Fluorescence Analysis of the Escherichia coli Transcription Regulator H-NS Reveals Two Distinguishable Complexes Dependent on Binding to Specific or Nonspecific DNA Sites*

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Here we report a structural investigation of the transcription factor H-NS and its DNA interaction. H-NS has a general effect on transcription by compacting DNA; but for a number of specific genes, it is known to act directly as repressor or activator. The homodimeric protein binds to the major groove of DNA in a sequence-nonspecific manner, recognizing a curved conformation of the target DNA. H-NS consists of 136 amino acids with a single tryptophanyl residue at position 108. To overcome the apparent lack of any other structural details, we took advantage of the intrinsic fluorescence of Trp-108. In addition, quenching experiments, suggest that H-NS shows two different complexes dependent on binding to specific or nonspecific DNA sites. These data, in correlation with acrylamide and iodide quenching studies, indicate a positively charged microenvironment and high degree of solvent exposure for Trp-108. In addition, quenching studies in the presence of the anionic quencher iodide are consistent with a hydrophilic environment and high degree of solvent exposure for Trp-108. Static and dynamic quenching constants obtained with the neutral quencher molecule acrylamide are consistent with a hydrophilic environment and high degree of solvent exposure for Trp-108. Static and dynamic quenching constants obtained with the neutral quencher molecule acrylamide are consistent with a hydrophilic environment and high degree of solvent exposure for Trp-108. In addition, quenching studies in the presence of the anionic quencher iodide indicate a positively charged microenvironment for the same amino acid residue. Specific and nonspecific H-NS-DNA complexes were studied by gel retardation and fluorescence analysis. While specific H-NS-DNA complex formation is accompanied by a clear enhancement of the tryptophan fluorescence intensity, interaction in the presence of the nonspecific competitor DNA poly(dI-dC) decreases the fluorescence quantum yield.

The Escherichia coli protein H-NS has originally been described as a histone-like, nucleoid-associated protein, considered to be important for cellular mechanisms like DNA compaction or alteration of DNA topology (1–5). More recently, it became clear that H-NS acts as a pleiotropic transcription factor involved in the regulation of several unrelated genes (6–10). As shown for some other transcription factors, H-NS is known to act as both activator and repressor, depending on the respective transcription unit it controls (6).

In line with the high binding preference to curved DNA (5, 7, 11, 12), H-NS-specific regulatory sequences have all been shown to contain a defined intrinsic curvature. Among the genes regulated by H-NS are those involved in osmoregulation and response to environmental stress conditions (8, 13, 14). In addition, the thermo-osmotic regulation of virulence gene expression in Shigella was shown to be influenced by H-NS (15). A common denominator for H-NS-dependent regulation of the unrelated group of genes appears to be certain extreme environmental conditions, i.e. cold shock, osmotic shock, or other kinds of stress situations.

The constantly growing number of genes whose expression is known to be affected by H-NS includes the most efficiently expressed RNAs of the cell, namely ribosomal RNAs (16). H-NS has been shown to act as a specific repressor of the ribosomal rrnB P1 promoter, thereby antagonizing the stable RNA transcription activator Fis under conditions of stationary growth (9, 17–19). The site of H-NS/P1 DNA interaction has been mapped for the rrnB promoter by high resolution footprinting techniques. The binding region extends from positions –18 to –89, relative to the P1 transcription start site, and shows an overlap with the known binding sites for Fis. Thus, in addition to the control of several other global regulatory networks, i.e. stringent or growth rate control, ribosomal RNA synthesis is linked to certain cellular stress conditions via H-NS-mediated repression (9, 16).

H-NS has a molecular mass of 15.5 kDa and consists of 136 amino acids (1, 2). Specific binding of the homodimeric protein is known to require bent DNA in a precise orientation with respect to the regulated promoters. Hence, the binding is DNA conformation-specific with no known sequence specificity (9, 20).

