THE BACTERIAL HISTONE-LIKE PROTEIN HU SPECIFICALLY RECOGNIZES SIMILAR STRUCTURES IN ALL NUCLEIC ACIDS: DNA, RNA AND THEIR HYBRIDS

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SUMMARY

HU, a major component of the bacterial nucleoid shares properties with histones, HMGs and other eukaryotic proteins. HU, which participates in many major pathways of the bacterial cell, binds without sequence specificity to duplex DNA but recognizes with high affinity DNA repair intermediates. Here, we demonstrate that HU binds to dsDNA, dsRNA, and linear DNA-RNA duplexes, with a similar low affinity. In contrast to this non-specific binding to total cellular RNA and to supercoiled DNA, HU specifically recognizes defined structures common to both DNA and RNA. In particular, HU specifically binds to nicked or gapped DNA-RNA hybrids and to composite RNA molecules, such as DsrA, a small non-coding RNA. HU, which modulates DNA architecture, could also play via its RNA binding capacity, additional key functions in the bacterial machinery. The simple, straightforward structure of its binding domain with its two highly flexible β-ribbon arms and an α-helical platform is an alternative model for the elaborate binding domains of the eukaryotic proteins that display dual DNA and RNA specific binding capacities.

Keywords: bacterial-nucleoid associated protein, histone-like protein, and structure-specific binding protein, DNA/RNA binding domain, DsrA RNA
INTRODUCTION

The Escherichia coli HU protein is a major component of the bacterial nucleoid (1-3). This small basic histone-like protein which can introduce negative supercoiling into a close circular DNA molecule in the presence of Topoisomerase I, is highly conserved and found in all bacterial species (4-7). HU plays a role in DNA replication, recombination and repair (8-10). It participates in Mu transposition (11) and regulation of gene transcription (12). HU has been shown to be important for optimal survival of the cells in stationary phase and under various stress conditions (13).

HU belongs to the family of architectural nuclear proteins that control DNA topology by introducing bends into double-stranded DNA (dsDNA) and stabilize higher-order nucleoprotein complexes. HU resembles eukaryotic proteins of the HMG class in its DNA-binding properties as it binds dsDNA with low affinity and no sequence specificity. In contrast, it displays high affinity for some altered DNA structures such as junctions, nicks, gaps, forks and overhangs even under stringent salt conditions (14-18). The DNA structural motif for HU recognition consists of either two dsDNA modules with propensity to be inclined or one dsDNA module adjacent to a ssDNA-binding module (19). X-ray crystallography and NMR studies established the structure of HU dimer in the absence of DNA (20-22). The two subunits are intertwined to form a compact α-helical hydrophobic core with two extended positively charged β-ribbon arms. Our recent studies suggest that HU contacts duplex DNA via the minor groove with its flexible arms while the high affinity binding to its specific binding motif requires an additional contact with HU body (19).

Like histones, HU has been shown to bind to poly (U) homopolymer (JRY, unpublished data) but the role of this abundant protein in RNA binding was underestimated. Recently we have shown that HU binds with high affinity to mRNA of rpoS, encoding the stress sigma factor of RNA polymerase, and stimulates its translation (20). Interestingly, in parallel to this work it was shown that HBsu, the HU protein of Bacillus subtilis, specifically binds the alu
domain of a small cytoplasmic RNA (scRNA), a homologue of mammalian SRP RNA (21). In the eukaryotic field, a growing body of evidence shows that a number of proteins - including transcriptional factors containing zinc-finger or RNA recognition motifs, are able to bind specifically to both DNA and RNA (22-30).

Although HU does not possess any sequence or structural homology to RRM or zinc-finger motifs, its small DNA-binding domain formed from two β-ribbon arms and a α-helical core is able to bind with high specificity to RpoS mRNA (31-33). In this work, we investigate the general RNA-binding features of HU. We measured HU affinity to total cellular RNA and found that HU binds to RNA as strongly as to supercoiled DNA, formerly believed to be the main target for the nucleoid associated HU (2). The characteristics of HU non-specific binding to double-stranded RNA and DNA as well as to simple linear DNA-RNA hybrids are shown to be similar. In contrast, HU binding to discontinuous DNA-RNA structures is much stronger than that to DNA and RNA duplexes and displays the same affinity as with nicked DNA, one of the structures that HU binds specifically. We searched for the structural determinant(s) of HU-RNA recognition using DsrA RNA, a small noncoding stable RNA which modulates the translation of several key transcriptional regulators such RpoS or H-NS (34-38). We found that DsrA RNA is one of the specific targets for HU binding. Truncation of DsrA RNA showed that HU bound with high affinity a RNA structure similar to that of a DNA overhang, one of its specific targets on DNA (19).
EXPERIMENTAL PROCEDURES

