indicate extended interwound helical protein fibres [5]. These findings were later confirmed using the recombinant protein from *S. solfataricus* [11], where it was further shown that the presence of the Alba2 protein caused subtle changes in the chromatin structures visualised. The crystal structure of Alba1 from *S. solfataricus* and Alba1 proteins from other species have highlighted a strongly conserved dimer:dimer interface centred on the Phe-60 residue, which stacks with its counterpart in the adjacent Alba1 dimer in the crystal lattice. It has been proposed that this interaction has biological relevance [14] and may relate to the fibre formation observed by electron microscopy [11]. It has also been suggested that one role of the Alba2 subunit is to weaken or disrupt the dimer:dimer interface in protein fibres, as this interface is not conserved in Alba2 [11].

In this paper we use NMR to map the amide backbone chemical shift changes of *S. solfataricus* Alba1 upon binding to dsDNA, ssDNA and RNA. This highlights differences between the three nucleic acid types, both in terms of the residues that are perturbed and the nature (size and shape) of the complexes formed. We show, using gel electrophoretic mobility shift experiments, that dsDNA is bound more tightly than either ssDNA or RNA. Site directed spin labelling (SDSL) is used as a sensitive probe of dimer:dimer formation on DNA binding, and the relevance of the Alba1 crystallographic dimer:dimer interface is confirmed using an F60A site directed mutant form of the Alba1 protein.

EXPERIMENTAL

Protein expression, purification and mutagenesis

Recombinant Alba1 proteins were expressed in *E.coli* BL21 *Rosetta* strain and purified as described previously [11, 15]. Protein concentrations were determined by measuring the optical absorbance at 280 nm. The F60A and R59C mutant versions of Alba1 were constructed using the Quikchange mutagenesis protocol (Stratagene), following the manufacturer's instructions. The oligos used for mutagenesis, F60Af; F60Ar; R59Cf; R59Cr, are available from the corresponding author on request. Mutated genes were sequenced fully to confirm that no spurious mutations had been introduced. The mutant proteins were purified as for the wild-type protein.

Oligonucleotides for binding studies

DNA and RNA oligonucleotides were purchased from MWG Biotech Ltd and Operon Biotechnologies, Cologne, Germany. The sequence of the 39mer ssDNA was 5'-CCTCGGTGCTAAGTTGATGCTGGTACTCGGAGTATCCCG-3' and that of the 16mer ssDNA and ssRNAs were 5'-CCCGGCGT(U)GCGGCCCG-3', where uracil (U) replaces thymine in the RNA oligomer. The 16 and 39mer ssDNA sequences correspond to one complimentary strand of the 16 and 39 bp DNA duplexes, respectively. 28mer polyadenine (28A) and 30mer polyuridine (30U) ssRNA oligonucleotides were also used.

DNA electrophoretic gel retardation assays

Binding assays were performed, and the data analysed as described previously [11]. Apparent dissociation constants for the interaction between wild-type Alba1 and a 39 bp DNA duplex, 39 mer ssDNA, 30U, 28A and 16 mer ssRNAs, and for the interaction between F60A Alba1 and 16 and 39 bp DNA duplexes were determined. Data were fitted to a two state binding model where the fraction bound = $1/(1 + K_d/[Alba1])^m$ and the exponent, m, corrects for deviations in slope resulting from cooperativity. Data-fitting and graphical representations were generated using the program KALIEDOGRAPH (Synergy Software).

NMR Spectroscopy

The backbone assignment of Alba1 was obtained as described previously [11]. A 420 μ M solution of 15 N labelled Alba1 in 20 mM Bis-Tris, 0.3 M NaCl, 1 mM NaN_3 , pH 6.5 containing 10 % D_2O was titrated with a solution of 1.2 mM 16 bp DNA duplex to give a final molecular ratio (duplex:Alba dimer) of 0.2. Similar titrations were performed with the 16 mer ssDNA 16 mer ssRNA and 28A ssRNA oligomers to final molecular ratios of 0.21, 0.33 and 0.14, respectively. Sub-stoichiometric quantities were used in order to maximise any potential contributions from additional protein-protein interactions formed upon binding of Alba1 to each oligonucleotide. After each addition the samples were allowed to equilibrate at 55 °C for ~15 min after which a ^1H - ^{15}N HSQC spectrum was recorded. Spectra were acquired on a 500 MHz Bruker DRX spectrometer. All spectra were referenced in the direct dimension to the carrier frequency at 4.75 ppm and to the relative gyromagnetic ratios of ^1H and ^{15}N in the indirect dimension. NMR data were processed using FELIX 2000 (Accelrys, San Diego).

