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Covalent binding of the natural antimicrobial peptide indolicidin to DNA abasic sites

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ABSTRACT

Indolicidin is a host defense tridecapeptide that inhibits the catalytic activity of HIV-1 integrase *in vitro*. Here we have elucidated its mechanism of integrase inhibition. Using crosslinking and mass spectrometric footprinting approaches, we found that indolicidin interferes with formation of the catalytic integrase-DNA complex by directly binding DNA. Further characterization revealed that the peptide forms covalent links with abasic sites. Indolicidin crosslinks single- or double-stranded DNAs and various positions of the viral cDNA with comparable efficiency. Using truncated and chemically modified peptides, we show that abasic site crosslinking is independent of the PWWP motif but involves the indolicidin unique lysine residue and the N- and C- terminal NH_2 groups. Because indolicidin can also inhibit topoisomerase I, we believe that multiple actions at the level of DNA might be a common property of antimicrobial peptides.

INTRODUCTION

Several oligopeptides have been reported to inhibit HIV-1 integrase (IN) [for review, see (1)]. We recently discovered that indolicidin (Figure 1), an antimicrobial cationic tridecapeptide, inhibits IN *in vitro* and that multimerization of this peptide greatly enhances its potency (2). Indolicidin has been isolated from the cytoplasmic granules of bovine neutrophils (3) and belongs to the cathelicidin family of the host defense peptides (HDPs) (4). HDPs play a key role in the innate host defense system and are thought to constitute the first line of defense against invading microorganisms. They represent an abundant and diverse group of molecules produced by a broad range of cells and tissues from invertebrate, plant and animal species [for review see (5)]. Cathelicidin HDPs have been found in many mammals including humans but also in primitive vertebrates such as hagfishes (6). Cathelicidin HDPs are mainly found in the cytoplasmic granules of circulating neutrophils. They are also expressed in non-myeloid cells in skin and mucosal surfaces. Human cathelicidin peptide (LL-37) deficiency in neutrophils has been correlated with Kostmann syndrome, a severe congenital neutropenia with chronic periodontal disease (6).

Cathelicidin HDPs exert their antimicrobial action through interactions with cell membranes and pore formation but other killing mechanisms based on interactions with internal microbial targets have also been reported. For instance, some cathelicidin HDPs interfere with DNA/RNA/protein synthesis [for review see (5,6)]. They can neutralize microbial endotoxins by direct binding, promote wound healing and modulate the immune response. Therefore, cathelicidin HDPs have been the focus of an increased interest as potential novel therapeutic agents (7).

Cathelicidin peptides share common features with other HDPs including a net positive charge and an overall amphipathic topology. They can be categorized according to their secondary structure and amino acid enrichment. They are either α-helical, β-hairpin or linear with enrichment in proline or tryptophan residues (6). Indolicidin is a linear natural 13mer cationic cathelicidin HDP containing five tryptophan (Trp) and two proline (Pro) residues, two of which are within Trp-Pro-Trp tandem repeats (PWWP motif). Indolicidin has a broad spectrum of activity; it is antibacterial (3), antifungal (8), antiparasitic (9,10), antiviral (11,12) and an inhibitor of aminoglycoside antibiotic resistance enzymes (13). The solution structure of indolicidin reveals multiple conformations of the peptide in aqueous solution and in membrane-mimicking environments suggesting that structural plasticity accounts for

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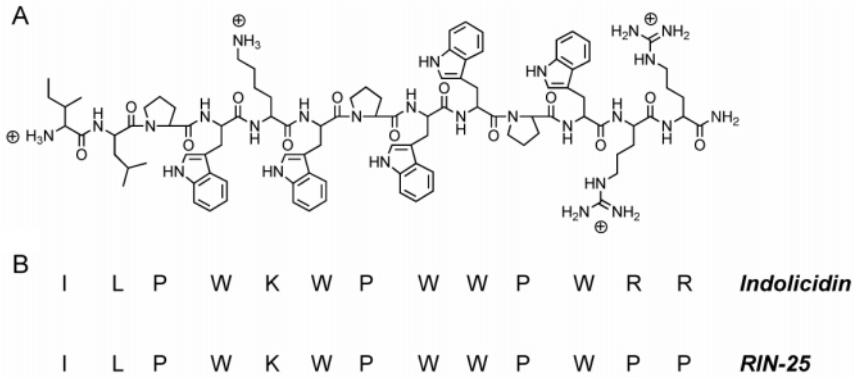

Figure 1. Structure and sequence of indolicidin and its derivative RIN-25. (**A**) Chemical structure of indolicidin. (**B**) Sequences of indolicidin and RIN-25. Amino acids are indicated as single letter code.

its multiple effects (14). Its mechanism of action has been related to cell membrane permeabilization (15–17), but indolicidin also inhibit DNA synthesis leading to *Escherichia coli* filamentation (18). Thus it is plausible that indolicidin exerts its antimicrobial activity by targeting nucleic acids.

In the present study, we demonstrate that indolicidin and its arginine (Arg)-free analog RIN-25 (2) (Figure 1) directly bind DNA and interfere with formation of the IN–DNA complex interfere with the formation of IN-DNA complexes. Both peptides also bind abasic site-containing DNA (abDNA). The efficiencies of crosslinking are comparable for double- and single-strand abDNA at different DNA positions. Using truncated and chemically modified peptides, we dissected the primary structural features of the peptide that are essential for efficient crosslinking to abDNA. Our results provide new insight into the mechanism of action of the natural antimicrobial indolicidin.

