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Specific photocrosslinking of DNA–protein complexes: Identification of contacts between integration host factor and its target DNA

(aryl azide/peptide sequence/DNA bend)

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ABSTRACT **Azide moieties have been specifically placed in the backbone of DNA by chemical coupling between azidophenacyl bromide and uniquely positioned phosphorothioate residues. The derivatized DNA forms specific complexes with a DNA-binding protein and, following irradiation with 302-nm light, makes specific crosslinks to the protein. Isolation of this covalent complex, followed by tryptic digestion and Edman degradation of the resulting crosslinked peptide, identifies the portion of the protein that is near the derivatized segment of the target DNA. We use this method to probe the interaction between a specific DNA sequence and integration host factor (IHF) protein. A single IHF heterodimer is known to contact >25 bp of DNA and thereby introduce a sharp bend. Two segments of a typical IHF site were derivatized with aryl azide. Although the segments were separated by only 5 bp, they crosslinked to different subunits of IHF. The locations of the crosslinks support our current view for the way IHF protein binds to and bends its specific targets.**

A large proportion of the vital transactions that affect the genome depend on specific protein–nucleic acid interactions. In a handful of cases the molecular structure of the complex is known (1). For the remainder, footprinting techniques (2) almost always provide strong clues to the residues of DNA that are contacted in the complex. However, the commonly used genetic and biochemical techniques leave one uncertain which residues of the protein contact the DNA and how the protein and nucleic acid are juxtaposed. The identification of specific crosslinks between a protein and its nucleic acid target is a powerful way to reduce the uncertainty in such speculation and to provide model-free answers to the question: What part of the protein is near what part of the target?

Crosslinking mediated by azides is particularly useful for identifying protein–DNA contacts because this moiety is stable unless exposed to light but then becomes very reactive (3). Moreover, some azide compounds, such as aryl azides, are activated by long-wavelength (300-nm) light that, by itself, produces little or no crosslinking. If one can incorporate such a moiety at a unique location on one component of the complex, the interpretation of the resulting crosslink becomes relatively straightforward. In the past, aryl azides have been successfully placed on both proteins and nucleic acids. Although many strategies have been successful (see refs. 4–6 and references therein), coupling of azidophenacyl bromide to sulfur residues (7) has been particularly versatile. For example, this compound has been used to attach an aryl azide to a unique cysteine in cAMP receptor protein (CRP) (8) and to a unique thiouridine in valyl-tRNA (9). Similarly, Hanna and Meares (10) showed that a thiophosphate at the 5′ end of an RNA was the preferred site of coupling for azidophenacyl bromide. They and others (11) used this trait to fashion end-derivatized RNAs which were then used to crosslink targets of interest. We have adapted this approach to the problem of protein–DNA interactions.

In the method devised for the present work, a DNA target is chemically synthesized to have internally positioned phosphorothioates; following coupling with azidophenacyl bromide, the derivatized DNA can be used to identify contact points between defined locations in the target sequence and a specific DNA-binding protein. We have explored this approach with the specific complex that is formed between integration host factor (IHF) protein and its DNA target. IHF is a small, basic, heterodimeric protein that was discovered as an accessory factor for integrative recombination by λ phage DNA (12). IHF binds to specific sites that are involved in λ integration and strongly bends its target DNA (13); binding and bending to similar targets presumably account for many of the uses for IHF in *Escherichia coli*. Several years ago our laboratory put forward a working model for the way IHF recognizes and bends DNA (14). This model combines (*i*) extensive footprinting analysis of several IHF targets that implicate the minor groove of DNA as the principal binding surface, (*ii*) stoichiometric determinations that indicate that a single promoter of IHF covers the target DNA, and (*iii*) the molecular structure of the closely related (but nonspecific) DNA-binding protein HU. Subsequently, genetic experiments identified residues that, when altered, either disrupt sequence-specific binding of IHF to DNA or relax the target specificity of binding (15–17). The identity of these residues is readily accommodated by our model. However, we were troubled to note that the molecular structure of the TATA-box-binding protein (TBP)–DNA complex (18, 19), a system that also involves minor-groove binding and deformation of DNA, revealed a global relationship between the protein and its target DNA site quite unlike that proposed for IHF. In the TBP–DNA complex, protein-induced kinks deflect the DNA away from the body of the protein, while in our proposal for IHF, deformation is associated with draping the DNA around the body of the protein. To provide new information that might resolve this dilemma, we have identified crosslinks between IHF and specific points in its target.