Plots of the average relative signal intensity as a function of mole fraction of

oligonucleotide were generated using KALIEDOGRAPH (Synergy Software). The initial intensity of each amide resonance was normalized to 1. In order to reflect only the effect on correlation time, data relating to residues with an associated chemical shift change (where $\sqrt{[(\Delta\delta n/10)^2 + \Delta\delta n^2]} > 0.015$) were excluded.

Site-directed spin-labelling (SDSL)

Site directed mutagenesis was used to convert residue Arg-59 to a cysteine residue, as described. As there were no cysteines in the native *S. solfataricus* Alba1 sequence, the R59C mutant could be directly modified with the thiol-specific methanethiosulfonate spin label MTSSL (Toronto Research Chemicals Inc.). The protein was purified as for the wild-type protein, and dialysed against 10 mM TES buffer pH 7.4, 100 mM NaCl overnight at 4 °C. The spin labelling reaction was carried out using 20 μ M R59C protein, 100 μ M MTSSL in 10 mM TES buffer pH 7.4 and 100 mM NaCl in a volume of 2.5 ml [16]. After incubation at 4 °C for 1 hr, the unincorporated label was removed from the protein sample using a sephadex G-25 minicolumn (GE Healthcare) according to the manufacturer's instructions. The purified protein was concentrated to achieve a final concentration of 100-150 μ M in 300 μ l total volume, and the incorporation of the spin label was confirmed by mass spectrometry. For experiments in the presence of DNA, a 16bp duplex assembled from the oligo 5'-CCCGGCGTGCGGCCCG-3' and its complement, was added to the protein sample to a final concentration of 100 μ M.

Electron Spin Resonance Spectroscopy

Samples were prepared for ESR by buffer-exchanging them into D₂O/deuterated ethylene-glycol media (TES pH 7.4, 100 mM NaCl). The spin-labelled protein was exchanged into D₂O (Aldrich) buffer and 20 % deuterated ethylene glycol-d⁶ (Cambridge Isotope Laboratories) by sequential concentration and dilution with a centricon spin concentrator. The protein solution was then transferred into a clear fused quartz ESR tube, 3 mm internal diameter under an Argon atmosphere. The samples in ESR-tubes were quick-frozen by immersion into an isopentane/hexane freezing mixture (~ -70 °C) and then stored under liquid nitrogen until use. Continuous wave electron spin resonance spectra (cw-ESR) were measured at X-band using a Bruker ESP 300 ESR spectrometer as described previously [17]. Spectra acquisition conditions are given in the figure legend. The pulsed DEER (Double Electron-Electron Resonance) experiments were carried out using a Bruker ELEXSYS E580 spectrometer operating at X-band with a dielectric ring resonator (ER 4118X-MD5-EN) and a Bruker 400U second microwave source unit. All measurements reported here were made at 50 K with an over-coupled resonator giving a Q factor of approximately 100. The measurements used the four pulse, dead-time free, sequence with the pump pulse frequency positioned at the centre of the nitroxide spectrum; the frequency of the observer pulses was increased by 70 MHz [16]. The observer sequence used a 32 ns π -pulse; the pump π -pulse was typically 30 ns. The resulting dipolar coupling evolution data was analyzed by Tikhonov regularization to a distance distribution using DeerAnalysis2006 developed and made freely available by Gunnar Jeschke (www.mpip-mainz.mpg.de/~jeschke/wpub.html) [18].

RESULTS

Comparison of nucleic acid binding affinities of Alba1

To compare the binding of Alba1 to dsDNA, ssDNA and ssRNA, we determined the binding

affinities by electrophoretic mobility shift assay using 39 bp dsDNA, 39mer ssDNA and 30U, 28A and 16mer ssRNA oligonucleotides (Fig. 1). The RNA oligonucleotides were designed to test for sequence-specific binding and to avoid the possibility of base pairing. The apparent dissociation constants were calculated from experiments carried out in triplicate. The data show that Alba1 binds about 5 fold less tightly to ssDNA (K_D 270 nM) and about 20 fold less tightly to ssRNA (K_D 960 nM) than it does to dsDNA (K_D 50 nM) (Table 1). In contrast, Guo *et al.* [8] reported that K_D 's for all three species were "similar" (~ 100-300 nM) although a rigorous quantification of the dissociation constants was not included in their paper. This difference can be accounted for by the fact that our experiments were conducted at relatively high salt (0.3 M vs 0.01 mM), which weakens the interaction with RNA [8], but does not affect the dsDNA binding affinity (data not shown). Higher ionic strengths are likely to be closer to the physiological condition. Notably, we also found the RNA binding affinity of Alba1 to be independent of the length of oligonucleotide in contrast to dsDNA, where binding to longer duplexes is tighter [11]. This suggests that the molecular nature of the interaction of Alba1 with ssRNA is also different from that with dsDNA. dsRNA, which adopts the A-form helical conformation, was not studied.