Due to a lack of detailed structural information on H-NS (for instance, three-dimensional crystallographic data or high resolution NMR spectroscopy), we took advantage of the intrinsic fluorescence of a single tryptophanyl residue at position 108, which we used as a structural indicator. The microenvironment of the fluorophor was investigated by fluorescence quenching studies. Furthermore, steady-state fluorescence measurements were performed to analyze the protein conformation in the free and DNA-bound states. The specific interaction of H-NS with a curved rrnB P1 promoter fragment was followed by fluorescence measurements and compared with the nonspecific interaction of H-NS with the competitor DNA poly(dI-dC). These studies demonstrate that the single H-NS tryptophanyl residue at position 108 is strongly affected upon DNA binding. The fluorescence emission intensity is enhanced after binding to specific DNA target sites. In contrast, the interaction of H-NS with nonspecific DNA decreases the Trp-108 fluorescence. These data, in correlation with acrylamide and iodide quenching experiments, suggest that H-NS shows two different complex conformations dependent on binding to specific or nonspecific DNA sites and closely links Trp-108 to this conformational change.

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1 While this manuscript was in the reviewing process, a NMR study of a C-terminal 47-residue tryptic H-NS fragment was published by Shindo et al. (32), which is fully compatible with the data presented here.
EXPERIMENTAL PROCEDURES

Materials—All common chemicals were of the highest purity and were purchased from Merck, Sigma, or Fluka. Ultrapure Tris was obtained from U. S. Biochemical Corp. Suprapure KCl and KI were products from Merck and Janssen, respectively. The competitor DNA poly(dI-dC) was a product of Boehringer Mannheim. For convenience and to allow quantitative comparison of poly(dI-dC), which is rather heterogeneous in length, with DNA fragments of defined length, the concentrations of all nucleic acids are given in units of molar base pairs. Acrylamide was purchased in a twice recrystallized form from Serva. KCl, KI, and acrylamide solutions were dissolved in assay buffer at the pH that was appropriate for each experiment.

DNA Fragment Preparation—A 267-bp rrnB P1 DNA fragment containing the ribosomal RNA P1 promoter, including the upstream activating sequence (UAS), was isolated after EcoRI-PstI digestion of the plasmid pUC18-2 (19).

Preparation and Purification of H-NS—H-NS protein was isolated and purified from E. coli MRE 600 cells as described recently (9). The purified protein was dialyzed against 50 mM Tris-HCl, pH 7.4. H-NS protein was homogeneous as judged by silver staining of polyacrylamide gel electrophoretic separations in the presence of sodium dodecyl sulfate. Protein concentrations were determined according to the Bradford microassay protocol (21) and according to the known extinction coefficients of 0.066 l mol⁻¹ cm⁻¹ (22).

DNA Binding and Gel Retardation—Standard binding assays and gel retardation experiments were performed as described (9). Binding conditions were precisely adapted to the ionic environment present during fluorescence spectroscopy. Routinely, samples were incubated for 20 min in buffer containing 50 mM Tris-HCl, pH 7.4, with 94 mM bp of radiactively labeled rrnB P1 promoter fragment, 35 mM bp of competitor DNA poly(dI-dC), and 86 mM H-NS in a total volume of 10 μl. The samples were mixed with 2 μl of 50% glycerol in TBE buffer (23). Gel electrophoretic dissection of free and bound DNAs was performed for 4 h at 250 V with native 5% polyacrylamide gels (46:1). Dried gels were autoradiographed for 12 h at −20 °C. The amounts of free and bound DNAs were determined by densitometric evaluation of unsaturated autoradiograms using a Zeineh SL-504-XL soft laser scanning densitometer and the software “Scan-Plot.” 3

Fluorescence Spectroscopy—All steady-state intrinsic fluorescence measurements of H-NS were made with an SLM 8000 photon-counting spectrofluorometer with a xenon arc lamp. A constant sample measurement temperature of 21 °C was maintained by a Julabo F20 circulating temperature bath. The excitation and emission slit widths were 8 and 4 nm, respectively. All emission spectra were recorded at an excitation wavelength of 282 nm to obtain maximum intrinsic H-NS tryptophan fluorescence. Emission spectra were recorded at wavelengths between 300 and 400 nm with 1-nm steps. Each data point of the spectra was the sum of all detected and counted photons in a time interval of 10 s. All measurements were corrected for dilution, and emission from the control was considered by recording subtraction spectra between sample and control probes. Since absorbance values did not exceed 0.01, corrections due to inner filter effects were not incorporated. In the case of protein-DNA complexes, where this value was exceeded, relative measurements as described below were performed, making corrections unnecessary.

