Volume 17 Number 3 1989 Nucleic Acids Research

The 43 residue DNA binding domain of γδ resolvase binds adjacent major and minor grooves of DNA

Vichien Rimphanitchayakit, Graham F.Hatfull+ and Nigel D.F.Grindley

Department of Molecular Biophysics and Biochemistry, Yale University Medical School, 333 Cedar Street, PO Box 3333, New Haven, CT 06510, USA

Received October 25, 1988; Accepted January 5, 1989

ABSTRACT

The carboxyl-terminal domain of γδ resolvase binds to each half of the three resolvase binding sites that constitute the recombination site, *res*. Ethylation inhibition experiments show that the phosphate contacts made by the C-terminal DNA binding domain are similar to those made by intact resolvase, with the exception of a single phosphate at the inside end of each contact region which is contacted solely by the intact resolvase. The DNA binding domain makes essentially identical contacts to all 6 half sites, whereas the intact resolvase makes slightly different contacts to each binding site. Despite its small size, only 43 amino acid residues, the resolvase C-terminal domain interacts with an unusually large segment of DNA. Phosphate contacts extend across an adjacent major and minor groove of DNA and about one third of the circumference around the helix. The minimal binding segment, determined experimentally, is a 12 bp sequence that includes the 9 base pair inverted repeat (common to all half sites), the adjacent 3 base pairs (towards the center of the intact resolvase binding site), and phosphates at both ends.

INTRODUCTION

The γδ transposon encodes a site-specific recombination system that consists of the *tnpR* gene product, resolvase, and its site of action, *res*. Intermolecular transposition of γδ promoted by the *tnpA* gene product, transposase, gives rise to a cointegrate intermediate, which contains 2 copies of γδ (and thus two *res* sites) in the same orientation. Resolvase-mediated recombination efficiently reduces this intermediate into the final products of transposition, 2 DNA circles each with a single *res*. The *res* site contains 3 resolvase binding sites, each consisting of inverted copies of an imperfectly conserved 9 base pair (bp) sequence (consensus TGTCYR$^{T}_{A}$TA) separated by a variable spacer (10, 16 and 7 bp at site I, site II and site III, respectively; for a review see reference 1).

In a recent study of the resolvase-*res* interaction, we have shown by ethylation inhibition that the phosphate "contacts" of resolvase extend across a segment of 14 bp within each half of a binding site (2). The contact region covers about one-third of the circumference around the DNA helix and includes

an adjacent major and minor groove. In separate studies of the resolvase protein, we have shown that the C-terminal chymotryptic fragment of 43 amino acid residues of resolvase has DNA-binding activity and binds non-cooperatively to each half of the 3 resolvase binding sites (3); no binding was detected with the 140 amino acid N-terminal fragment. The C-terminal domain contains a region which is similar in sequence to the helix-turn-helix structural motif found in many other sequence-specific DNA binding proteins (3,4). Presumably this part of the C-terminal domain binds to the portion of the DNA contact region that covers the major groove.

There are 3 reasons to ask whether all the phosphate contacts identified in the binding studies with the intact resolvase are actually made by the C-terminal domain or whether some of them are specific to the N-terminal domain. First, the contact region is unusually large, covering both a minor groove and a major groove. Second, the small size of the C-terminal domain suggests it might be too small to cover all the phosphate positions contacted by intact resolvase. Third, in our collection of resolvase mutants, a number of those that fail to bind *res* contain amino acid substitutions in the C-terminal portion of the N-terminal domain (G.F.H. and N.D.F.G., unpublished data, see ref. 5). This suggests the possibility that this portion of the N-terminal domain may make specific contacts with the DNA.

In this paper we have compared the contacts made by the C-terminal domain to those of the intact protein by examining the inhibition of binding that results from phosphate ethylation. In addition, we have determined the minimal size of the DNA segment bound by the C-terminal domain. Our results indicate that the C-terminal domain is responsible for nearly all the binding contacts made by the intact resolvase.

MATERIALS AND METHODS

γδ res plasmids and preparation of DNA substrates

Plasmids containing single complete resolvase binding sites were pRW33 (site I), pRW115 (site II) and pRW118 (site III). They were constructed from the deletions *resΔ24R*, *resΔ26LΔ82R*, and *resΔ48L*, respectively (6); the relevant segments are shown in Fig. 5A. In addition to site III, pRW118 contains the right half of site II. The plasmids were digested with appropriate restriction enzymes to obtain DNA fragments each with an individual binding site. The DNA fragments were labeled at the 5′ end using T4 polynucleotide kinase and $\gamma[^{32}P]$ATP. The sizes of the fragments used in ethylation inhibition experiments were 94 bp (SalI-EcoRI fragment) for the analysis of

site I top and bottom strands, 65 bp (EcoRI-BamHI fragment) for site II top strand and 83 bp (BamHI-Sau96I) fragment for site II bottom strand, 75 bp (EcoRI-MaeIII fragment) for site III top strand, and 108 bp (TaqI-EcoRI fragment) for site III bottom strand. Construction of the *resΔ24R* plasmid, pRW33, has given rise to a HpaII site at the deletion end point. In the hydroxyl radical cleavage experiment, SalI-HpaII (91 bp) and HindIII-SspI (63 bp) fragments were used,