MATERIALS AND METHODS

DNA oligonucleotides, HIV-1 integrase and peptides

Oligonucleotides were purchased from IDT Inc. (Coralville, IA) and purified on a 20% (19:1) denaturing polyacrylamide gel using UV shadow. Purified oligonucleotides were 5′ end labeled by T4-polynucleotide kinase (New England Biolabs, Inc. Beverly, MA) as described previously (19). The wild-type recombinant IN was expressed and purified as described previously (20). The synthesis of indolicidin analogs has been described in detail separately (2).

Schiff base assay

The Schiff base assay was performed as described previously (19,21). Briefly, uracil-containing oligonucleotides corresponding to the 21 last bases of the HIV-1 U5 long terminal repeats (LTR) were 5′ end labeled and annealed to their complementary strand. The resulting duplexes were then treated by 1 U of uracil DNA glycosylase (UDG) (Gibco BRL/Life Technologies) for 1 h at 37°C in order to generate an abasic site.

Peptides were incubated with 500 nM IN for 15 min at room temperature in a total volume of 9 µl with a buffer containing 25 mM MOPS, pH 7.2, 5 mM NaCl, 7.5 mM $MgCl_2$, and 14.3 mM 2-mercaptoethanol. Then 1 µl of 5′ end labeled abDNA (20 nM final) was added and the reaction was incubated for an extra 30 min at room temperature.

When IN was not present in the reaction, peptides were incubated with 20 nM of 5′ end labeled abDNA in the same volume and buffer for 15 min at room temperature.

IN-DNA crosslinks were reduced by 1 µl of 1 M sodium borohydride ($NaBH_4$, 0.1 M final). After 5 min at room temperature, samples were treated with one volume of 2× SDS–tricine gel-loading buffer, heated 5 min at 95°C and loaded onto 12–20% tricine–SDS polyacrylamide gels (Invitrogen Corp., Carlsbad, CA). Gels were exposed overnight and analyzed using a Molecular Dynamics Phosphorimager (GE Healthcare, Waukesha, WI). Densitometric analyses were performed using ImageQuant from the Molecular Dynamics software package. Each lane was quantified for crosslinking product formation, which was expressed as a fraction of the total DNA per lane and plotted using Prism 4.0 (Graphpad Software Inc., San Diego, CA).

Mass spectometry

Peptides in their free form and complexed with either DNA or IN were subjected to modification with *N*-hydroxysuccinimidobiotin (NHS-Biotin) (Pierce, Rockford, IL). NHS-Biotin reacts specifically with primary amines on peptides resulting in covalent addition of a biotin molecule (226 Da). After incubation at 37°C for 30 min, the reactions were quenched with 10 mM lysine in its free amino acid form. The peptides were purified by ZipTip according to the manufacturer's instructions (Millipore Corporation, Bedford, MA) and subjected to mass spectrometric (MS) analysis. MS spectra were recorded with a Shimadzu Axima-CFR MALDI-TOF instrument (Shimadzu Scientific Instruments, Columbia, MD) using α-cyano-4-hydroxycinnamic acid as matrix.

Topoisomerase I (Top1) assay

For the Top1-mediated DNA relaxation assay, reaction mixtures (10 µl final volume) contained 0.3 µg supercoiled ϕX174 DNA in reaction buffer [10 mM Tris–HCl, pH 7.5, 50 mM KCl, 5 mM $MgCl_2$, 0.1 mM EDTA, and 15 µg/ml bovine serum albumin (BSA)] and 2 U of Top1 (22). Reactions were performed at 37°C for 30 min with Top1 in the absence or presence of varying concentrations of indolicidin. The reactions were terminated by the addition of 0.5% SDS

and 0.5 mg/ml proteinase K. Samples were incubated for 30 min at 50°C. Next, 1.2 µl of 10× gel-loading buffer (20% Ficol 400; 0.1 M Na_2EDTA, pH 8.0, 1.0% SDS, and 0.25% bromphenol blue) was added and reactions mixtures were loaded onto 1% agarose gels made in 1× TBE buffer. Gels were run in 1× TBE. After electrophoresis, DNA bands were stained in 10 µg/ml of ethidium bromide and visualized by transillumination with ultraviolet light (300 nm).

In the ϕX174 DNA unwinding assay, reactions were performed in a 10 µl final volume at 37°C in reaction buffer (see above) with 0.3 µg supercoiled ϕX174 DNA and 2 U of Top1 per reaction. First, the reactions were performed with Top1 alone for 30 min followed by incubation in the presence or absence of varying concentrations of indolicidin for another 30 min. The reactions were terminated by addition of 0.5% SDS and 0.5 mg/ml proteinase K and processed as described in the relaxation assay. After electrophoresis, DNA bands were stained in 10 µg/ml of ethidium bromide and visualized by transillumination with ultraviolet light (300 nm).