Fluorescence Quenching Analysis—All quenching experiments were made as steady-state acrylamide and KI quenching procedures at 21 °C. 86 mM H-NS protein in 50 mM Tris-HCl, pH 7.4, was quenched in the presence of increasing amounts of acrylamide (25–200 mM) or KI (10–50 mM). The fluorescence measurements were performed as described above, as continuous emission spectra between 300 and 400 nm; or as fixed point measurements with emission recordings at 337 nm. The excitation wavelength was 282 nm.

The classical Stern-Volmer equation relates the drop in fluorescence to the concentration of a collisional quencher as shown in Equation 1:

\[ \frac{F_0}{F} = 1 + K_{SV}Q \]  

(Eq. 1)

where \( F_0 \) is the fluorescence in the absence and \( F \) is the fluorescence in the presence of the quencher (Q). \( K_{SV} \) is the Stern-Volmer constant for the collisional quenching process (24). According to the fluorescence quenching data obtained from acrylamide quenching, the following

modified Stern-Volmer equation was used to estimate the relevant quenching constants:

\[ \frac{F_0}{F} = 1 + K_{SV}(Q)e^{2Q/V} \]  

(Eq. 2)

where \( K_{SV} \) is the dynamic quenching constant and \( V \) is the additional static quenching constant (24). To determine \( K_{SV} \) and \( V \), the collected data were fitted to Equation 2 using the nonlinear least-squares program “FITLS32.”

Fluorescence Analysis of Nonspecific and Specific H-NS/DNA Interactions—Fluorescence measurements were performed under conditions in which specific binding of H-NS to the upstream sequence of the rrnB P1 promoter fragment had been demonstrated by footprint analysis (9). In the presence of 35 μM bp of poly(dI-dC) as competitor DNA, varying amounts of rrnB P1 promoter fragment (0.473, 94.6, 141.9, 189.2, 236.5, and 283.8 M bp) were incubated in a total volume of 800 μl of 50 mM Tris-HCl, pH 7.4, with 86 mM H-NS. In contrast, nonspecific DNA interactions were investigated by replacing the specific rrnB promoter fragment with the corresponding identical amounts of poly(dI-dC). Fluorescence emission measurements were performed and corrected for control samples in the absence of protein as indicated above.

RESULTS

Steady-state Fluorescence Analysis of H-NS—The small neutral protein H-NS contains a single tryptophan residue at position 108. This offers a convenient way for a possible conformational analysis of the protein by measuring the intrinsic tryptophan fluorescence. The fluorescence intensities of H-NS, recorded between 300 and 400 nm, yield a typical emission spectrum of a tryptophan-containing protein when excited at 282 nm (see insets in Figs. 1 and 2). The emission peak is broad with a maximum in the range of 377–340 nm and shows a somewhat slower decay at higher wavelengths than the increase in fluorescence intensity at shorter wavelengths.

Effect of Acrylamide or Iodide on H-NS Fluorescence Emission—The influence of the effective fluorescence quencher molecules acrylamide and iodide on the tryptophan fluorescence of H-NS is shown in Figs. 1 and 2, respectively. Both acrylamide and KI quench the fluorescence intensity of H-NS without significant spectral shift, but in a different manner.

FIG. 1. Stern-Volmer plot of the acrylamide quenching of H-NS fluorescence emission in the presence of 0, 0.025, 0.05, 0.075, 0.1, 0.125, 0.15, 0.175, and 0.2 M acrylamide ( ). The inset shows complete H-NS fluorescence emission spectra in the presence of 0 and 0.2 M acrylamide recorded between 300 and 400 nm. To obtain a maximum of graphical clearness, fluorescence emission spectra at 337 nm with different acrylamide concentrations (indicated above) are shown as crossed circles. λex = 282 nm, 21 °C.

4 FITLS32 is available via anonymous ftp from the server tui.cri.nz in the directory/pub/gle/gle32.
Fluorescence Analysis of H-NS

The Stern-Volmer plot of the iodide quenching of H-NS fluorescence emission in the presence of 0, 10, 20, 30, 40, and 50 mM KI (trio) is shown in Fig. 2. The inset shows complete H-NS fluorescence emission spectra in the presence of 0 and 50 mM KI recorded between 300 and 400 nm. To obtain a maximum of graphical clearness, fluorescence emission spectra at 337 nm with different KI concentrations (indicated above) are shown as crossed circles. $\lambda_{em} = 282$ nm, 21 °C.