Residues involved in binding dsDNA, ssDNA and ssRNA

Initial ^1H 1D experiments, where Alba1 was titrated with a 28 bp duplex, resulted in the formation of a very large complex suggestive of a DNA-templated assembly process involving multiple protein-protein interactions (data not shown). A much smaller complex was formed upon binding to a shorter duplex (16bp) under the same conditions (0.3 M NaCl, pH 6.5, 55 °C). Residues perturbed upon binding this duplex and the 16 mer ssDNA and ssRNA oligomers were then determined by measuring the changes in chemical shift of fast exchange peaks observed in the ^1H - ^{15}N HSQC spectrum of Alba1 upon titration with the relevant oligonucleotide. It was found that amide resonances, corresponding to G4 and R86, were not present in the ^1H - ^{15}N HSQC spectrum at pH 6.5, presumably due to intermediate exchange with solvent. In addition, no peaks were observed for K16, R44 or Q80 regardless of pH. In Figure 2 the summed, weighted changes in ^1H - and ^{15}N chemical shifts are plotted against residue number. These changes cannot unequivocally be attributed to a direct interaction between protein and oligonucleotide, but their localised nature implies that large changes in protein conformation are not involved. Where the sum chemical shift changes (ϵ 0.015) are plotted onto a surface representation of Alba1, the general binding regions can be clearly identified. It is clear that the basic surface predicted to interact with DNA following the initial structural studies [7] does constitute the major portion of the binding interface. It is apparent that the residues involved in binding the DNA duplex and ssDNA are the same whilst only a subset of these are involved in binding to the equivalent ssRNA. Additionally, there is a distinct region (residues 10-15) that appears to be associated more specifically with RNA binding as well as a DNA specific region (residues 19-21). Data obtained from a titration of Alba1 with 28 poly-A ssRNA were very similar to those obtained with the 16mer ssRNA, suggesting that the changes observed were not strongly sequence dependent.

The NMR acquisition parameters, processing and experimental conditions for the titrations of Alba1 with DNA and RNA were identical. The degree to which peaks are attenuated upon addition of the various oligonucleotides therefore provides further evidence that the interaction of Alba1 with DNA is distinct from that with RNA (Fig. 3). Provided that contributions from chemical and conformational exchange resulting from a direct binding interaction are removed, the degree of line-broadening reflects the tumbling rate (i.e the size and shape) of the complex. The greater degree of line-broadening indicates that a larger complex is formed upon binding of Alba1 to the 16mer ss- and duplex DNAs than to 16mer

ssRNA. These data clearly show that the increase in correlation time upon binding to DNA is larger than that upon binding to RNA. This indicates that the DNA complex is larger and/or more elongated than the RNA complex and is consistent with the formation of additional protein-protein interactions.

Importance of the dimer:dimer interface for DNA binding.

Upon binding of Alba1 to DNA, chemical shift changes were observed for residues associated with a conserved crystallographic dimer-dimer interface (Figure 2). These observations are consistent with the formation of additional protein-protein interactions in the Alba1-DNA complex that may indicate a mechanism for the assembly of nucleoprotein filaments. Crystal structures of the Alba1 protein from several species highlight the conserved dimer:dimer interface [14]. In contrast, the *S. solfataricus* Alba2 protein is quite divergent in this region, and this interface is not present in the crystal structure [11]. Phe-60 is central to the dimer:dimer crystallographic interface of Alba1, where it forms a π - π stacking interaction with an adjacent dimer. Mutation of this position (F54R) in *Archaeoglobus fulgidus* Alba1 resulted in a qualitative decrease in the apparent binding affinity for plasmid DNA [14]. The F60A mutant version of Alba1 was made to test the significance of this residue with respect to DNA binding. The apparent binding affinities of the mutant with 16 and 39 bp duplexes were determined by electrophoretic mobility shift assay and compared with the wild-type protein (Fig. 4 and Table 1). The F60A mutant bound the 16 mer and 39 mer duplexes more weakly than the wild-type protein (3.5-fold and 9-fold, respectively), resulting in an equal affinity for both duplexes. This was consistent with the hypothesis that the dimer:dimer interface is involved in the assembly of Alba1-DNA nucleoprotein complexes and suggests that additional protein-protein interactions formed upon binding of the wild-type protein to longer duplexes are not present in the F60A complexes. These data therefore support the assertion that a protein-protein interaction surface, similar to the crystallographic dimer:dimer interface is involved in the assembly of Alba1 nucleoprotein filaments.