Preparation of the C-terminal DNA binding domain of resolvase

The C-terminal domain of resolvase was purified using the procedure of Abdel-Meguid *et al.* (3) with a few modifications. Purified intact resolvase (4-6 mg/ml) was dialyzed at 4°C against 20 mM Tris.HCl, pH 7.5, containing 1.5 M NaCl and 2.5 mM EDTA. 2 ml of dialysate (~8 mg) was incubated with N-α-tosyllysine chloromethyl ketone-treated α-chymotrypsin (Worthington) for 30 min. The final concentration of the α-chymotrypsin was 0.03 U/ml. The reaction was stopped by adding 1 ml freshly prepared 3 mM phenylmethylsulfonyl fluoride (PMSF, Sigma), and the sample was immediately passed through a Sephadex G-50 column (2.5 x 30 cm) preequilibrated with the eluting buffer, 20 mM Tris.HCl, pH 7.5, containing 1 M NaCl, 2.5 mM EDTA, 25 mM 2-mercapto-ethanol, 0.1 mM PMSF and 0.02% NaN_3. The protein fractions were located by monitoring at 280 nm, by SDS-polyacrylamide gel electrophoresis and by DNA binding activity as described below. Since the C-terminal DNA binding domain contains 1 tyrosine and 1 tryptophan, we estimated the concentration of the peak fraction to be 12 μM, using $\epsilon = \epsilon_{trp} + \epsilon_{tyr} = 6756\ M^{-1}cm^{-1}$. The peak fraction of the C-terminal domain of resolvase was used directly in DNA binding experiments.

Ethylation interference

The ethylation interference experiments were as described by Siebenlist and Gilbert (7). About 1-5 μg of 5'[^{32}P]-labeled DNA fragment was resuspended in 100 μl of 50 mM sodium cacodylate, pH 8.0, and mixed with an equal volume of ethanol saturated with ethylnitrosourea. The reaction was incubated for 30-45 min at 50°C, stopped by addition of 20 μl of 3M sodium acetate and 200 μl of 95% ethanol, and the DNA was precipitated at -20°C. The DNA was resuspended and reprecipitated 3 more times, rinsed with 70% ethanol and dried. Ethylated DNA was then used in the binding assay as described below.

Primer extension experiment

The primer extension experiments were done according to the procedure of Liu-Johnson *et al.* (8). The templates and primers are shown in Fig. 6A for the extension of the bottom strand, and in Fig. 6B for the extension of the

top strand. About 4 ng of each template was annealed to about 2 ng of the 5'-labeled complementary primer in 20 μl reactions containing 10 mM Tris.HCl, pH 8.0, and 5 mM $MgCl_2$ at 50°C for 30 min. The samples were divided into 4 aliquots, one for each of the four dideoxy-sequencing reactions (T,C,G, and A). The reactions were incubated for 5 min at 25°C and were stopped by adding EDTA to a final concentration of 2.5 mM. All 4 reactions were mixed together, phenol extracted and ether extracted 3 times. Protein-DNA complexes were purified and analyzed as described below.

Hydroxyl radical cleavage

Hydroxyl radical cleavage reactions were done according to the method described by Tullius and Dombroski (9). The Fe(II)-EDTA-H_2O_2 solution was prepared as follows: 10 μl of a solution containing equal volumes of 20 mM $Fe(NH_4)_2SO_4$ and 20 mM EDTA was added to 50 μl of 6% H_2O_2, 20 μl of 100 mM L-ascorbic acid, and 20 μl of distilled water. 10 μl of this mix was added to 40 μl of DNA solution containing about 5 μg of DNA labeled either at a 3' end (with α-[^{32}P]dATP and the Klenow fragment of DNA polymerase I) or at a 5' end in 20 mM Tris.HCl, pH 7.5, 100 mM NaCl, 10 mM $MgCl_2$ and 1 mM dithiothreitol. The reaction was incubated for 15 min at room temperature and was terminated by adding 5 μl of 100 mM thiourea, 5 μl of 500 mM EDTA, 5 μl of 3 M NaOAc and 200 μl of ethanol. DNA was precipitated, washed with 70% ethanol, dried and resuspended in a suitable volume of 10 mM Tris.HCl, pH 8.0, and 1 mM EDTA.

Separation and analysis of protein-DNA complexes

End-labeled and modified DNA fragments were mixed with resolvase (~0.1 μM) or the C-terminal domain (1.2μM) in 20 μl reactions containing 20 mM Tris.HCl, pH 7.5, 100 mM NaCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, 2 μg of sonicated calf thymus DNA and 2 μg of bovine serum albumin. Reactions were incubated at 37°C for 10 min (for resolvase) or 25 min (for the C-terminal domain), and then at room temperature for 5 min. The samples were loaded onto native polyacrylamide gels (generally 5% for intact resolvase, 20% for the C-terminal domain) in 100 mM Tris borate, pH 8.3, 1 mM EDTA (10,11). DNA bands were identified by autoradiography, eluted, phenol extracted and ethanol precipitated. Ethylated DNA samples were cleaved at modified positions by resuspending the pellets in 5-10 μl of 10 mM sodium phosphate, pH 7.0, containing 1 mM EDTA and 150 mM NaOH and incubating at 90°C for 30 min. Appropriate amounts of samples were mixed with 2 volumes of formamide containing 10 mM EDTA and 0.1% xylene cyanol and bromophenol blue. The samples were analyzed by polyacrylamide gel electrophoresis (acrylamide:bis-acrylamide, 20:1) in 50 mM Tris borate, pH 8.3, 1 mM EDTA and 8 M Urea.

RESULTS AND DISCUSSION

Isolation of complexes between the resolvase C-terminal domain and DNA.

Resolvase-DNA complexes can be separated readily from unbound DNA by non-denaturing polyacrylamide gel electrophoresis (2,11). We have used this technique to isolate complexes of intact resolvase, or its C-terminal domain, with partially ethylated *res* DNA fragments. Complexes with intact resolvase were separated using 5% polyacrylamide gels; those with the C-terminal domain, because of their small shift in mobility, were separated using 10% or 20% polyacrylamide gels. Since the C-terminal domain binds independently to each half of a resolvase binding site (3), one might expect to see a number of complexes equal to the number of half-sites in the DNA. In preparative gels (Fig. 1) we observed 2 complexes with site I DNA, only one complex with site II DNA and three complexes with the fragment containing site III (which included II-R, the right half of site II). Analysis of the complexes (as described below) showed that the single site II complex was with II-L.