The Stern-Volmer plots of the collected acrylamide and iodide quenching data of H-NS show opposite tendencies: the Stern-Volmer plot of H-NS in the presence of acrylamide follows an upward curved exponential function, which can be fitted according to Equation 2. The acrylamide quenching data yield apparent static and dynamic quenching constants of $K_{SV} = 4.3 \times 10^6$ M$^{-1}$ and $K_{SV} = 11.7 \times 10^3$, respectively. In contrast, the ratio $F_0/F = \alpha - (a - 1)\times e^{-b}$, where $F_0$ is the fluorescence intensity at 340 nm. The downward curvature of such a dependence in quenching experiments can be explained with the hypothesis that a net positive charge exists in the microenvironment of the tryptophanyl residue, for example, due to protonation of nearby amino acid side chains. After saturation of this positive charge with increasing amounts of iodide anions, following quenching, interactions with the fluorophor can only occur in a weaker manner (24). As we shall see, the notion of a positively charged microenvironment of Trp-108 is essential for the considerations below.

Specific and Nonspecific Interactions of H-NS with the rRNA P1 Promoter Fragment—Detailed analyses of H-NS-DNA complexes have revealed that specific H-NS binding requires a strong DNA curvature, usually provided by a defined number of appropriately spaced AT clusters (9, 11–13). Such specific binding had been shown, for example, to the upstream sequences of ribosomal RNA P1 promoters (UAS region). However, the protein has also a low affinity for random DNA sequences, and nonspecific binding is known to occur to synthetic polynucleotides, such as poly(dI-dC), which is widely used as a competitor DNA in binding studies. For analysis of specific binding, e.g. footprinting studies, stringent binding conditions suppressing nonspecific interactions have been elaborated. Stringent binding conditions imply the simultaneous presence of specific target DNA and an excess of nonspecific competitor (9). The binding constant for H-NS and the P1 fragment measured according to Fried (25) has been determined as $3.8 \times 10^6$ M$^{-1}$ under standard binding conditions. This number differs by a factor of 100 compared with the $K_f$ for poly(dI-dC), assuming a single binding site for the specific fragment and a number of binding sites corresponding to the concentration of molar base pairs of the nonspecific DNA. Fig. 3 shows the interaction of H-NS with the P1 promoter UAS fragment as a function of the addition of increasing amounts of nonspecific competitor DNA poly(dI-dC). Binding was followed by gel mobility shift analysis. With increasing amounts of poly(dI-dC), a loss of specific H-NS-P1 complex formation can be observed (Fig. 3, H-NS$^{sp}$, lanes 1–10). A second band representing complexes with lower gel mobility is visible in lane 1, to which no competitor DNA was added. Obviously, the second complex was formed due to additional nonspecific interactions of H-NS with the rRNA P1 promoter fragment.

Fluorescence Emission Spectra of H-NS-rRNA P1 Nucleoprotein Complexes—To study possible protein conformational changes related to the binding of H-NS to specific DNA sites, fluorescence analysis of H-NS-rRNA P1 promoter complexes was performed. As outlined above, specific binding of H-NS to the P1 promoter fragment, as for footprint analysis, was routinely performed in the presence of high concentrations of competitor DNA. We attempted to maintain the same conditions for the fluorescence measurements. Reactions were designed to compare the effect on fluorescence emission when, in the presence of competitor DNA, H-NS samples were titrated with either increasing amounts of specific binding P1 promoter fragment or nonspecific competitor DNA poly(dI-dC). We sought to determine if the environment of Trp-108 would be affected differently under conditions of specific or nonspecific interaction. The results of such experiments are exemplified in Figs. 4 and 5, where H-NS was titrated with increasing amounts of poly(dI-dC) or rRNA P1 fragment, respectively. From these samples, the H-NS fluorescence emission spectra were recorded. All samples had constant amount of competitor DNA poly(dI-dC) (35 $\mu$M bp), but contained different amounts of specific rRNA P1 DNA (0–189.2 M bp; Fig. 4) or additional poly(dI-dC) at the same concentrations (Fig. 5). In each case, there was a change in fluorescence intensity at 340 nm. Thus, the fluorescence intensity at this wavelength was suitable to monitor conformational differences upon the addition of specific or nonspecific DNA to H-NS complexes.