RESULTS

Indolicidin and RIN-25 inhibit HIV-1 integrase-DNA binding

To investigate the influence of indolicidin and its Arg-free analog RIN-25 (Figure 1) (2) on IN-DNA binding, we used the Schiff base crosslinking method (19,21) that can trap IN chemically onto its DNA substrate (Figure 2A). In this assay, an abasic site is first generated in an uracil-containing DNA molecule using uracil DNA glycosylase. The abasic site then reacts with an ε-amino group of a Lys residue present in its vicinity to generate an imine. This imine is subsequently stabilized by reduction with sodium borohydride ($NaBH_4$) into a covalent complex.

The data depicted in Figure 2 indicate that both indolicidin and RIN-25 inhibit IN-DNA crosslinking. Titration of increasing concentrations of the peptides in the reaction mixture correlates with a decrease of the gel band intensity corresponding to the IN–DNA complex and with a concomitant increase of the band intensity corresponding to a peptide–DNA complex (Figure 2B and C). Unlike indolicidin, RIN-25 fully suppressed IN-DNA crosslinking at the peptide concentration range of 37–333 µM (Figure 2C). These IN-DNA binding results correlate well with the inhibition profiles of IN catalytic activity by these two peptides (2).

These results demonstrate that both indolicidin and its analog RIN-25 inhibit IN catalytic activity by competing with the enzyme for DNA binding.

Indolicidin crosslinks with a similar efficiency single- and double-stranded abDNA at different DNA positions

We next investigated the crosslinking of indolicidin and its derivative RIN-25 to abDNA in the absence of IN. In addition

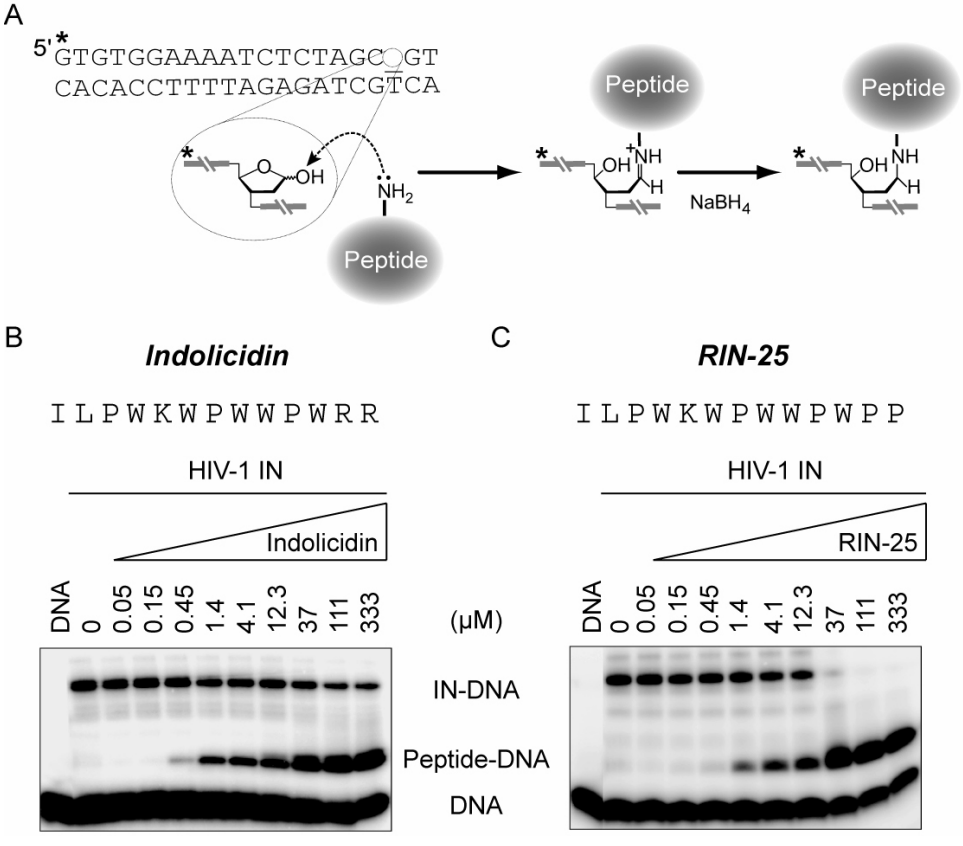

Figure 2. Inhibition of integrase-DNA binding by indolicidin and RIN-25. (**A**) Schematic representation of the Schiff base assay mechanism (19,21). (**B** and **C**) Gel images of representative experiments performed with indolicidin and RIN-25.

to the duplex abDNA (Figure 2), a single-stranded oligonucleotide containing an abasic site at the same nucleotide position was tested. Figure 3 shows that both indolicidin and RIN-25 crosslink efficiently single- and double-stranded abDNA (Figure 3A, left panel and data not shown for RIN-25). Crosslinking can only be observed after reduction of the complex by $NaBH_4$ treatment (Figure 3A, right panel and Figure 2A). Crosslinking products appear at submicromolar concentrations of peptide (Figure 3A) and as fast as in 30 s (Figure 3B). The rate of crosslink formation is similar for single- and double-stranded abDNA (Figure 3B).

We next wanted to investigate the influence of the abasic site position on the efficiency of crosslinking by indolicidin. A single abasic site was introduced in the duplex oligonucleotide at positions ranging from −1 to −12 from the IN cleavage site (Figure 3C). Indolicidin and RIN-25 (data not shown) crosslink each oligonucleotide with similar efficiency suggesting that abDNA crosslinking by indolicidin and RIN-25 is independent of the abasic site position on the DNA sequence. We also found that indolicidin crosslinks abDNA independently of the presence of divalent metal (data not shown).