DNA templates and RNA synthesis - The DNA fragments corresponding to DsrA RNA and DsrA deletion mutants RNA C, D and E were amplified by PCR using the pDDS164 plasmid provided by S. Gottesman (34) as a template and appropriate primers. In the case of the smallest RNA-F, two complementary DNA oligonucleotides were annealed. The resulting DNAs were cloned under the control of the T7 promoter into the EcoRI and HindIII sites of pGem3Z (Promega). The [α-32P]-RNAs were synthesized from a plasmid linearized by HindIII by in vitro transcription. Before use, the RNAs were renatured by incubation at 65°C for 5 min and cooling on ice. For construction of RNA duplex the DNA synthetic oligonucleotides X1 and Y1 were annealed with their complementary DNA strands and cloned under the control of the T7 promoter into EcoRI and HindIII sites of pGem3Z. The corresponding [α-32P]-labeled X and non-labeled Y RNA were prepared from plasmid linearized by HindIII by in vitro transcription. These complementary RNAs were annealed in A buffer [20mM Tris-HCl, pH 7.5, 100mM NaCl] by incubation at 80°C for 3 min followed by slow cooling. For the construction of the DNA-RNA hybrid, the synthetic DNA oligonucleotide H was annealed with [α-32P] - labeled X RNA in A buffer as described above.

X1: (AATTCGGGTAGGAGCCACCTTATGAGGAATTCGCCCA)
Y1: (AATTCCTCATAAGGTGGCTCCTACCCGAATTCGCCCA)
H: (TGGGCGAATTCCTCATAAGGTGGCTCCTACCCGAATTCGCCCA)
X RNA: (GGGCGAATTCGGGTAGGAGCCACCTTATGAGGAATTCGCCCA)

DNA construction - Duplex DNA was constructed from oligonucleotide h and a complimentary oligonucleotide. DNA containing a nick was constructed from oligonucleotides x, c and d. 3’-DNA overhang was constructed from oligonucleotides-x and c. DNA-RNA nick and 3’-DNA-RNA overhang were constructed as DNA structures, but oligonucleotide c was replaced by the corresponding oligoribonucleotide. Oligonucleotide x
and h were 5’-labeled and annealed with appropriate oligonucleotides or oligoribonucleotide in A buffer by incubation at 80°C for 3 min followed by slow cooling.

X: (AGTCTAGCAGTTGAGTCCTTGCTAGGACGGATCCCT),
C: (A CTCAACTGCAGTCTAGACT, 5’-phosphorylated)
D: (AGGGATCCGTCCTAGCAAG G).

Gel mobility shift assay - Binding assays were carried out as described previously (20) in high salt buffer [20 mM Tris-HCl, pH 7.5, 200 mM NaCl, 10% glycerol] or in low salt buffer which was the same except 10 mM NaCl. In the case of low salt buffer, electrophoresis was carried out in 22.5 mM Tris-borate, pH 8.6.

Competition assay with non-labeled nucleic acids - Total RNA was prepared by hot phenol extraction of E. coli K-12 C600 cells grown in LB medium to exponential phase. E. coli bulk tRNA was a gift from A. Rak. The supercoiled form of the plasmid pNB1 (Biolabs) was purified on agarose gel and electroeluted. To prepare the linear DNA sample, the same plasmid was linearized with ScaI. HU protein at a final concentration of 13nM was mixed with 2 fmol of [32P]-labeled nicked DNA and varying concentrations of non-labeled nucleic acids were added in 15 µl of high salt buffer. Samples were analyzed as described previously (20).