MATERIALS AND METHODS

Oligonucleotides. The IHF binding sites with phosphorothioate substitutions in one strand were constructed by annealing the following pairs of oligonucleotides: oHN-x3 (5′-ATAAAAsAsAGCATTGCTTATCAATTTGTTG-CAAGG-3′) and oHN-x4 (5′-CCTTGCAACAAATTGATA-AGCAATGCTTTTTTAT-3′);oHN-x7(5′-CCTTGCAACA-AATTGATAAGCsAsATGCTTTTTTAT-3′) and oHN-x8

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Abbreviations: IHF, integration host factor; TBP, TATA-box-binding protein.

(5'-ATAAAAAAGCATTGCTTATCAATTTGTTGCA-AGG-3'). For oHNx3 and oHNx7, "s" indicates the position at which aryl azides were coupled to phosphorothioates that had been introduced during chemical synthesis (20). Control duplexes were made by annealing nonphosphorothioate oligonucleotides whose sequences were identical to those shown above or a pair of complementary oligonucleotides, oHN68 and oHN69 (21), that comprise a 35-bp duplex lacking an IHF site.

Coupling of the Azidophenacyl Moiety to Oligonucleotides. Previously described methods (7, 10, 11) were used with some modifications. An aliquot of 100 nmol of oHN-x3 or oHN-x7 (20 pmol of which had been 5' radiolabeled) was resuspended in 20 ml of 20 mM sodium bicarbonate (pH 9.0) containing 45% (vol/vol) dimethyl sulfoxide and 5.0 mM azidophenacyl bromide (Fluka); the mixture was incubated at room temperature for 1 hr. Free azidophenacyl bromide was extracted four times with isobutyl alcohol and then 100 nmol of the complementary oligonucleotide (dissolved in 5.0 ml) was added and annealed at room temperature for 10 min. The duplex was collected by ethanol precipitation and dissolved in 40 ml of 10 mM Tris·HCl (pH 8.0) containing 1 mM Na_2EDTA. We found that addition of dimethyl sulfoxide instead of methanol (10) improved the coupling, estimated by retardation of the oligonucleotide in urea/PAGE, by a factor of ≈1.3 to a final yield of about 80%.

Reactions for Photocrosslinking Analysis. In a 96-well "U-form" tissue culture plate (Costar), samples of the 5'-end-labeled duplex DNA (25 nM) were incubated with IHF (75 nM) in a 20-μl mixture containing bovine serum albumin (40 μg/ml), 45 mM Tris·HCl (pH 8.0), 70 mM NaCl, 11 mM Tris borate (pH 8.9), 1 mM EDTA, and 10% (vol/vol) glycerol. After 20 min in the dark at room temperature, the mixture was placed on ice and irradiated with a UV light source (model UVM-57, 302 nm; Ultraviolet Products, San Gabriel, CA) at 5.0 cm for 2 min. Short-wavelength light (<300 nm) that could cause crosslinks in the absence of photoagent was filtered out by the polystyrene cover of the culture plate. After addition of 4 μl of loading buffer (2% SDS) and heating at 85°C for 3 min, the reaction mixture was applied to a Tris/tricine/SDS/12% polyacryamide gel (22).

Large-Scale Preparation and Purification of Photocrosslinked IHF–DNA Complex. Reaction mixtures like those described in the preceding section were scaled up to 100 ml, except that the amounts of IHF and duplex were increased disproportionately to final concentrations of 800 nM and 1000 nM, respectively. The increase in concentration facilitated the generation of sufficient complex for further analysis. Because the dissociation constants of IHF for specific and nonspecific targets are so different (2 nM and 12 μM, respectively; unpublished observations), we calculate that even at these concentrations, >95% of the complexes between IHF and DNA are specific. Aliquots (10 ml) of this mixture were transferred in turn to a Petri dish (Falcon 1029; Becton Dickinson), kept on ice for 3 min, covered, and irradiated for 2 min with 302-nm UV light as described above. Sodium phosphate (pH 5.5), urea, and NaCl were then added to a final concentration of 25 mM, 1.5 M, and 0.25 M, respectively. A pool of five aliquots was applied to a Mono Q column (1.0-ml bed volume; Pharmacia) which had been equilibrated with 25 mM sodium phosphate (pH 5.5) containing 0.25 M NaCl. After washing with 10 ml of the same buffer, a linear gradient (30 ml) was then developed from 0.25 M to 1.0 M NaCl in 25 mM sodium phosphate (pH 5.5). The crosslinked IHF–DNA complexes were identified by PAGE, recovered by ethanol precipitation, and dried *in vacuo* for 2 min. The complexes from two pools were suspended (350-μl total volume) in 50 mM Tris·HCl (pH 7.6) containing 0.05% SDS; the detergent was necessary to solubilize the precipitated material.