These results demonstrate that indolicidin and its derivative RIN-25 bind with similar efficiency to single- and double-stranded abDNAs.

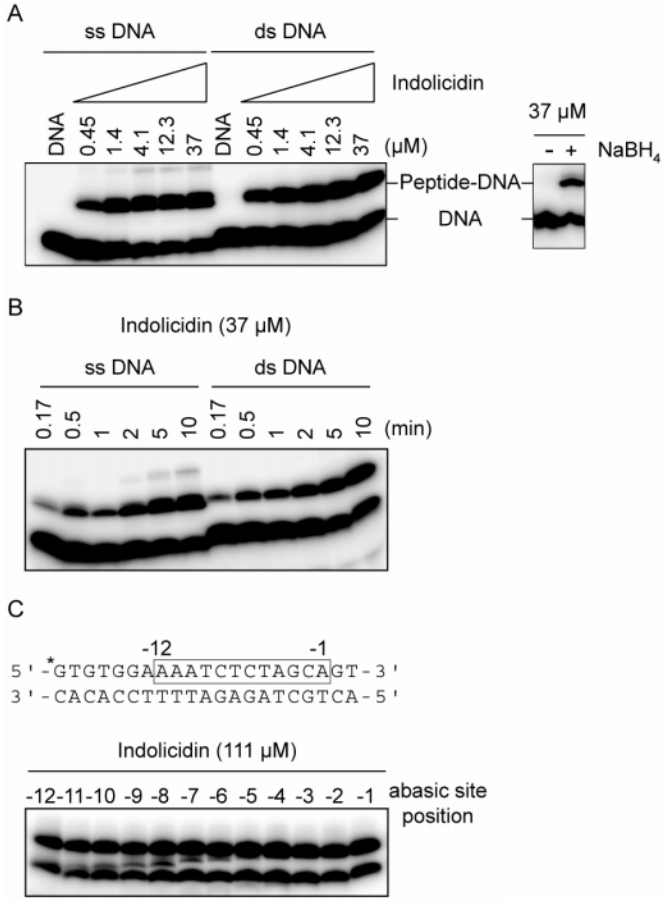

Figure 3. Concentration response and kinetics of crosslinking of indolicidin to an abDNA. (**A**) left panel: Representative gel of a typical dose-response experiment; right panel: Gel of a control experiment showing that reduction by sodium borohydride is required for crosslinking formation. (**B**) Representative gel of a typical kinetics experiment in the presence of indolicidin at 37 μM. (**C**) upper panel: DNA sequence of the oligonucleotides used. The box indicates the DNA position range where a single abasic site has been introduced. Asterisk indicates labeling with ^{32}P at the 5′ end of the DNA; lower panel: representative gel of a crosslinking experiment on DNA oligonucleotides containing a single abasic site at the indicated 12 different positions. An intermediate minor band corresponding to the crosslink of indolicidin to the oligonucleotide cleaved at the abasic site after β-elimination appears on the gel from positions −6 to −11.

The N-terminus region of indolicidin is required for DNA binding

We next wanted to determine the structural requirements of indolicidin for DNA binding. We studied the efficiency of several indolicidin derivatives for abDNA crosslinking (Figure 4A). Replacing the two C-terminal protonated Arg residues of indolicidin by two Pro residues in RIN-25 (Figure 4A) slightly reduced crosslink formation (Figure 4B). However, when the Pro-Trp-Trp-Pro (PWWP) motif of indolicidin is removed by C-terminal truncation, RIN-69 (Figure 4A) remains as potent as the natural peptide (Figure 4B) suggesting that the PWWP motif and the two Arg residues present at the C-terminus of indolicidin are not necessary for abDNA crosslinking. In contrast, when the peptide is truncated from the N-terminus to leave part of the PWWP motif and two Pro residues at the C-terminus, the resulting RIN-66 peptide fails to crosslink abDNA (Figure 4A). This demonstrates that the N-terminal portion of indolicidin is required for DNA binding.

Finally, we wanted to evaluate the importance of the single lysine residue of indolicidin. The peptide RIN-85 is derived from RIN-25 by a single Lys to Gly mutation (Figure 4A).

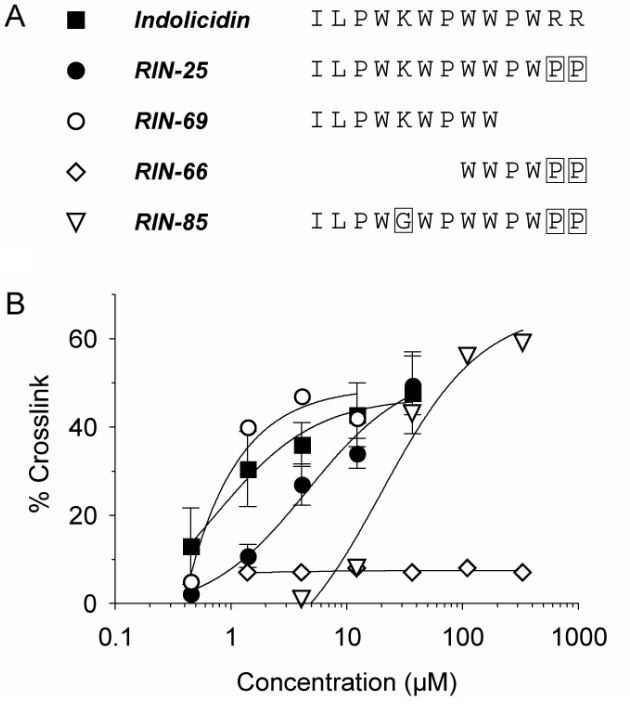

Figure 4. The amino acid portion of indolicidin and RIN-25 containing the lysine residue is critical for abasic site crosslinking. (**A**) sequences of the peptides used. (**B**) Crosslinking formation curves of dose-response experiments. Error bars indicate SD for at least three independent experiements.