Determination of dissociation constant - At equilibrium, the dissociation constant of the complex formed by one nucleic acid molecule and one HU dimer is given by the equation:

\[ K_d = \frac{[\text{protein}_{\text{free}}] \times [\text{nucleic acid}_{\text{free}}]}{[\text{nucleic acid}_{\text{bound}}]} \]

where [protein\text{free}] is the concentration of HU not bound to nucleic acid, [nucleic acid\text{free}] is the concentration of the protein binding sites, [nucleic acid\text{bound}] is the concentration of the nucleic acid molecules occupied by the protein and [protein\text{free}] = [protein\text{total}] - [protein\text{bound}] and [protein\text{bound}] = [nucleic acid\text{bound}]. Finally, [nucleic acid\text{bound}] = [nucleic acid\text{total}] \times b / [(f+b) \times (\text{number of protein binding sites on nucleic acid molecule})].
For a DNA, N bp long, the number of binding sites for the ligand which covers L bp there are “N-L+1” binding sites per DNA molecule. The values f and b measured in the experiment are proportional to the concentration of free and bound nucleic acids, respectively, quantified in arbitrary units. The radioactivity of the bands corresponding to free (f) and bound (b) fractions was determined by PhosphorImager analysis of dried gels. Thus, the final equation for calculation of the dissociation constant of the first protein-nucleic acid complex is:

\[ K_{d, \text{non-specific}} = \frac{[\text{protein}_{\text{total}}] \times (f/b) \times (N-L+1) - [\text{nucleic acid}_{\text{total}}] \times (f/(f+b)) \times (N-L+1)}{[\text{protein}_{\text{total}}] \times (f/b) \times (N-L+1) - [\text{nucleic acid}_{\text{total}}] \times (f/(f+b)) \times (N-L+1)} \]  

(1)

Notice that this equation is adequate for non-specific binding when the affinity of the protein is the same for any L bp binding site of N bp long nucleic acid. For a nucleic acid molecule containing one specific binding site, if non-specific interaction is negligible, the equation is:

\[ K_{d, \text{specific}} = \frac{[\text{protein}_{\text{total}}] \times f/b - [\text{nucleic acid}_{\text{total}}] \times f/(f+b)}{[\text{protein}_{\text{total}}] \times f/b - [\text{nucleic acid}_{\text{total}}] \times f/(f+b)} \]

The best fit over several protein concentrations was taken as \( K_d \). Equation (1) serves for evaluation of HU dissociation constant for the first complex. Binding of the second HU dimer might be facilitated by the protein-protein interactions which can be measured by the factor of cooperativity \( \omega \). The factor \( \omega \) is determined as \( K_{d,1} / K_{d,2} \), where \( K_{d,1} \) and \( K_{d,2} \) are the dissociation constants of the first and second complexes, respectively. For binding of HU to double-stranded DNA, RNA and to DNA-RNA hybrid the factor of cooperativity \( \omega \) has been determined as described previously (15, 18).

The apparent dissociation constant of HU to non-labeled nucleic acids was calculated as following:

\[ K_{d,a} = \frac{[P_{\text{free}}] \times [A_{\text{free}}]}{[P_A]} \]

where \([A_{\text{free}}]\) is the concentration of free non-labeled nucleic acid. In assumption that binding is non-specific and the nucleic acid is much longer than a protein binding site, \([A_{\text{free}}]\) is equal to the concentration of base pairs of free nucleic acid. \([P_{\text{free}}]\) is the concentration of the free protein and \([P_A]\) is the concentration of the protein bound to non-labeled nucleic acid.
The protein concentration in the tube \([P_{\text{total}}] = [P_a] + [P_n] + [P_{\text{free}}]\) where \([P_a]\) is the concentration of the protein bound to labeled nicked DNA. Since nicked DNA concentration is much less than that of non-labeled nucleic acid, then \([P_a] = [P_{\text{total}}] - [P_{\text{free}}]\). The concentration of total non-labeled nucleic acid \([A_{\text{total}}] = [A_{\text{free}}] + [A_{\text{bound}}]\). As \([A_{\text{bound}}] = [P_a] \times L\), where \(L\) is the length of the binding site of the protein in bp, then \([A_{\text{free}}] = [A_{\text{total}}] - [P_a] \times L\)