Peptide Sequence of the Crosslinked Complex. The purified crosslinked IHF–DNA complexes (200 μl, about 2 nmol) were heated at 60°C for 10 min to denature the protein and kept on ice for 2 min. After incubation at 37°C for 5 min, modified trypsin (2 μg; Promega) and 2 μl of 100 mM $CaCl_2$ were added. The mixture was incubated at 37°C for 2 hr and for another 1 hr at room temperature. The resulting peptide–DNA complexes were ethanol precipitated and subjected to 2 M urea/12% PAGE in 0.5× TBE (45 mM Tris/45 mM boric acid/1.25 mM EDTA). The resulting band containing the peptide–DNA complexes was localized by autoradiography, excised, and passively eluted overnight with 1 ml of 50 mM Tris·HCl (pH 8.0) containing 1 mM EDTA. The eluate was passed through a gel filtration column (PD-10; Pharmacia) equilibrated with HPLC-grade water. The amount of crosslinked peptide–DNA complex was estimated by converting DNA concentration determined from A_{260}. The eluate was dried completely and peptide sequences in the material were identified by automated Edman degradation (23).

RESULTS AND DISCUSSION

Experimental Strategy. When IHF binds specifically to a typical target DNA, it protects a region that encompasses about 30 bp from attack by various reagents (14). Moreover, the affinity with which IHF binds to a 30-bp duplex is close to that for binding to a much larger DNA fragment (unpublished observations). Accordingly, crosslinking probes can be conveniently made by (*i*) chemically synthesizing a 30-mer in which one or more specific backbone phosphodiesters are replaced by phosphorothioate and (*ii*) mixing this oligonucleotide with azidophenacyl bromide. As shown in Fig. 1, nucleophilic attack by sulfur displaces bromine and couples the aryl azide moiety to the DNA backbone. The derivatized DNA is then annealed to a complementary oligonucleotide, incubated with IHF, and irradiated.

In choosing which positions of the DNA target to derivatize, we were guided by the pattern of conservation of bases among IHF sites. In one half of the site, lying on the right as conventionally written, several bases are moderately well conserved, leading to the consensus WATCAANNNNTTR (12). In the other half of the 30-bp site, there are important DNA contacts and a nonrandom pattern of base use but no clear consensus can be discerned (24). The genetic studies generally appear to identify residues of IHF that are involved in contacting the conserved half of the target (15–17). To provide new information, we derivatized phosphodiester positions in the nonconsensus half of the IHF target. Rather than rely on a single site of derivatization, we synthesized

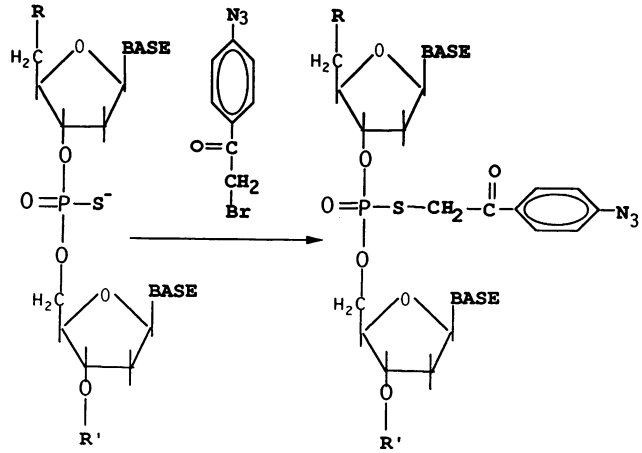

FIG. 1. Chemical coupling of azidophenacyl bromide to a phosphorothioate residue in DNA. See text for details.

DNA in which two adjacent phosphodiester positions were replaced by phosphorothioate, but we do not know whether this strategy significantly enhances the yield of crosslinks.