Inhibition of binding by ethylation of phosphates

Treatment of DNA with ethylnitrosourea results predominantly in ethylation of the backbone phosphates (7). In addition, and to a lesser extent, purines are ethylated. Cleavage of 5'-labeled ethylated DNA by alkali then gives rise to 3 labeled products: DNA fragments which terminate with 3'-OH, 3' ethylated phosphate or 3' phosphate. If these ends are far from the labeled end, they migrate as a broad single band slightly slower than the corresponding band cleaved by a Maxam-Gilbert chemical sequencing reaction. In some of our experiments we used short DNA fragments and detected all three products of alkali cleavage (see Fig. 3 for example).

The effects of phosphate ethylation on complex formation by intact resolvase (lanes marked R) and the small C-terminal fragment (lanes marked SF) are compared for the three binding sites, I, II and III in Fig. 2-4 respectively. The patterns of interference within each of the six half sites in *res* are very similar to one another and a summary is shown in Fig. 5 B and C. In the following discussion the phosphate positions are described relative to the consensus half-site shown in Fig. 5B using *italicized* numerals and either *t* or *b* to indicate top or bottom strands respectively. The chief results are as follows.

1. The only major differences between the two patterns of inhibitory phosphate ethylations for intact resolvase (black symbols) and the C-terminal domain (open symbols) occur at the innermost region of each half-site, at positions *13t* and *14t*. (a) Ethylation of the phosphate 5' to position *14t* of

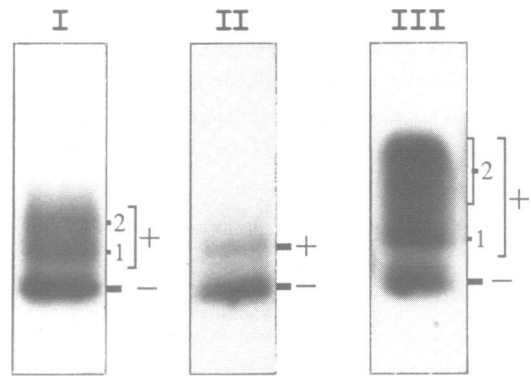


Figure 1. Polyacrylamide gel electrophoresis of complexes of the resolvase DNA binding domain with labeled DNA fragments containing sites I, II or III. The fastest migrating species (-) are the unbound DNA fragments; complexes are marked (+). 1 and 2 refer to the bands analysed in Figs. 2-4.

the consensus generally inhibits binding of intact resolvase (but not always, see below) but has no effect on the binding of the C-terminal fragment. The reduced intensity of the band labeled *14t* in the site I-resolvase complexes (Fig. 2A and B, R+ lanes) is partially masked by an enhanced band running at its leading edge. The enhanced band results from tighter binding of resolvase to site I DNA depurinated at positions -1 or +1 (= consensus position *14*) (V.R., unpublished results, see ref. 5). In other experiments and in our earlier work (2) the inhibition by the *14t* ethylation in site I can be seen clearly. (b) Ethylation of the phosphate 5′ to position *13t* of the consensus does not inhibit, and may slightly enhance, binding of intact resolvase. However, this same ethylation is seen to inhibit slightly the binding of the small domain to each half of site I and to site II-L (but not to either half of site III). We conclude that while the phosphate contacts of the intact protein stretch from positions *1-14* of a half-site, those of the C-terminal domain extend only to positions *12* or *13*. The contact between the intact resolvase and the phosphate 5′ to position *14t* either could involve the N-terminal domain directly, or could involve the C-terminal domain in an N-terminal-dependent manner. For example, interactions between the two domains of the intact protein could influence the conformation of the C-terminal domain, or dimerization (which is dependent upon the N-terminal domain) could distort the interaction between the C-terminal domain and a binding site. In either case, our results suggest that the C-terminal domain is oriented with its amino terminus towards the center of each resolvase binding site.