Site-directed spin-labelling (SDSL) as a probe for DNA-templated protein assembly.

In the absence of DNA, Alba1 exists as a dimer in solution, and Arg-59 in one subunit is separated from the same residue in the second subunit of the dimer by a distance of approx 40 Å (Figure 5A). The optimum form of ESR spectroscopy to measure interspin distances depends on the separation of the spin labels: below 20 Å, dipolar splittings observed by conventional continuous wave (cw) ESR can be used; above 20 Å more sophisticated ESR methods are required. We used pulsed DEER spectroscopy (pulsed Double Electron-Electron Resonance) to measure the distance between spin labels in the Arg-59C SDSL sample, estimating an average inter-spin distance of 41 Å with a half height distribution of \pm 3.5 Å (Figure 4B) in good agreement with the crystal structure (Figure 5A).

When 16bp duplex DNA was added to the spin labelled Alba R59C to a final concentration of 100 μ M a dramatic change in the spin-spin interaction between spin-labels on adjacent monomers occurred. A splitting of the cw-ESR spectrum due to dipolar interaction between close paramagnets was observed. In Fig. 4C the cw-ESR spectrum of the spin-labelled Alba R59C is shown in the absence (A-top spectrum) and the presence of 16 bp duplex DNA (B-middle spectrum) or 21mer ssDNA (C-bottom spectrum). Splitting of the spectrum due to dipolar interaction between closely adjacent paramagnets was induced by dsDNA binding (these are indicated in Figure 4 and also shown at x10 amplification). Not all

the Alba R59C present was expected to exhibit this interaction as the 16 bp duplex is only long enough to bind three dimers and there was also more DNA present than required for saturation of binding. Therefore the ESR spectrum shows a mixture of the interacting and non-interacting species because some of the spin-label will be at an exposed 'free-end' and not involved in a close dipolar interaction with an adjacent dimer. The dipolar splitting observed is in the order of 72 Gauss peak to peak (7.2×10^{-3} Tesla) which corresponds to an inter-spin distance of approx. 7.5 Å [16]. This confirms the close assembly of two spin labels, as would be expected if consecutive Alba dimers stack end-to-end on DNA binding as predicted from the crystal structure. The addition of ssDNA (21 bases) appeared to have a grossly similar but less well resolved effect to the addition of dsDNA. There are splittings due to interactions between close spin-labels, but these are broader and less well resolved, indicating the structure is more heterogeneous, perhaps due to a greater flexibility in the Alba-ssDNA complexes.

DEER spectra could not be readily obtained in the presence of dsDNA or ssDNA because the close dipolar interactions enhance the transverse relaxation times, which shortens the available experimental timing window to an extent that the technique can't be readily used. Direct measurement of the transverse relaxation (not shown) confirmed a considerable enhancement of the relaxation due to the close interactions between spin labels when bound to DNA.

DISCUSSION

The majority of sequenced archaeal genomes contain at least one gene encoding the Alba protein [3], making it perhaps the most conserved nucleic acid binding protein in the archaea. Although Alba clearly binds dsDNA *in vitro*, there has been some debate about its true role *in vivo*. On the one hand, UV crosslinking data has suggested that an interaction with RNA might be more physiologically relevant [8]. On the other, chromatin immunoprecipitation experiments have demonstrated that Alba1 is associated with dsDNA [10]. Alba1 from *S. solfataricus* has been shown to stabilize dsDNA against DNA melting by the cognate single stranded DNA binding protein, consistent with a role in the stabilization of dsDNA *in vivo* [19]. Our data demonstrates that Alba1 binds dsDNA more tightly than either ssDNA or RNA *in vitro*, and suggests that differences exist in the interface with DNA and RNA species that are still not fully understood. The stabilization of RNA as well as DNA is likely to be particularly important in thermophiles, where chemical and physical damage to nucleic acid is accelerated by elevated growth temperatures [20]. A recent study of nucleic acid binding proteins in the crenarchaeote *Thermoproteus tenax*, which lacks any clear ssDNA binding protein encoding gene, revealed that the two main detectable ssDNA binding proteins in this organism were the Alba protein and a novel protein named CC1 [21]. Both proteins bound ssDNA and dsDNA. Thus, it is possible that archaea utilize a number of highly-expressed general nucleic acid binding proteins, such as Alba, to stabilize all classes of nucleic acid *in vivo*.