The crosslinked complex can be separated from the other components in the incubation mixture by column chromatography. This is followed by digestion of the purified complex with a protease and isolation of the peptide that remains covalently joined to the DNA. The amino-terminal sequence of this peptide indicates which portion of the protein is near the derivatized residues on DNA, and the point of interruption of the peptide sequence can provide clues as to the actual amino acid(s) involved in the linkage. The following sections present the results of specific steps of the method.

Formation of Specific Crosslinked IHF–DNA Complex. Using duplexes that were derivatized as shown in Fig. 2, an experiment was carried out to determine the efficiency of specific crosslinking between IHF and one of its typical targets, the H′ site that is found in the attachment region of bacteriophage λ (14). Under the conditions of Fig. 2, in which IHF protein is present at a concentration well above its dissociation constant (≈2 nM; unpublished observations) and in 3-fold excess over DNA, 5–10% of the labeled DNA becomes crosslinked. In preliminary experiments that scanned other positions in the backbone of the H′ site, no higher level of crosslinking was observed. Controls presented

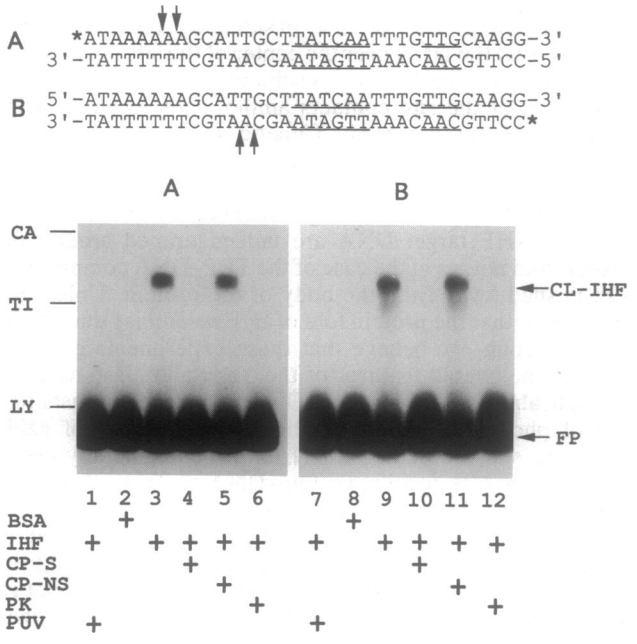

FIG. 2. Specific formation of covalent crosslinked IHF–DNA complexes. (*Upper*) Crosslinking probes A and B were made by coupling of the azidophenacyl moiety at two adjacent phosphate backbone positions of an oligonucleotide (arrows), followed by annealing to the complementary strand. The consensus sequence for IHF binding and the positions of radiolabel of the resulting 34-bp duplex are indicated by underlining and asterisks, respectively. (*Lower*) The ^{32}P-labeled duplexes were incubated with IHF or bovine serum albumin (BSA) as indicated. After irradiation, the reaction mixtures were subjected to SDS/PAGE and the labeled complexes were visualized by autoradiography. The positions of the crosslinked IHF–DNA complexes (CL-IHF) and free DNA probe (FP) are indicated at right; the positions of color-labeled carbonic anhydrase (CA), trypsin inhibitor (TI), and lysozyme (LY) (Amersham) are indicated at left. PUV indicates samples in which the radiolabeled probes were irradiated to inactivate the crosslinker before incubation with IHF; PK indicates samples that had been treated with proteinase K (50 μg/ml for 30 min at 37°C) after irradiation. Samples to which 2500 nM specific or 12,500 nM nonspecific competitor duplex had been added prior to irradiation are noted by CP-S and CP-NS, respectively.

in Fig. 2 indicate that the observed crosslinking involves IHF and specific DNA in a 1:1 complex, and other controls (data not shown) demonstrate the requirement for derivatization and photolysis.

Isolation of Crosslinked IHF Complexes. The photocrosslinking reaction described above was scaled up in order to generate sufficient amounts of the IHF–DNA complex for peptide analysis. After irradiation, noncovalent interactions were disrupted by addition of urea and the crosslinked IHF–DNA complex was separated from free IHF and free DNA by anion-exchange chromatography. Free IHF flows through the column and free DNA is strongly retained. As seen by SDS/PAGE analysis of fractions from this column (Fig. 3*A*), the crosslinked IHF–DNA complexes are eluted earlier than free DNA, presumably as a consequence of the neutralization of some negative charge in DNA by IHF. Most of the IHF–DNA complexes in these fractions remain covalently crosslinked throughout sample preparation and electrophoresis. The small amount of free probe seen in fractions 14 and 15 may reflect noncovalent complexes that survive denaturation or a low degree of heat lability of the covalent joint.