A typical H-NS fluorescence emission spectrum between 300 and 400 nm can be recorded when the control samples without the P1 fragment or additional poly(dI-dC) are excited at 282 nm. The addition of the specific binding P1 UAS fragment results in a clear increase in fluorescence intensity, indicative of a change in the environment of the fluorophor, caused either by an alteration of the protein conformation or by the close proximity of the DNA (Fig. 4). In contrast, the addition of nonspecific DNA under the same conditions leads to a decrease

FIG. 2. Stern-Volmer plot of the iodide quenching of H-NS fluorescence emission in the presence of 0, 10, 20, 30, 40, and 50 mM KI (trio). The inset shows complete H-NS fluorescence emission spectra in the presence of 0 and 50 mM KI recorded between 300 and 400 nm. To obtain a maximum of graphical clearness, fluorescence emission spectra at 337 nm with different KI concentrations (indicated above) are shown as crossed circles. $\lambda_{em} = 282$ nm, 21 °C.

FIG. 3. Gel mobility shift analysis of H-NS-rRNA P1 complexes in the presence of different amounts of nonspecific competitor DNA. Lanes 1–10, 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10, respectively. In contrast, the ratio $F_0/F = \alpha - (a - 1)\times e^{-b}$, where $F_0$ is the fluorescence intensity at 340 nm. The downward curvature of such a dependence in quenching experiments can be explained with the hypothesis that a net positive charge exists in the microenvironment of the tryptophanyl residue, for example, due to protonation of nearby amino acid side chains. After saturation of this positive charge with increasing amounts of iodide anions, following quenching, interactions with the fluorophor can only occur in a weaker manner (24). As we shall see, the notion of a positively charged microenvironment of Trp-108 is essential for the considerations below.

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Apart from its early characterization (1, 22), little is known about the structure and mode of DNA interaction of the transcription regulator H-NS. The neutral chromatin-associated protein shows a rather nonspecific affinity for nucleic acids including double-stranded DNA of random sequence, single-stranded DNA, or even RNA (27). Preferential binding of H-NS with much higher affinity and specificity has been demonstrated to intrinsically curved DNA (8–10, 12). In those cases, the protein apparently acts as a specific transcription factor. However, no common DNA sequence motif for specific binding is known, and the available evidence indicates that interaction depends exclusively on specific DNA conformations (curvature) (9, 12, 20). This unusual binding property is reflected in the complete absence of peptide motifs commonly found in DNA-binding proteins, i.e. helix-turn-helix, zinc fingers, or other DNA-binding elements. To understand the binding mechanism, structural information about the protein and protein-DNA complexes is required. Because the protein contains a single tryptophan residue, we performed fluorescence analysis as the method of choice.

Fluorescence quenching studies have provided valuable information concerning the exposure of the tryptophanyl residues of proteins in the past (24). Comparison with quenching data on other proteins helps to determine the degree of exposure of the fluorophor. The magnitude of the static and dynamic acrylamide quenching constants (V = 4.3 m⁻¹ and KSV = 11.7 m⁻³) obtained for H-NS indicates that the microenvironment of the H-NS tryptophanyl residue at position 108 is totally exposed to the solvent. For instance, in the case of the galactose repressor protein GalR (V = 0.3 m⁻¹ and KSV = 5.3 m⁻³), Brown et al. (28) postulated some steric shielding of the tryptophan residue. In this case, the microenvironment is not totally protected from the solvent (28). In contrast, the quenching constants for glucagon (V = 1.0 m⁻¹ and KSV = 10.5 m⁻³) are greater than those found for GalR. Here, the tryptophan residue of glucagon is considered to be solvent-exposed (29).

The magnitude of the quenching values for H-NS presented in this study are similar or greater than the values for glucagon, indicating that the tryptophanyl residue of H-NS is, as in glucagon, totally exposed to the solvent.

The Stern-Volmer plot of the iodide quenching experiments exhibits a downward curved exponential saturation. Because both the fluorescence acrylamide and iodide quenching studies of H-NS show a significant drop in fluorescence emission intensity without spectral shift and since DNA-binding proteins are almost always symmetrical multimers, we do not favor the possible explanation that the tryptophan residues of one H-NS dimer are in different environments. Rather, we want to point out the more likely fact that the saturation function is due to the circumstance that a net positive charge exists in the microenvironment of the tryptophanyl residue because of the protonation of nearby amino acid side chains. After neutralization of this positive charge with increasing amounts of iodide anions during quenching, interactions with the fluorophor become progressively weaker (24). This explanation is in good agreement with the high degree of tryptophan solvent exposure demonstrated by the acrylamide quenching results. It is obvious that a region with such positive charge either must be shielded intramolecularly through other negatively charged groups or must be exposed to the environmental medium. The apparently positive charge of the microenvironment of the H-NS tryptophanyl residue at position 108 and its high degree of solvent exposure offer the first indications of a possible interaction during binding of negatively charged DNA to this protein domain.