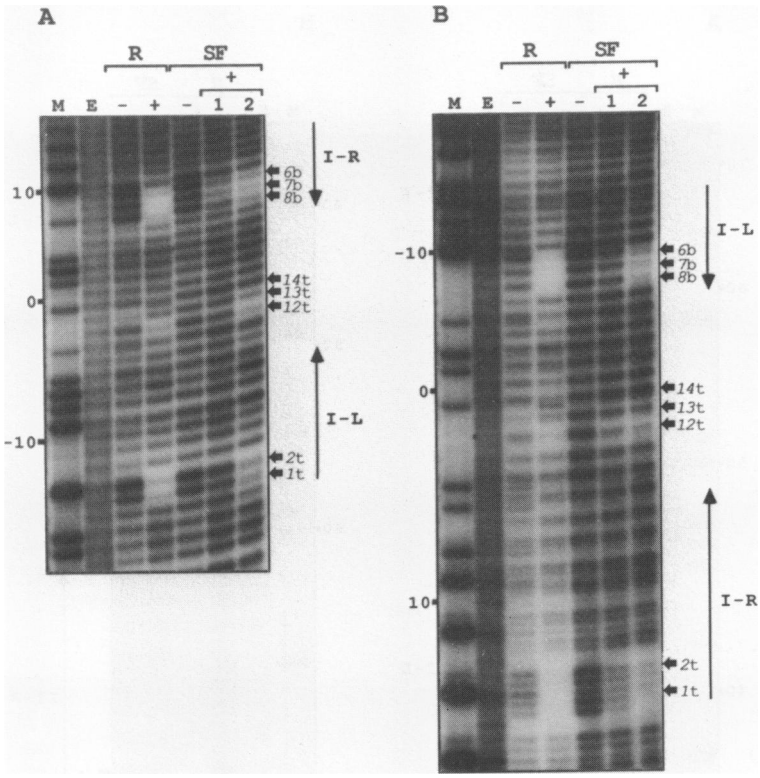


Figure 2. Inhibition of complex formation by ethylation of phosphates in site I. Analysis of the 'top' strand (**A**) and the 'bottom' strand (**B**) as shown in Fig. 5A. Autoradiograms show the positions of ethylated phosphates present in complexes (+) and unbound DNA (-) from binding reactions with resolvase (R) or its small C-terminal fragment (SF). M, marker lane of the G>A Maxam-Gilbert sequencing reaction (25). E, ethylated DNA. 1 and 2 indicate the complexes visible in Fig. 1. The arrows indicate the major positions of inhibitory phosphate ethylations, labeled with the position relative to the consensus half-site shown in Fig. 5B. Thus, *2t* corresponds to the phosphate 5′ to base *2* on the top strand (Fig. 5B), *7b* corresponds to the phosphate 5′ to base *7* on the bottom strand.

2. As suggested previously (2) the interaction of the intact resolvase with each binding site is very similar. However, there appear to be some significant differences. The most notable is that ethylation of the phosphate 5′ to position 57 (bottom strand) does not inhibit binding of resolvase to site II (see Fig. 3B, position *14t*). This is the only example of ethylation of the consensus position *14t* being non-inhibitory and it suggests that the interaction with the right half of site II is a little distorted, either as a

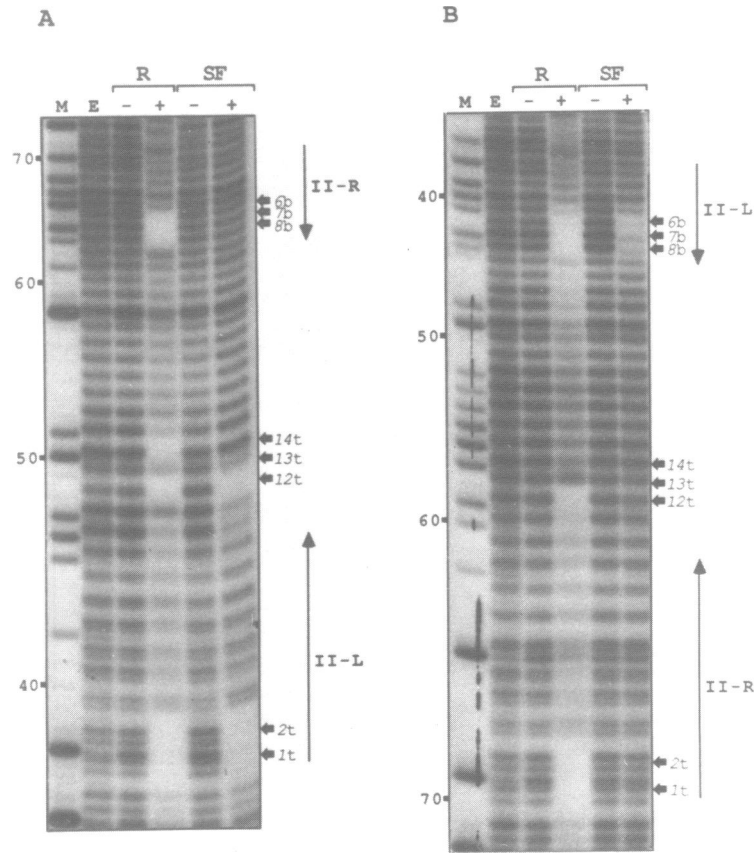


Figure 3. Inhibition of complex formation by ethylation of phosphates in site II. See legend to Fig. 2 for explanation of labels.

result of the large spacer that separates the two halves of site II or because the interaction between the C-terminal domain and site II-R is unusually weak.

3. We can infer the order of binding of the C-terminal domain to each DNA fragment from the positions of the inhibitory ethylations in complex 1 versus complex 2 (Fig. 2-4). With the site I fragment, site I-L is only filled in the second complex, indicating that the C-terminal domain has a higher affinity for I-R. Similarly the affinity for III-L is greater than for III-R. As noted above, only a single complex was observed with site II DNA; as can be seen from Fig. 3, only II-L is occupied in this complex. These relative affinities agree with those determined by DNase I footprinting (3). Although a complex with site II-R was not observed with the site II DNA

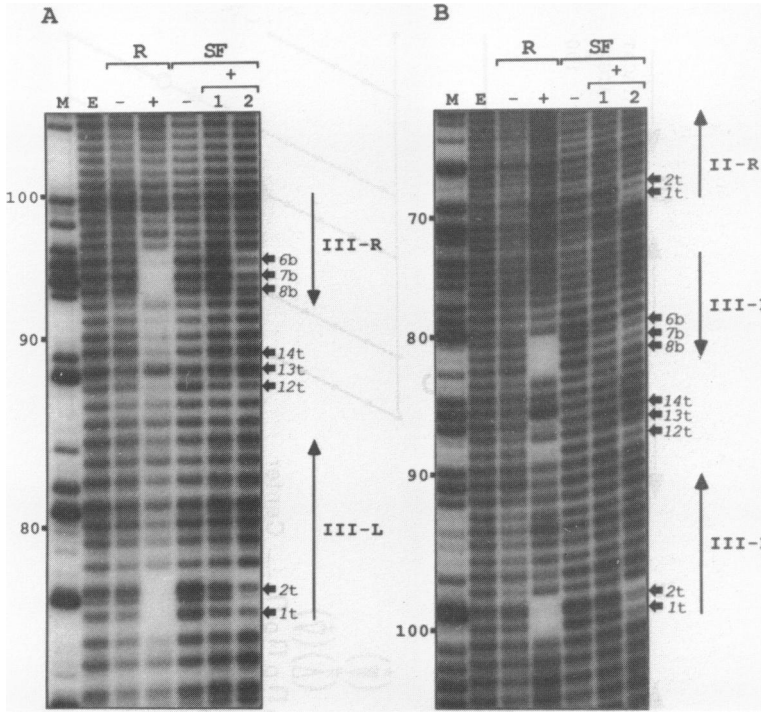


Figure 4. Inhibition of complex formation by ethylation of phosphates in site III. See legend to Fig. 2 for explanation of labels.