Isolation of Peptides Crosslinked to DNA. The IHF–DNA complexes purified by column chromatography were subjected to extensive digestion by trypsin. The degree of the digestion was monitored by analytical PAGE in the presence of 2 M urea. As typified by the example shown in Fig. 3*B*, digestion converted most of the crosslinked IHF complex to a unique species whose mobility falls between that of the undigested complex and that of free DNA. The treatment also apparently liberated a small amount of the DNA probe from covalent attachment to the protein. The principal product of the limit digest was purified by preparative urea/PAGE. About 400 pmol of the putative crosslinked peptide, more than 10 times the amount needed for amino acid sequencing, was recovered.

Identification of the Amino Acid Sequences of the Crosslinked Peptides. We isolated peptides crosslinked to the two duplexes shown in Fig. 2. In each case, automatic Edman degradation produced a single principal amino acid sequence (Table 1), and in each case, the sequence matched a portion of one of the subunits of IHF (Fig. 4). Thus, although the sites of derivatization of duplexes A and B are separated by only a few nucleotides (Fig. 2), they contact different subunits of IHF. The peptide attached to the A duplex, LSGFGNFDLR, precisely matched amino acid residues 45–54 of the α subunit of IHF. The decrease in the yield of the relevant amino acid at cycle 5 and all following cycles indicates that the major site of photocrosslinking for duplex A is at or near residue G49. The tryptic peptide crosslinked to duplex B, TGDKVELEGK, matches residues 66–75 of the β subunit of IHF. Based on interference with trypsin digestion and low yield, the most probable position(s) of crosslinking to the B duplex involve the R68 and/or K69 residues.

Structural Basis for the IHF–DNA Interaction. The results of this work provide independent evidence that both subunits of IHF are in contact with target DNA. It has long been known that both subunits are needed for IHF function *in vivo* and that the protein purifies as an apparent heterodimer (12, 13). Although it has been recently demonstrated that the individual purified subunits of IHF can bind specifically to DNA (25, 26), apparently as homodimers, such binding is weak and is probably not of physiological significance. These results do, however, raise the possibility that all the contacts with DNA could be made by a single subunit. However, our crosslinking results provide strong evidence that the natural heterodimer uses contact surfaces from both subunits. This result agrees with the inference of genetic studies, which showed that mutants affecting each subunit can alter DNA binding (15–17). However, the effects caused by mutation could have been indirect—i.e., a consequence of changes in