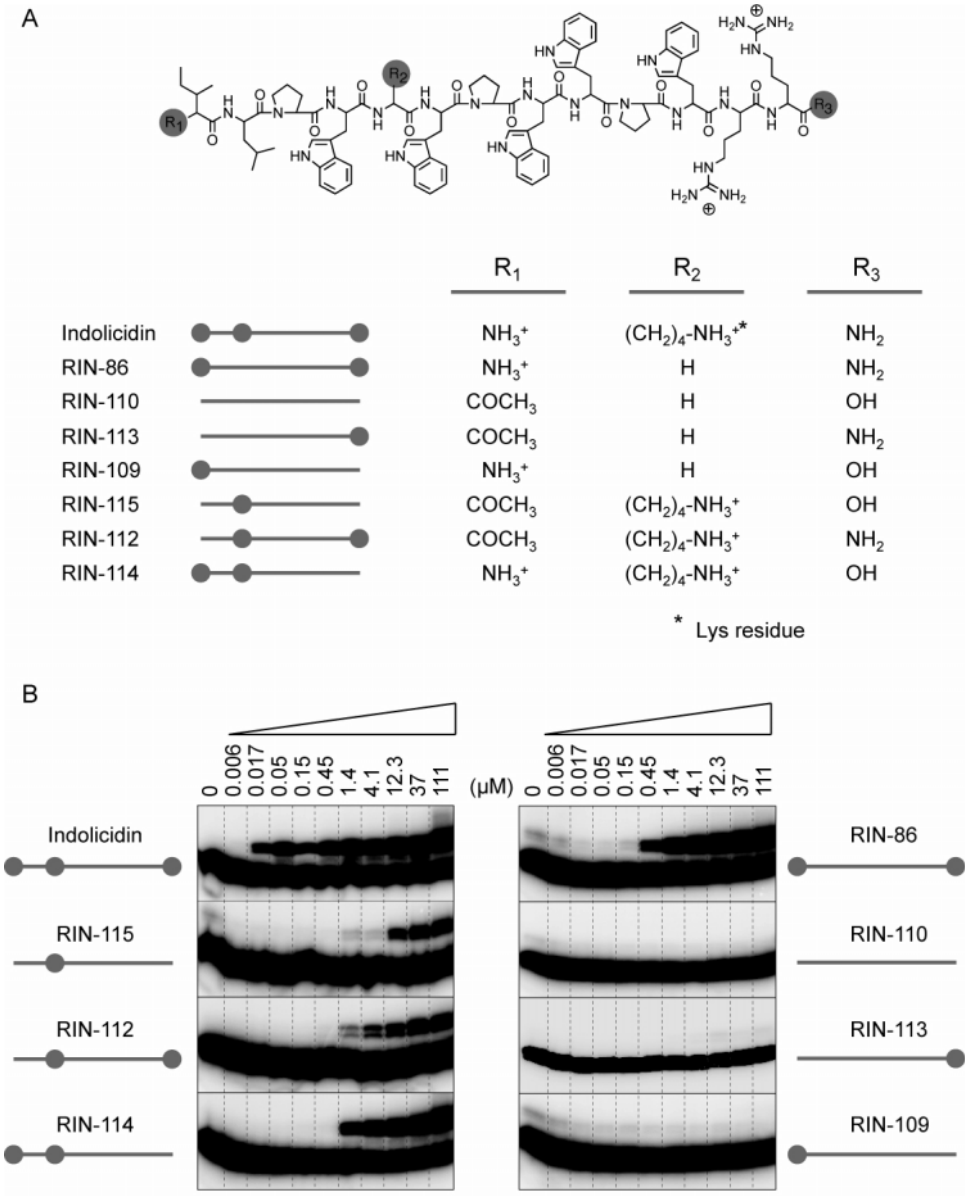

Figure 5. The lysine ε-amino group and the terminal NH_2 groups all contribute to the crosslinking of indolicidin to the DNA abasic site. (**A**) schematic representation of the reactive NH_2 groups of indolicidin. (**B**) representative gels of dose-response experiments are shown with corresponding peptides. The presence of NH_2 groups are schematized by grey circles.

RIN-85 is still able to crosslink abDNA, albeit with ~20-fold reduction in efficiency as compared to RIN-25 (Figure 4B). These results suggest that the Lys residue significantly facilitates but is not absolutely required for crosslinking.

Our data demonstrate that the N-terminal sequence of indolicidin is required for DNA binding and that the presence of the single Lys residue promotes DNA binding.