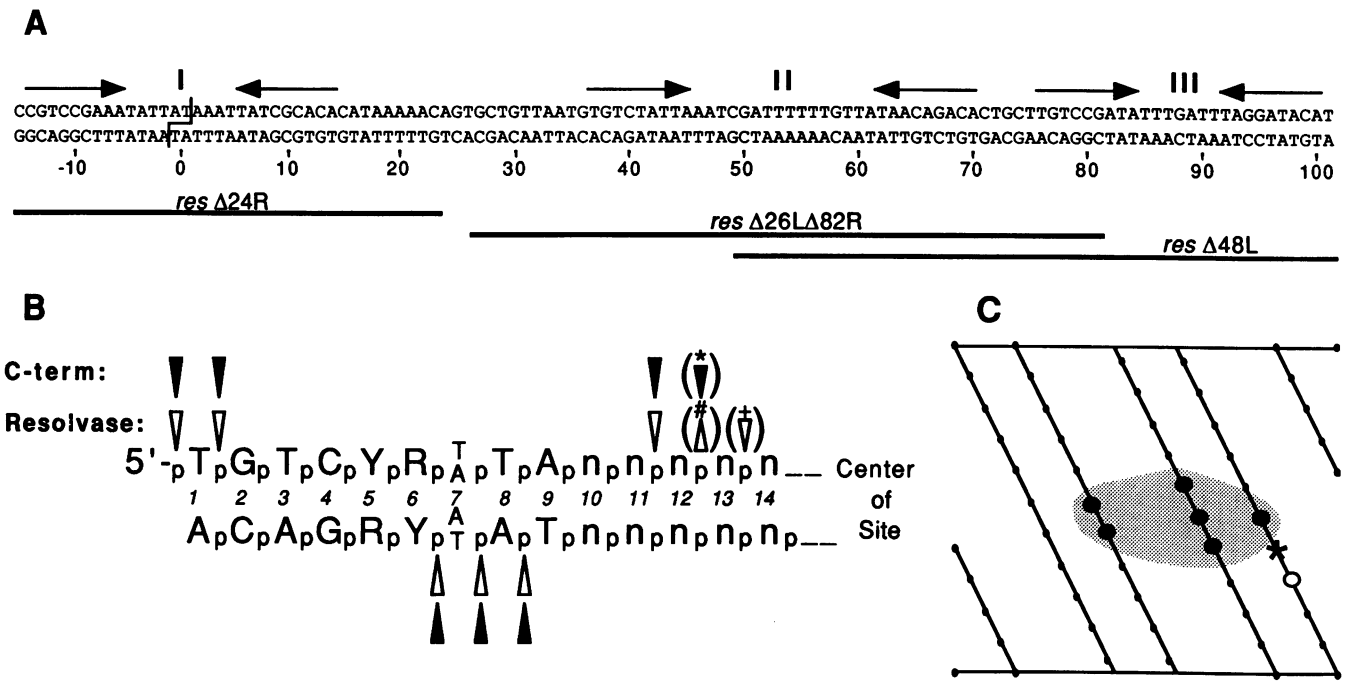
fragment, the data in Fig. 4 clearly show that II-R is bound in the third complex with the site III fragment. We note that we have seen a complex between the C-terminal domain and II-R only when site III is present. Since II-R and III-L are very close to one another, it is possible that there are cooperative interactions between the C-terminal domains at these adjacent sites which assist binding to II-R.

Extent of the DNA segment bound by the C-terminal domain

We have used two approaches to determine the minimum length of the DNA segment required for binding by the small C-terminal domain of resolvase.

a) Elongated primer selection

Gronostajski *et al.* (12) and Liu-Johnson *et al.* (8) have described a procedure which involves extension of a specific primer (5'-labeled) through a protein binding site in the presence of dideoxynucleoside triphosphates. This generates a set of DNA fragments in which the duplex segment is terminated at random positions. The comparison of the distribution of these fragments


A
I
II
III
CCGTCCGAAATATTATAAATTATCGCACACATAAAAACAGTGCTGTTAATGTGTCTATTAAATCGATTTTTTGTTATAACAGACACTGCTTGTCCGATATTTGATTTAGGATACAT
GGCAGGCTTTATAATATTTAATAGCGTGTGTATTTTTGTCACGACAATTACACAGATAATTTAGCTAAAAAACAATATTGTCTGTGACGAACAGGCTATAAACTAAATCCTATGTA
-10
0
10
20
30
40
50
60
70
80
90
100
res Δ24R
res Δ26LΔ82R
res Δ48L
B
C-term:
Resolvase:
5'-pTpGpTpCpYpRpTApTpApnpnpnpnpnpn__ Center
1 2 3 4 5 6 7 8 9 10 11 12 13 14
of
ApCpApGpRpYpTApApTpnpnpnpnpnpnp__ Site
C

between unbound and protein-complexed DNA locates the boundary of the binding site.