The DNA interaction region as determined by NMR is only partially consistent with previous binding models [7, 14] and differs significantly from that determined by Cui et al [22]. Our experiment is different in that sub-stoichiometric quantities of duplex were used in order to maximize the contribution from protein-protein interactions, which were not evident at the 3:1 excess of duplex used previously [22]. We also found widespread changes in chemical shift upon the addition of salt, which we measured prior to commencing our titrations. We found that whilst the basic, positively charged surface is highlighted, the extended α -loop region (residues 78-84), previously thought to bind in the minor groove of the double helix does not appear to be involved. The evidence for this is two-fold; first the

lack of changes in chemical shifts in this region and second, that the loop remains flexible upon binding. This flexibility is exemplified by R83, at the tip of the loop region which gives rise to a distinctive sharp resonance throughout the titrations (not shown). The NMR data also provided evidence that higher order complexes of Alba1 are a feature of DNA binding. Oligomerization of Alba1, through a dimer-dimer interface, into linear rod-like structures has been observed in all but one of the 5 crystal structures published to date [7, 14, 23]. This has been argued to be of functional importance as the residues involved at the oligomerisation interface are highly conserved within the archaea [14]. The NMR data suggest that this protein-protein interface might indeed be important for the assembly of the Alba:DNA nucleoprotein complex. This was confirmed by site-directed mutagenesis of the central residue of the interface, Phe-60, which resulted in weaker DNA binding.

Additionally, we have shown using SDSL that Alba1 dimers assemble on DNA with consecutive dimers binding end-to-end, consistent with the crystal structures. This presumably relates to the extended helical protein fibres observed by electron microscopy when Alba1 is complexed with plasmid DNA [5, 11]. An attractive hypothesis is that the dimer:dimer interface may provide a mechanism by which DNA could be rapidly sequestered into high order structures. The next challenge is to formulate a molecular description of the chromatin structure formed by DNA-bound Alba1. This represents, to the best of our knowledge the first example of the use of spin labeling to study nucleoprotein formation, and demonstrates the utility of SDSL to study this type of molecular interaction. One advantage of SDSL over FRET is that only a single type of reporter group is required in SDSL, whereas donor and acceptor dyes are required for FRET studies, which can pose a technical challenge.

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Table 1 Comparison of the binding affinity of Alba1 for DNA and RNA

Oligonucleotide	Apparent K_d (nM)	m^a
16 bp (dsDNA)	200 ± 4^b (710 ± 20)	1.3 ± 0.1 (0.9 ± 0.1)
39 bp (dsDNA)	56 ± 2^b (520 ± 13)	1.9 ± 0.1 (1.4 ± 0.1)
39mer (ssDNA)	270 ± 13	2.0 ± 0.2
30U (ssRNA)	900 ± 38	1.6 ± 0.1
28A (ssRNA)	960 ± 30	2.1 ± 0.1
16mer (ssRNA)	920 ± 69	2.2 ± 0.4

Values are mean \pm SEM for triplicate measurements

Values obtained for the F60A mutant are shown in parentheses.

^aThis parameter was used to improve the fit and reflects the steepness of the curve.

^bThese values are reproduced from [11].

Figure legends

Figure 1. DNA and RNA binding by Alba1.

Binding Curves were obtained by gel electrophoretic retardation analysis. Data relating to binding of Alba to 39 bp dsDNA*, 39 mer ssDNA and 30U ssRNA, 28A ssRNA and 16 mer ssRNA are denoted by open circles, open squares, closed triangles, closed squares and closed circles, respectively. Standard error bars are shown for each data point and curves obtained from fitting the data to the binding equation described in the text are represented as solid black lines.