The indolicidin lysine residue and both terminal NH_2 groups all contribute to DNA binding

We next investigated the influence of indolicidin NH_2 groups on the efficiency of abDNA crosslinking. Dose-response crosslinking experiments were performed with several indolicidin analogs (Figure 5A). Indolicidin crosslinks abDNA at concentrations as low as 17 nM (Figure 5B, indolicidin). Removal of the ε-amino group of the single Lys by mutation to a Gly residue reduced crosslinking efficiency by ~20-fold (Figure 5B, RIN-86, see Figure 4). This suggests that the ε-amino group of single Lys is important but not absolutely required for abDNA crosslinking.

RIN-110, a Gly mutant in which the terminal NH_2 groups were removed by acetylating the N-terminus and changing the C-terminal amide to a carboxylic group, produced no DNA-crosslink (Figure 5B, RIN-110). Hence, the NH_2 groups present at the sequence termini play a role for DNA binding. Crosslink formation cannot be rescued by re-introduction of either the C-terminal or the N-terminal NH_2 groups alone (Figure 5B, RIN-113 and 109, respectively) suggesting a cumulative effect of both termini. The only

NH_2 groups that can restore crosslink is the ε-amino group of single Lys (Figure 5B, RIN-115) but the efficiency of crosslinking is reduced by almost 1000-fold as compared with indolicidin (Figure 5, compare RIN-115 and indolicidin). These results confirm the synergistic effect of the terminal NH_2 groups on the global crosslinking efficiency. Restoring the C-terminal amide group or the N-terminal amine increases crosslinking efficiency by ~10-fold (Figure 5B, RIN-112 and 114, respectively) but the crosslinking level remains ~100-fold lower than the one obtained with indolicidin (Figure 5, compare RIN-112 and 114 to indolicidin). The guanidino groups of the two Arg residues do not seem to influence crosslinking efficiency since similar results were obtained with a series of RIN-25 derivatives that contains two Pro residues in place of the two Arg residues (data not shown).

Together, these results suggest that the NH_2 groups present on the Lys residue and on both termini play a synergistic role in the crosslinking of indolicidin to abDNA.

Indolicidin interacts with DNA in the absence of an abasic site

To demonstrate that indolicidin also interacts non-covalently with native DNA, the MS footprinting approach was employed (23–25). This method uses the primary amine-modifying reagent NHS-biotin to compare surface topologies of free peptide versus the peptide-ligand complex.

To obtain plausible footprinting results, it was important to perform NHS-biotin treatments under mild conditions where the integrity of the peptide–ligand complexes would be preserved. Our previous experience indicated that the optimal concentration range for NHS-biotin is from 50 to 400 μM (23–25). Incubation of free indolicidin (p1, Figure 6A) with 50 μM reagent results in peak p2 corresponding to the addition of one biotin molecule to the free peptide (Figure 6B). MS/MS analysis of p2 indicates that the single Lys residue is the major modified site (data not shown). An additional modification is detected at the N-terminus when the NHS-biotin concentration is significantly increased in the reaction mixture. These observations are consistent with our previous findings that Lys side chains exhibit significantly higher chemical reactivity compared with N-terminal NH_2 groups (26).

We next analyzed indolicidin interactions with native DNA (21mer duplex IN substrate). Figure 6C indicates a strong protection of the modified p2 peak due to direct DNA binding of the peptide. For a control, we used a peptide of random sequence (HDMNKVLDL) that like indolicidin has a single Lys residue but does not inhibit IN activity. Extents of Lys modification in free peptides and peptide-DNA mixtures (Figure 6E and F) are comparable suggesting that the control peptide does not form a complex with DNA.

Finally, we used the same technique to address the question whether indolicidin can directly interact with IN. In the control experiments, indolicidin is incubated with BSA. Since both IN and BSA contain surface exposed Lys residues which provide alternative reactive sites, NHS-biotin concentration was increased to 200 μM to achieve detectable modification of indolicidin in the presence of the proteins. The results in Figure 6G and H indicate that the extents of modification of indolicidin in the presence of IN and BSA are very similar. Taken together, the MS footprinting results demonstrate that indolicidin binds non covalently to native DNA but not to IN, suggesting that the indolicidin inhibits IN catalytic activity by interfering with the formation of IN–DNA complex.

Indolicidin inhibits topoisomerase I-mediated DNA relaxation

To further examine the binding of indolicidin to native DNA, we tested whether indolicidin could interfere with human DNA topoisomerase I activity (Top1). Top1 is ubiquitous and essential as it relaxes DNA supercoiling during replication and transcription [for a review see (27,28)]. Relaxation of ϕX174 DNA by Top1 is reduced by indolicidin when compared with the Top1-mediated ϕX174 DNA reaction in the absence of drug (Figure 7A). This inhibition of Top1 is indolicidin concentration-dependent. Therefore, indolicidin inhibits Top1 catalytic activity.

An unwinding assay using supercoiled DNA in the presence of Top1 is a simple procedure to detect DNA intercalation (22). Figure 7 (Panel B) shows that indolicidin has no detectable effect on Top1-mediated unwinding of ϕX174 DNA. These results suggest that indolicidin is not a DNA intercalator up to a concentration of 37 μM.