We have used two primer-template combinations to analyze the interaction of the resolvase DNA-binding domain, one to determine the bottom strand 3' boundary (Fig. 6A), the other to locate the top strand 3' boundary (Fig. 6B). Protein-DNA complexes were separated from unbound DNA as described earlier and the band corresponding to complex 1 (Fig. 1, I-1) was analysed on a sequencing gel (Fig. 6). Complex 1 contains the C-terminal domain bound predominantly to site I-R (Fig. 2); thus the bottom strand extension (Fig. 6, A and C) will define the inside boundary of site I-R and the top strand extension (Fig. 6, B and D) the outside boundary of site I-R. Fig. 6C shows that the bound complexes include only DNA extended through the 9 bp inverted repeat plus an additional 3 bases (weakly represented) and beyond (bands equally represented in bound and unbound). In Fig. 6D, extension through the inverted repeat plus one base (weakly represented) and beyond (equally represented) is sufficient for binding. (A small amount of complexes between the C-terminal domain and site I-L accounts for the weak bands within the site I-R sequence). We conclude that complex formation between the C-terminal domain and site I-R requires the 9 bp inverted repeat plus 1-2 bp on the outside and 3-4 bp on the inside of the repeat. Similar results have been obtained with site III (data not shown).

b) Selection of randomly cleaved (and gapped) DNA

A potential disadvantage of using primer extension to generate the nested set of DNA fragments is that only the two 3' boundaries of a binding site can be determined since extension occurs only in the 5'-3' direction. We have

Figure 5. **A.** The $\gamma\delta$ *res* sequences showing the three resolvase binding sites Numbering is from the center of the crossover point in site I. The segments carried on plasmids pRW33, pRW115 and PRW118 are shown by the bars below the sequence. **B.** Consensus sequence of a half-site showing the phosphate ethylations that inhibit binding of intact resolvase (▽) and the C-terminal domain (▼). Ethylation of the phosphate 5' to position *13t* results in weak inhibition of binding of the C-terminal domain (*) to site I (L and R) and site II-L (but not to site III), but causes somewhat enhanced binding of intact resolvase (#) to all three sites. (+) Ethylation of the phosphate 5' to *14t* in all half-sites except II-R inhibits binding of intact resolvase; binding of the C-terminal domain is not affected by this ethylation. **C.** Planar representation of a half-site showing the contact region of the C-terminal domain (stippled area). ●, positions at which phosphate ethylation inhibits binding of both the C-terminal domain and intact resolvase; ○, the phosphate ethylation that inhibits binding of only the intact resolvase; *, the phosphate ethylation that generally inhibits binding of the C-terminal domain but enhances binding of intact resolvase. The center of the binding site lies to the right.

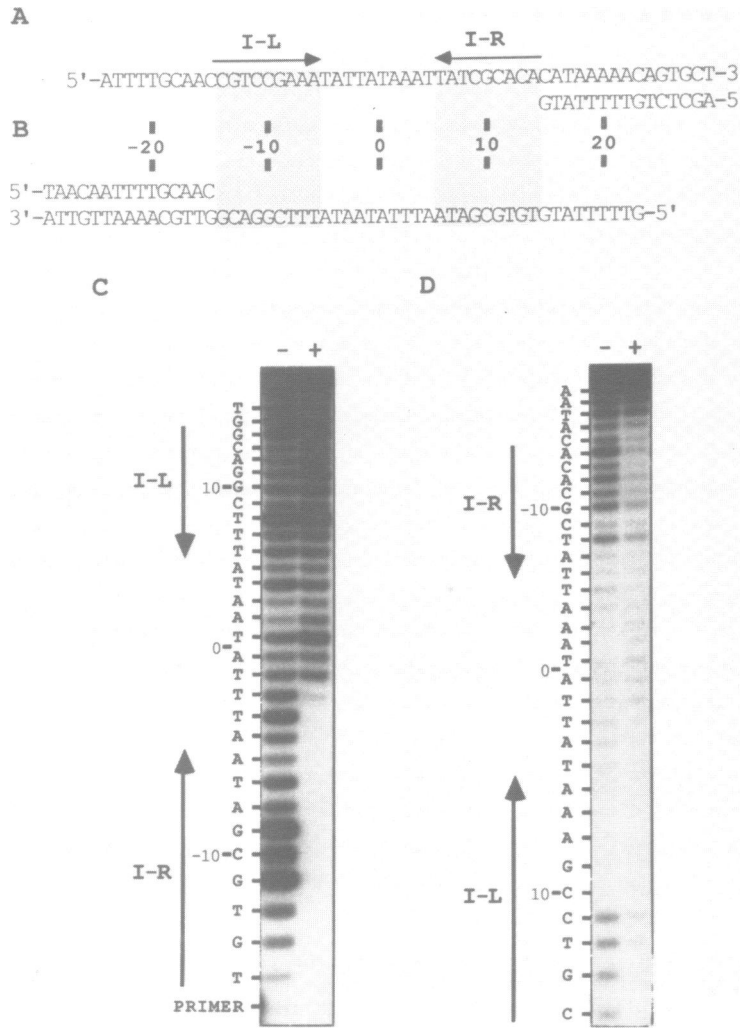


Figure 6. Mapping the DNA segment bound by the resolvase C-terminal domain using randomly elongated primers. **A** and **B**. Primer-template combinations which were elongated in the presence of dideoxynucleoside triphosphates. The primers were 5' end-labeled. **C** and **D**. Autoradiograms showing analysis of complexed (+) and unbound (-) DNA from the elongated primer-template mixtures shown in **A** and **B** respectively. The elongated mixtures were incubated with the C-terminal domain, complexes were separated from unbound DNA and were analysed on DNA sequencing (denaturing) gels. See text for further details.

generated similar sets of DNA fragments by cleaving randomly with hydroxyl radical [EDTA.Fe(II); ref. 9] and selectively removing the DNA to one side of the cut by denaturation (see below and Materials and Methods.). Not only does