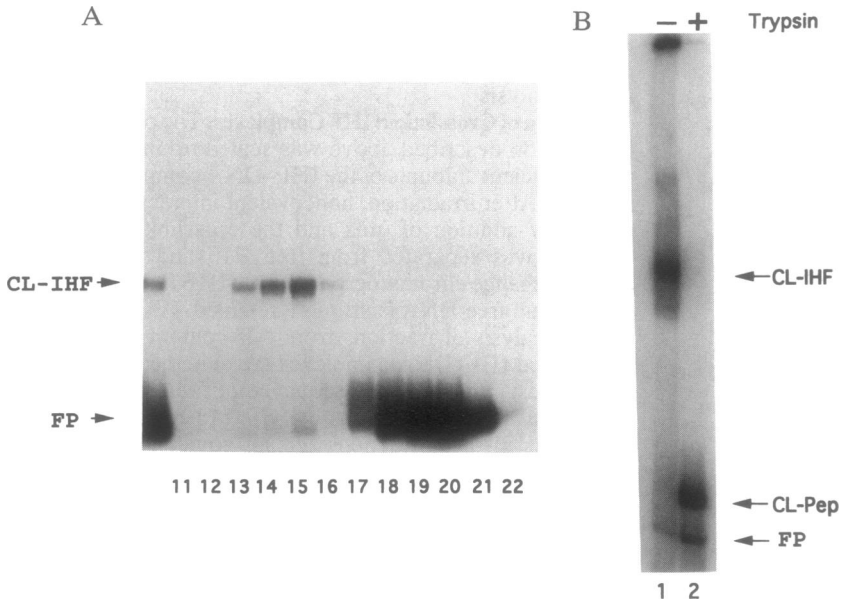

FIG. 3. Purification of crosslinked complexes and isolation of crosslinked peptides. (*A*) Autoradiogram of an SDS/12% polyacrylamide gel. IHF–DNA complexes were formed by incubation of 80 nmol of IHF with 100 nmol of 5′-end-labeled probe (duplex A of Fig. 2) followed by UV irradiation. The crosslinked complexes were purified by column chromatography (*Materials and Methods*). An aliquot (5 μl of 1.0 ml) of each indicated fraction from the column was analyzed by SDS/PAGE. An irradiated sample that was not subjected to chromatography is shown in the left-most lane. (*B*) Autoradiogram of a 2 M urea/12% polyacrylamide gel after electrophoresis in 0.5× TBE. Lane 1, 0.5 μl of the IHF–DNA complex purified in *A*; lane 2, 0.5 μl of a tryptic digest of this material. The position of the crosslinked peptide–DNA complex (CL-Pep) is shown.

the overall structure of the protein but not the DNA-binding surface. In addition, the genetic studies focused on residues that are the most conserved between IHF sites whereas, by design, the present work examined contacts that are made with less-well-conserved residues. That both the genetic studies and the present work provide compatible answers greatly enhances the strength of the conclusion that distinct parts of each subunit contact specific portions of this target.

It is gratifying that the present crosslinking data agree reasonably well with the Yang and Nash model (14). If one uses genetic data (15) to assign an orientation of the IHF heterodimer onto DNA, the model makes specific predictions for the regions of IHF that are close to the two segments of DNA that we have tested. In both cases the points of crosslinking we observe are quite consistent with the predictions of the model (Fig. 5). A distance of 11.0 Å separates a phosphorus atom and the reactive nitrogen of an aryl azide coupled to it. In the model, the phosphorus atom between bp 23 and 24 (highlighted to the right of the letter A in Fig. 5) is 12.5 Å from the α-carbon of αG49 of IHF; thus, the model readily accounts for the crosslink observed with duplex A. The model is less perfect in accounting for the crosslinking of duplex B: distances of 16.1 Å and 16.3 Å lie between the closest phosphorus (highlighted to the left of letter B) and the α-carbons of βD68 and βK69, respectively. However, it should be noted that, in construction of the model, no attempt was made to fit the arms of IHF into the minor groove; modest adjustments in their relative position would accommodate the observed crosslink. In summary, our data support the view that the DNA near the center of the site contacts the two-stranded "arm" of the β subunit, and the DNA at the distal part of the site contacts amino acids located on the "flanks" of the α subunit. Genetic studies have strongly suggested that both central and distal nucleotides from the well-conserved half make complementary contacts with IHF (15). Taken together, these experiments convince us that the 30 bp of IHF target DNA are indeed draped around the protein and not, as in the case of the TBP–DNA complex (18, 19), pushed away from the body of the protein. Unless the contention that the protein fold of IHF resembles that of HU proves wrong, we believe that these experiments go far in proving the overall features of the Yang and Nash model.

Table 1. Amino acid sequence of crosslinked peptides

Cycle	Duplex A		Duplex B	
	AA	pmol	AA	pmol
1	Leu	17.1	Thr	9.1
2	Ser	16.2	Gly	2.8
3	Gly	9.0	Asp	0.8
4	Phe	9.1	Lys	1.3
5	Gly	0.0	Val	2.8
6	Asn	0.0	Glu	1.3
7	Phe	0.0	Leu	2.8
8	Asp	1.7	Glu	1.0
9	Leu	0.2	Gly	1.5

The sequence of the primary peptide detected is shown together with the yield of the corresponding amino acid (AA), corrected by subtraction of the yield of that amino acid in the previous cycle. For the sample attached to duplex B, no secondary peptide was detected. For the sample attached to duplex A, a minor amount of a tryptic peptide that spans residue R81 of the α subunit of IHF was detected. The data shown were collected under contract by the W. M. Keck Biotechnology and Resource Laboratory (New Haven, CT), using ≈25 pmol of material for each sample.