It is known that at sufficiently high concentrations, H-NS interacts with any double-stranded DNA (30), which explains its function in the compaction of DNA and changing DNA topology (2, 4). Strong and specific binding, however, requires DNA with an intrinsic curvature (11, 12). In accordance, genes under direct repression or activation of H-NS exhibit curved DNA target sequences, to which the protein is known to bind (9, 13, 14). Specific binding can be prevented in the presence of the drug distamycin, which is known to bind to the minor groove of AT tracts, thereby straightening out curvature (9, 11, 31). For instance, strong and specific binding to H-NS has been demonstrated for the curved upstream sequence of the ribosomal RNA P1 promoter region, where the interacting sequences have been mapped by footprinting (9). Therefore, we have studied protein
DNA interaction with the P1 UAS region as an example of strong DNA binding in comparison to the weak interaction of H-NS with nonspecific synthetic DNA poly(dI-dC). Nevertheless, at a high excess of H-NS and in the absence of competitor, nonspecific binding can also be observed to the P1 UAS DNA after all specific sites have been saturated (see, for instance, Fig. 3, lane 1).

Studies on the salt-dependent binding of H-NS to the rrnB P1 promoter fragment confirm previous findings that H-NS is involved in the transcription regulation of several important systems. Under high competitor concentrations, stable binding occurs between 50 and 125 mM KCl, consistent with stable interactions under in vivo conditions, where the intercellular K+ concentration is about 100 mM (26). At KCl concentrations above 130–150 mM, a typical loss of binding affinity by a factor of 2 can be noticed (data not shown). This modulation, produced by differences in ionic strength, may in part underline the linkage of H-NS activity changes with its regulatory implications under osmotic shock or stationary phase conditions.

In this paper, we describe for the first time molecular details of two distinguishable H-NS binding states depending on the interaction with specific or nonspecific DNA target sites. In the presence of 35 μM bp of competitor DNA, the addition of P1 DNA increases the fluorescence emission intensity dramatically. Under these conditions, the fluorescence results indicate that there must be a specific complex structure that is accompanied by a steric shielding of the tryptophan residue. The increase in the fluorescence emission intensity upon P1 DNA binding indicates more than simple electrostatic interactions and is very likely due to a hydrophobic contact of Trp-108 with staggered base pairs of the DNA major groove. The results do not imply intercalation of Trp-108 into the DNA double strand since this would apparently cause quenching rather than enhancement of the fluorescence intensity. An alternative explanation could be a drastic protein conformational change, shielding Trp-108 and simultaneously exposing domains for specific DNA binding. Both events may not be mutually exclusive.

A possible scenario explaining our data could be as follows. Binding of the protein to the specific P1 target site involves interaction of the positively charged tryptophan microenvironment with the negatively charged DNA phosphate backbone. Thus, the tryptophan residue gets closer to the apolar base pairs and is shielded from solvent influence, which results in an increase in fluorescence emission. This process occurs only if the binding partner of H-NS is a specific DNA ligand and if the salt conditions are suitable for this specific protein/DNA interaction. Obviously, a stringent prerequisite for this specific contact is an intrinsic curvature or high flexibility of the target DNA. It is conceivable that only appropriately curved DNA is able to provide the steric requirements necessary for a close interaction with the H-NS binding domain, which very likely includes tryptophan 108.

In contrast, if the target DNA does not adopt the right conformation due to the lack of curvature or bendability, only weak, almost exclusively electrostatic interactions can be performed. Nonspecific binding is therefore much more prone to increase in ionic strength or competition by heparin. Apparent under such conditions, the H-NS tryptophanyl residues are more exposed to collisional quencher molecules from the environmental solvent, which causes a drop in fluorescence intensity.

In summary, Trp-108 is a suitable sensor responding to specific and nonspecific binding conditions. It is thus conceivable to assume that Trp-108 constitutes part of the H-NS/DNA binding domain.

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