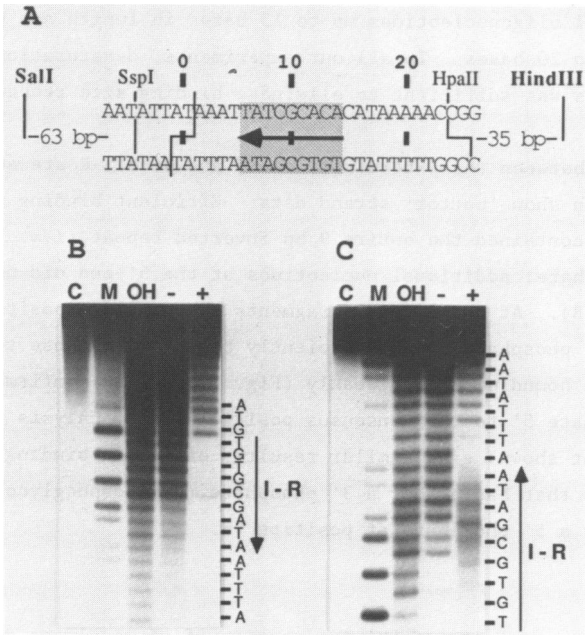


Figure 7. Mapping the DNA segment bound by the resolvase C-terminal domain using randomly gapped DNA. **A.** Sequence of Site I-R showing the conserved 9 bp segment (stippled region) and relevant restriction sites. The DNA fragments used were SalI-HpaII (labeled at the SalI site), or HindIII-SspI (labeled at the HindIII site). 3′ and 5′ labeled fragments were analysed separately. DNA fragments were cleaved with hydroxyl radical and small terminal gaps were generated (see text). **B.** Autoradiogram showing the 5′ boundary of the bottom strand. The DNA used was the 3′-labeled SalI-HpaII fragment. **C.** Determination of the 3′ boundary of the bottom strand, using the 5′-labeled HindIII-SspI fragment. + and -, hydroxyl radical-treated DNA that was bound by the C-terminal domain or remained unbound, respectively. C, untreated DNA. OH, hydroxyl-radical cleaved DNA. M, marker lane with G>A Maxam-Gilbert sequencing reaction. The sequence marked at the side is shifted upwards by half a band to emphasize that the bands terminate with the adjacent phosphate or phosphoglycolate (5′ in **B**, 3′ in **C**). [The origin of the smeary bands in **C**(+) within the inverted repeat (vertical arrows) is unknown; these bands do not comigrate with the products of hydroxyl radical cleavage and do not appear in repeat experiments.]

this procedure allow analysis of the 5′ boundaries, but it also complements the primer extension procedure since it leaves a phosphate (or phosphoglycolate) at the 3′ ends (13) rather than a dideoxynucleotide. Selective loss of the DNA 5′ or 3′ to a hydroxyl radical cleavage site was achieved by having the binding site very close to the unlabeled end of the DNA fragment. The manipulations of the fragment after cleavage (ethanol precipitation, preparative gel electrophoresis and fragment extraction) resulted in quantitative

loss of terminal oligonucleotides up to 15 bases in length and partial loss of those from 16 to 20 bases. In all our experiments, denaturation of a segment of 12 bp or less was sufficient to eliminate binding site recognition (see Fig. 7A).

Complexes between the C-terminal domain and site I-R are analyzed in Fig. 7 B and C, which show 'bottom' strand data. Efficient binding was found with fragments that contained the entire 9 bp inverted repeat (*i.e.*, position +14) with a 5′ phosphate; additional nucleotides at the 5′ end did not increase binding (Fig. 7B). At the 3′ end, fragments that include position +3 (terminated with a 3′ phosphate) were efficiently bound while those terminated at position +4 are bound much more weakly (Fig. 7C). This confirms the contact with the phosphate 5′ to the consensus position *13t*. Analysis of the top strand (data not shown) gave similar results: efficient binding was observed with a fragment that has either a 3′ phosphate (or phosphoglycolate) at position +14 or a 5′ phosphate at position +3.

CONCLUSIONS

Our previous analysis of the interaction of $\gamma\delta$ resolvase with its DNA binding sites defined a contact region at each half-site that covered nearly one third of the circumference of the DNA helix and included adjacent major and minor grooves (about 14 bp)(2). What portion of this region is contacted by the small C-terminal DNA binding domain? The experiments described in this paper define the minimum binding site for the small domain. It contains positions *1* to *12* of the consensus half-site shown in Fig. 6 and includes the contacted phosphates at each end (5′ to *1t* and 3′ to *12t*). Six of the seven phosphates identified as contacts with intact resolvase are also contacts with the small domain. Only the phosphate 3′ to *13t* is contacted exclusively by intact resolvase and this phosphate lies outside the minimal segment bound by the C-terminal domain. The contact region of the small DNA binding domain still covers an adjacent major and minor groove and is surprisingly large, particularly since the domain has only 43 amino acid residues. The domain is thought to contain a helix-turn-helix structural motif (3,4). In other proteins this structural motif has been shown to bind to DNA with the second α-helix in the major groove providing sequence-specific protein-DNA contacts (4, 14-17). Typically these contacts are limited to a segment of 4 to 6 bp because the path of the major groove follows a convex curve while the α-helix is straight (4). From the planar projection of the DNA helix shown in Fig. 5B, it appears that a subset of bp *1-7* should be recognized by the recognition

α-helix of the C-terminal domain of resolvase. Consistent with this, we have shown previously that methylation of guanines (at N7 in the major groove) at half-site positions *2* (top strand) and *4-7* (bottom strand) inhibits binding of intact resolvase to complete binding sites (2). However, the minimal binding site of the small domain extends a further 5-6 bp (towards the center of the binding site for intact resolvase), and an additional strong phosphate contact (5′ to *12t*) indicates that the domain stretches across the minor groove. The conservation of bp *8* and *9* suggests either that the DNA binding domain contacts specific base pairs (in the minor groove) or that contacts with the phosphate 5′ to *12t* depend on the precise helical pitch of the intervening DNA which is determined by its nucleotide sequence (18).