Applicability of the Method. Our experience suggests that use of phosphorothioates to direct the coupling of azido groups to specific places in DNA will be a generally useful method to assess specific protein–DNA contacts. The preparation of the derivatized DNA was facile, the derivatized DNA bound avidly to its specific protein, the yield of crosslinked complexes was adequate for further analysis, and, most importantly, the crosslinks were specific. That is, for each marked position of the target DNA, the derivatized DNA not only identified a unique subunit as the principal partner for crosslinking but identified a unique peptide within

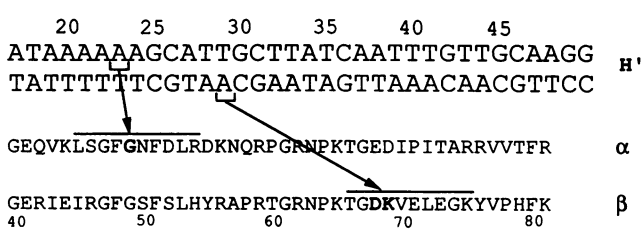

FIG. 4. Summary of crosslinking experiments. At the top, the sequence of the 34-bp IHF site used as a probe in these experiments is listed with brackets to indicate the sites of derivatization; the numbers are the coordinates of this site relative to the core of the bacteriophage λ attachment site. Below, the amino acid sequences of portions (residues 40–81) of the α and β subunits of IHF are presented with the single-letter code. Overlining indicates the peptide crosslinked to each probe, and the residues judged to be the most likely site of crosslinking are indicated with boldface letters.

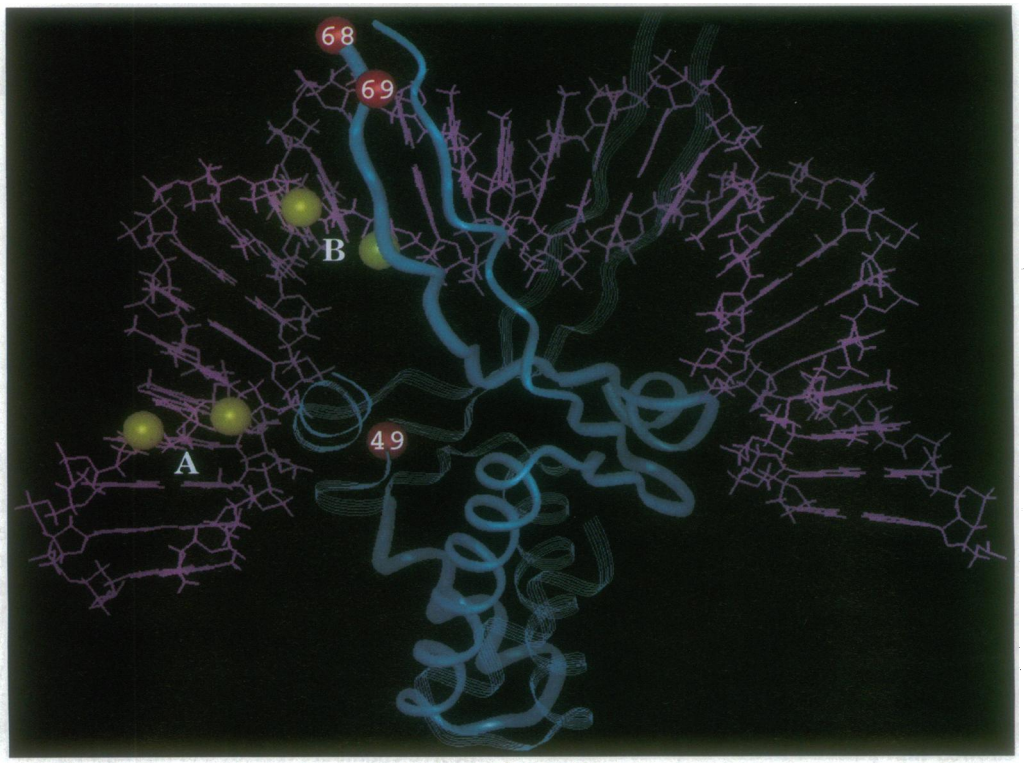

FIG. 5. Model for the IHF–DNA complex. The model is redrawn from ref. 14 with assignments of probable orientation of the α and β subunits taken from refs. 15 and 17. The 28-bp H′-site DNA is colored pink and the phosphates of the duplex to which the photosensitive crosslinker was coupled in sites A and B are highlighted in green. The α and β subunits are shown as linear and tubular blue ribbons, respectively. The amino acids that are the probable point of major crosslinking to the A and B sites are highlighted in red. The position of the secondary peptide that is crosslinked at lower yield to duplex A is within the strand just above αG49.