DISCUSSION

We previously reported that the natural cationic tridecapeptide indolicidin inhibits HIV-1 IN catalytic activity *in vitro* (2). The present study elucidates the mechanism of action of indolicidin. Our crosslinking and MS footprinting experiments indicate that indolicidin interferes with the formation of the IN–DNA complex by directly binding to DNA and not to IN. Indolicidin also interferes with Top1-mediated DNA relaxation without unwinding DNA, suggesting that indolicidin may inhibit a large variety of DNA processing enzymes through DNA binding. It is generally admitted that cathelicidin HDPs (including indolicidin) exert their broad range of biological activities through interactions with cell membranes (15–17); but it is becoming increasingly clear that they can also interfere with other cellular mechanisms. It is possible that formation of plasma membrane 'pores' would contribute to cellular entry. Our study and others [for a review, see (5)] strongly suggest that cathelicidin HDPs can inhibit biological processes by targeting nucleic acids.

In addition to inhibiting DNA processing enzymes, indolicidin binds directly to abDNA. Our mass spectroscopy (Figure 6) and Top1 inhibition results (Figure 7) are consistent with a recently published study showing that indolicidin binds to native double-stranded DNA (14). However, our dose-response and kinetics experiments demonstrate that indolicidin crosslinks both double- and single-stranded abDNA with similar efficiency at various DNA positions on the HIV-1 U5 LTR DNA and without requirement for divalent metal. The preferential binding of indolicidin to normal double-stranded DNA was observed by surface plasmon resonance (SPR) (14), which measures affinity after equilibrium is reached. In our crosslinking experiments, the system does not reach equilibrium since the generation of covalent complexes is extremely fast. Thus, the strong affinity of