Presumably, a second portion of the C-terminal domain, distinct from the helix-turn-helix structural motif, makes contacts over this inner DNA segment. The most likely portion of the domain is its amino terminus. We have argued above, from the proximity of the phosphate (5′ to *14t*) contacted only by the intact resolvase, that the amino terminus of the small domain lies towards the center of a resolvase binding site. More direct evidence comes from an experiment of Sluka *et al.* (19) with the resolvase-related recombination protein, Hin. They have shown that, when EDTA is coupled to the amino terminal residue of the Hin DNA binding domain, Fe(II)-dependent DNA cleavage occurs near the center of the Hin binding site with a cleavage pattern indicative of a minor groove location of the EDTA.Fe(II).

Secondary portions of other DNA binding proteins of the helix-turn-helix class have also been implicated in specific interactions with their cognate binding sites. The extreme amino terminus of λ repressor wraps around the center of its operator making sequence specific contacts in the major groove (no additional phosphate contacts have been identified) (20, 21). A distinct portion of the cyclic AMP receptor protein appears to contact phosphates across the minor groove at the *outside* of the binding site, apparently inducing a substantial bend in DNA in the process (8,22,23). Finally, the AraC protein has a pattern of phosphate contacts (very similar to that of resolvase) which span the inside minor groove at each half-site and it has been proposed that a structure other than its helix-turn-helix motif is responsible (24). Whether these additional contacts are an intrinsic property of a DNA-binding domain of AraC is not known.

ACKNOWLEDGEMENTS

This research was supported by NIH grant GM28470 and by a postgraduate

studies scholarship from Chulalongkorn University, Thailand, to Vichien Rimphanitchayakit.

+Present address: Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260, USA

REFERENCES

1. Grindley, N.D.F. and Reed, R.R. (1985) Ann. Rev. Biochem. **54**, 863-896.
2. Falvey, E. and Grindley, N.D.F. (1987) EMBO J. **6**, 815-821.
3. Abdel-Meguid, S.S., Grindley, N.D.F., Templeton, N.S. and Steitz, T.A. (1984) Proc. Natl. Acad. Sci. USA **81**, 2001-2005.
4. Pabo, C. and Sauer, R. (1984) Ann. Rev. Biochem. **53**, 293-321.
5. Hatfull, G.F., Salvo, J.J., Falvey, E.F., Rimphanitchayakit, V. and Grindley, N.D.F. (1988) In "Transposition" (Kingsman A., Kingsman, S. and Chater, K., eds.) Soc. Gen. Microbiol. Symp. **43**, Cambridge Univ. Press, pp. 149-181.
6. Wells, R.G. and Grindley, N.D.F. (1984) J. Mol. Biol. **179**, 667-687.
7. Siebenlist, U. and Gilbert, W. (1980) Proc. Natl. Acad. Sci. USA **77**, 122-126.
8. Liu-Johnson, H., Gartenberg, M.R. and Crothers, D.M. (1986) Cell **47**, 995-1005.
9. Tullius, T.D. and Dombroski, B.A. (1986) Proc. Natl. Acad. Sci. USA **83**, 5469-5473.
10. Fried, M. and Crothers, D. (1981) Nucleic Acids Res. **9**, 6505-6525.
11. Hatfull, G.F. and Grindley, N.D.F. (1986) Proc. Natl. Acad. Sci. USA **83**, 5429-5433.
12. Gronostajski, R.M., Adhya, S., Nagata, K., Guggenheimer, R.A. and Hurwitz, J. (1985) Molec. Cell. Biol. **5**, 964-971.
13. Hertzberg, R.P. and Dervan, P.B. (1984) Biochemistry **23**, 3934-3945.
14. Hecht, M.H., Nelson, H.C.M. and Sauer, R.T. (1983) Proc. Natl. Acad. Sci. USA **80**, 2676-2680.
15. Wharton, R.P., Brown, E.L. and Ptashne, M. (1984) Cell **38**, 361-369.
16. Anderson, J.E. Ptashne, M.and Harrision, S.C. (1985) Nature **316**, 596-601.
17. Wharton, R.P. and Ptashne, M. (1985) Nature **316**, 601-605.
18. Otwinowski, Z., Schevitz, R.W., Zhang, R.-G., Lawson, C.L., Joanchimiak, A., Marmorstein, R.Q., Luisi, B.F. and Sigler, P.B. (1988) Nature **335**, 321-329.
19. Sluka, J.P., Horvath, S.J., Bruist, M.F., Simon, M.I. and Dervan, P.B. (1987) Science **238**, 1129-1132.
20. Pabo, C.O., Krovatin, W., Jeffrey, A. and Sauer, R.T. (1982) Nature, **298**, 441-443.
21. Eliason, J.L., Weiss, M.A. and Ptashne, M. (1985) Proc. Natl. Acad. Sci. USA **82**, 2339-2343.
22. Majors, J.E. (1977) Ph.D. Thesis, Harvard University, Cambridge, MA.
23. Warwicker, J., Engelman, B.P. and Steitz, T.A. (1987) Proteins **2**, 283-289.
24. Hendrickson, W. and Schleif, R. (1985) Proc. Natl. Acad. Sci. USA **82**, 3129-3133.
25. Maxam, A. and Gilbert, W. (1980) Meth. Enzymol. **65**, 499-560.