that subunit. This suggests that the rigid nature and relatively short length of the crosslinking substituent attached to DNA restrict its access to a small segment of the bound protein. Mayer and Barany (27) and T. S. Heuer and P. O. Brown (personal communication) have independently devised methods for crosslinking that are nearly identical to ours, with comparable success. Although these studies have focused on identifying the amino acids involved in the crosslink, if one's goal is to simply stabilize and detect protein–DNA complexes, the method can be made even more facile. Indeed, this has been used to assess the effect of metals on complex formation in the Mu transposition system (28). Regardless of whether the goal is detection or analysis of complexes, the method should be widely applicable because it can be exploited with proteins that interact with the minor groove or backbone of DNA, as well as proteins that interact with the major groove.

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1. Harrison, S. C. & Sauer, R. T. (1994) *Curr. Opin. Struct. Biol.* **4**, 1–2.
2. Sauer, R. T., ed. (1991) *Methods Enzymol.* **208**, 1–651.
3. Knowles, J. R. (1972) *Acc. Chem. Res.* **5**, 155–160.
4. Bartholomew, B., Kassavetis, G. A., Braun, B. R. & Geiduschek, E. P. (1990) *EMBO J.* **9**, 2197–2205.
5. Capson, T. L., Benkovic, S. J. & Nossal, N. G. (1991) *Cell* **65**, 249–258.
6. Sylvers, L. A. & Wower, J. (1993) *Bioconjugate Chem.* **4**, 411–418.
7. Hixson, S. H. & Hixson, S. S. (1975) *Biochemistry* **14**, 4251.
8. Pendergrast, P. S., Chen, Y., Ebright, Y. W. & Ebright, R. H. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 10287–10291.
9. Schwartz, I. & Ofengand, J. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 3951–3955.
10. Hanna, M. M. & Meares, C. F. (1983) *J. Am. Chem. Soc.* **22**, 3546–3551.
11. Burgin, A. B. & Pace, N. R. (1990) *EMBO J.* **9**, 4111–4118.
12. Friedman, D. I. (1988) *Cell* **55**, 545–554.
13. Nash, H. A. (1990) *Trends Biochem. Sci.* **15**, 222–227.
14. Yang, C. C. & Nash, H. A. (1989) *Cell* **57**, 869–880.
15. Lee, E. C., Hales, L. M., Gumport, R. I. & Gardner, J. F. (1992) *EMBO J.* **11**, 305–313.
16. Mengeritsky, G., Goldenberg, D., Mendelson, I., Giladi, H. & Oppenheim, A. B. (1993) *J. Mol. Biol.* **231**, 646–657.
17. Granston, A. E. & Nash, H. A. (1993) *J. Mol. Biol.* **234**, 45–59.
18. Kim, J. L., Nikolov, D. B. & Burley, S. K. (1993) *Nature (London)* **365**, 520–527.
19. Kim, Y., Geiger, J. H., Hahn, S. & Sigler, P. B. (1993) *Nature (London)* **365**, 512–520.
20. Iyer, R. P., Phillips, L. R., Egan, W., Regan, J. B. & Beaucage, S. L. (1990) *J. Org. Chem.* **55**, 4693–4699.
21. Burgin, A. B. & Nash, H. A. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 9642–9646.
22. Schägger, H. & von Jagow, G. (1987) *Anal. Biochem.* **166**, 368–379.
23. Edman, P. & Begg, G. (1967) *Eur. J. Biochem.* **1**, 80–91.
24. Goodrich, J. A., Schwartz, M. L. & McClure, W. R. (1990) *Nucleic Acids Res.* **18**, 4993–5000.
25. Zulianello, L., de la Gorgue de Rosny, E., van Ulsen, P., van de Putte, P. & Goosen, N. (1994) *EMBO J.* **13**, 1534–1540.
26. Werner, M. H., Clore, G. M., Gronenborn, A. M. & Nash, H. A. (1994) *Curr. Biol.* **4**, 477–487.
27. Mayer, A. N. & Barany, F. (1994) *Gene*, in press.
28. Baker, T. A. & Mizuuchi, K. (1992) *Genes Dev.* **6**, 2221–2232.