*Data reproduced from [11]

Figure 2. NMR chemical shift analysis of Alba1 interaction with nucleic acids. The weighted sum of nitrogen and proton chemical shift changes ($\sqrt{[(\Delta\delta_n/10)^2 + \Delta\delta_{nh}^2]}$) upon titration of Alba with **A** 16 bp dsDNA **B** 16 mer ssDNA **C** 16 mer ssRNA and **D** 28A ssRNA are plotted against residue number. Vertical dashed lines show residues associated with a conserved crystallographic dimer-dimer interface. Those residues with a sum chemical shift change >0.015 are plotted. These are highlighted in red on the corresponding surface representations of Alba1 in order to indicate the regions that are perturbed upon binding. K16 and R44, which are in intermediate exchange, are shown in grey. K16 has been shown previously to be involved in binding DNA [7] and R44 is likely to be involved given its position and charge. The structural representations were generated using MacPymol (© 2008 Delano Scientific LLC).

Figure 3. Global NMR line broadening effects during titration of Alba1 with nucleic acid. Alba1 was titrated with a 16 bp duplex (circles), a 16 mer ssDNA (squares) and the corresponding 16 mer ssRNA (triangles). For each point in the titration, the average relative signal intensity for peaks in the ^{15}N - ^1H HSQC spectrum of Alba1 was plotted with standard error bars. Residues with an associated chemical shift change were omitted from the calculation to remove contributions from chemical and conformational exchange resulting from direct binding interactions. Plots of the average relative signal intensity as a function of mole fraction of oligonucleotide were generated using KALIEDOGRAPH (v 3.6 Synergy

Software). Standard error bars were calculated for each experimental condition, where only cross-peaks satisfying the criterion $\sqrt{[(\Delta\delta n/10)^2 + \Delta\delta n h^2]} > 0.015$ were included in the analysis (corresponding to a total of 61, 58 or 67 cross-peaks in dsDNA, ssDNA and ssRNA experiments, respectively).

Figure 4. The F60A Alba1 mutant binds DNA more weakly than the wild-type protein.

Alba-DNA Binding Curves Obtained by Gel Electrophoretic Retardation Analysis. Open and closed symbols are used to denote binding of Wild-type* (circles) and F60A (squares) Alba1 to either a 39 bp or 16 bp duplex respectively. The sequence of the 39mer ssDNA was 5'-CCTCGGTGCTAAGTTGATGCTGGTACTCGGAGTATCCCG, which was annealed with a complementary strand to produce the 39 bp duplex. Gel-shift analysis was carried out in triplicate and analysed as described previously [11]. Standard error bars are shown for each data point. The apparent dissociation constants measured for the wild-type and F60A mutant proteins to the 39 bp duplex were $56 \pm 2 \mu\text{M}$ and $520 \pm 13 \mu\text{M}$, respectively. The equivalent values for the 16 bp duplex were $100 \pm 4 \mu\text{M}$ and $710 \pm 20 \mu\text{M}$. A significant increase in apparent binding affinity (K_d) for the 39 bp duplex when compared to the 16 bp duplex is observed for the wild-type but not for the F60A mutant (Table 1).

*Data for the wild-type protein are reproduced from reference [11], but were measured at the same time as those for the F60A mutant.

Figure 5. SDSL confirms the DNA-dependent assembly of consecutive Alba dimers

- A. Overlaid cartoon and surface representations of three Alba1 dimers in the end-to-end arrangement found in various crystal structures are shown in grey. The side chains of R59 and F60 for each molecule, coloured blue and orange respectively, are shown as sticks. The distance in Angstroms between two R59 guanidinium N atoms belonging to the same dimer unit is highlighted in black. This image was generated using MacPymol (© 2008 Delano Scientific LLC).
- B. The interspin distance profile is obtained from transformation of the background corrected DEER spectrum, using Tikhonov regularisation of the four pulse DEER spectrum of spin labelled Alba R59C (no DNA). The minor peak is not significant. The normalized background corrected DEER spectrum is also shown (insert), with the best fit by Tikhonov regularisation shown in red. The experimental conditions and spectral analysis procedure are outlined in the Methods section. A four pulse DEER sequence was used at X-band frequencies (approx. 9.5 GHz) and a sample temperature of 50K.
- C. First derivative cw-ESR spectra of spin labelled Alba R59C are shown in the presence and absence of 16 bp DNA and 21 mer ssDNA. The black trace shows the cw-ESR spectrum in the absence of DNA, the green trace the spectrum in the presence of 16 bp DNA, and the red trace in the presence of 21 mer ssDNA. The inserts show an amplification (x10) of salient features of the spectrum. ESR conditions: temperature, 140 K; modulation frequency, 100 KHz; modulation amplitude, 0.4 mT, microwave frequency 9.5 GHz and microwave power 0.2 mW.