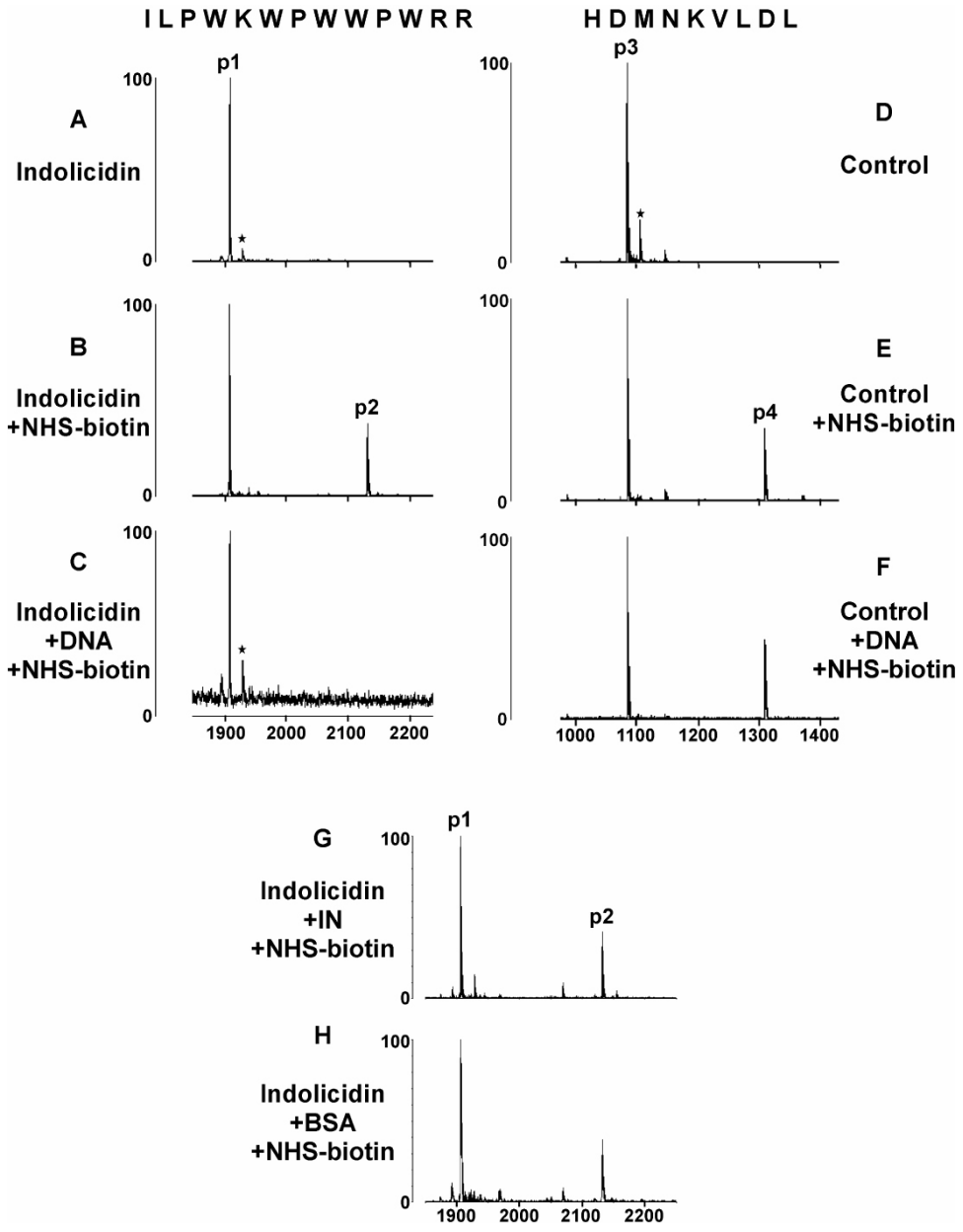

Figure 6. MALDI-TOF data depicting mass spectrometric footprinting results on indolicidin interactions with DNA and IN. In parallel experiments, interactions of indolicidin (**A–C**) and interactions of a control peptide (**D–F**) with DNA were compared. (**A** and **D**) unmodified peptides. (**B** and **E**) 20 μM peptides were modified with 50 μM NHS-biotin. (**C** and **F**) peptides were first incubated with 40 μM DNA and then exposed to the NHS-biotin treatment. (**G** and **H**) indolicidin interactions with IN and BSA were compared. (**G**) 20 μM indolicidin was pre-incubated with 50 μM IN and then exposed to 200 μM NHS-biotin. (**H**) 20 μM indolicidin was pre-incubated with 50 μM BSA and then exposed to 200 μM NHS-biotin. Peaks p1 and p2 correspond to native and biotinylated indolicidin, respectively. Peaks p3 and p4 correspond to native and biotinylated control peptide, respectively. Sodium adducts of indolicidin and the control peptide are indicated by 'star' signs.

indolicidin for abasic sites may mask the differential affinity for single versus double-stranded DNA.

Indolicidin contains a PWWP amino acid motif, which creates a center of symmetry in the peptide. PWWP motifs are found in nuclear proteins such as hepatoma derived growth factors (HDGF) and DNA metyltransferase proteins. PWWP domains are involved in DNA binding (29,30). Another example of a PWWP domain-containing protein is the lens epithelium-derived growth factor (LEDGF/p75) (31), which directly binds HIV-1 IN (32) and facilitates its tethering to chromosome [for review see, (33)]. Truncation of the PWWP motif from the C-terminus portion of indolicidin did not affect crosslinking efficiency demonstrating that both the PWWP motif and the two Arg residues present at the C-terminus of indolicidin do not influence crosslinking to abDNA. In contrast, the N-terminal portion of indolicidin is required for efficient binding to abasic sites. The single Lys residue is directly involved in binding to native DNA as indicated by our MS footprinting results (Figure 6). It is also involved but not absolutely critical in the binding to

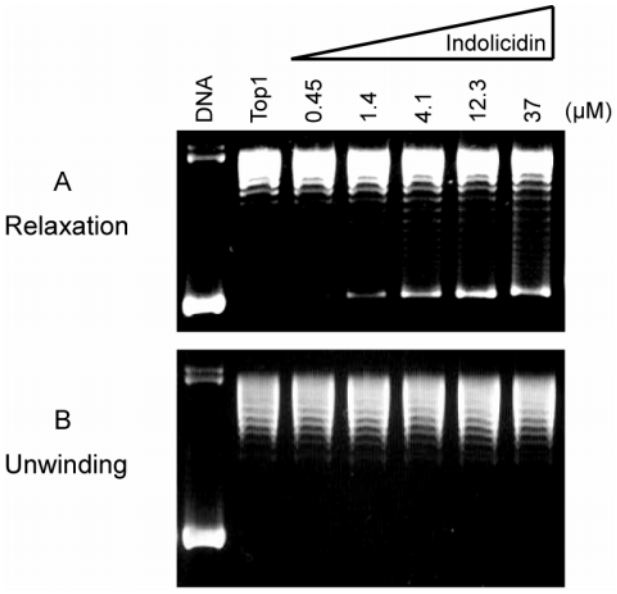

Figure 7. Indolicidin inhibits Top1 catalytic activity but does not intercalate into DNA. (**A**) native supercoiled ϕX174 DNA (lane 1) was incubated with Top1 in the absence of indolicidin (lane 2) or in the presence of indicated concentrations of indolicidin (lanes 3–7). (**B**) Native supercoiled ϕX174 DNA (lane 1) was first reacted with excess Top1 to fully relax the DNA in the absence of indolicidin (lane 2). Samples were then further incubated with the indicated concentrations of indolicidin (lanes 3–7).

abDNA. Its replacement by a Gly residue results in a ~20-fold reduction of crosslinking efficiency.

In addition to the influence of the single Lys residue, the N- and C-terminal NH_2 groups also contribute to the binding to abDNA. Removal of one of these three NH_2 groups results in a ~20- to 100-fold reduction in the crosslinking efficiency. Removal of two of them abrogates crosslinking completely except when the single Lys residue remains in the peptide. This peptide with no terminal NH_2 groups crosslinks ~1000-fold less efficiently than indolicidin. Therefore, the presence of each of these three NH_2 groups has a synergistic effect on the binding to abDNA.

SIGNIFICANCE

HDPs are presently under a focus of interest as they may offer a novel therapeutic alternative to current anti-infective therapies. Our study brings new insights on the mechanism of action of indolicidin with the possibility that this natural peptide may target DNA processing enzymes. In particular, the ability of indolicidin to bind to abDNA represents a novel feature that may play a role in the biological activity of this cathelicidin HDP. Abasic sites are considered to be the most frequent DNA lesions in mammalian cells with an estimated frequency of 10 000 events per day per cell (34). Therefore further investigations on the potential interference of indolicidin with DNA repair mechanisms are warranted.

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Conflict of interest statement. None declared.

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