The dissociation constant of HU with nicked DNA “\(K_N = \frac{[P_{\text{free}}]}{f/b}\) where \(f\) and \(b\) are proportional to the concentrations of free and bound nicked DNA, respectively, and the radioactivity of the bands corresponding to free (f) and bound (b) fractions was determined by PhosphorImager analysis of dried gels. Thus, \([P_{\text{free}}] = K_N \times \frac{b}{f}\). Finally,

\[
K_{d,a} = \frac{[P_{\text{free}}] \times [A_{\text{free}}]}{[P_A]} = \frac{[P_{\text{free}}] \times ([A_{\text{total}}] - [P_a] \times L)}{[P_A]} = \frac{[A_{\text{total}}]}{[P_A] - L}.
\]

\[
K_{d,a} = \frac{[P_{\text{free}}] \times ([A_{\text{total}}] - \frac{[P_{\text{free}}]}{K_N \times \frac{b}{f}} \times L)}{([P_{\text{total}}] - [P_{\text{free}}]) - L}, \text{ where } [P_{\text{free}}] = K_N \times \frac{b}{f} \text{ or }
\]

\[
K_{d,a} = (K_N \times \frac{b}{f}) \times \frac{[A_{\text{total}}]}{([P_{\text{total}}] - K_N \times \frac{b}{f} - L)}.
\]

These equations were obtained in assumption that the number of the binding sites is equal to the length of the nucleic acid. This is the case when DNA is far from saturated with the protein. The saturation of the DNA can be evaluated according to Mc Ghee-von Hippel equation (39). We estimated that under experimental conditions used, DNA is far from saturation. Indeed, in our experiments the amount of the competitor added is 100-1000 bp per one HU dimer.
RESULTS

The binding of HU to supercoiled and linear DNA, is similar to its binding to total RNA

and tRNA - HU is one of the most abundant DNA binding proteins in the bacterial cells. In
contrast to its low affinity, salt sensitive binding to duplex linear DNA, HU binds with high
affinity to DNA damage and repair intermediates under high salt conditions (19). We have
also found, recently, that HU specifically recognizes the mRNA of rpoS and stimulates its
translation (20). HU affinity to rpoS mRNA fragment is as high as that to nicked DNA, which
is 1000-fold higher than that for double-stranded DNA. Since it appears that HU is able to
bind DNA and RNA, both nucleic acids may serve as HU binding targets in the cell. This
finding prompted us to further investigate the RNA-binding properties of HU.

We first measured the affinity of HU to the major DNA and RNA species present in
the bacterial cell. HU binding to supercoiled and linear DNA as well as to total cellular RNA
and tRNA was compared. Gel mobility shift assays can not be applied directly to separate free
and bound high molecular weight nucleic acids, because plasmid DNA is too bulky to be gel-
shifted by HU and total RNA too disperse in size to form sharp bands in the gel. To tackle the
problem we applied an assay where one nucleic acid of interest competes with labeled 40-bp
nicked DNA for HU binding. HU forms a single complex with the nicked DNA with an
apparent dissociation constant $K_d$ of 10 nM under stringent salt conditions (16-18). HU was
mixed with labeled nicked DNA and the complex was challenged with increasing amounts of
the non-labeled nucleic acid of interest. The progressive decrease in HU-nicked DNA
complex upon increase in the concentration of non-labeled DNA, or RNA, reflects the
decrease in the concentration of free HU in the solution as a result of binding to the non-
labeled nucleic acid competitor. The comparison of the ratio of complexed and free nicked
oligonucleotides in the absence or presence of the competitor provides the concentration of
HU bound to the non-labeled nucleic acid. Thus, the dissociation constant of the non-labeled
nucleic acid can be calculated on the basis of the known parameters of the HU-nicked DNA interaction as explained in Experimental Procedures.

As a control of this experimental approach, the same nicked DNA was used as a competitor of the labeled nicked DNA (Fig 1A). The apparent dissociation constant of HU to nicked DNA found in this experiment is 12 nM, a value very close to the $K_d$ of 10 nM derived from the classical protein titration experiment (16-18). Using this methodology, we successively measured the dissociation constants for linear and supercoiled DNAs, then to tRNA and total RNA (Fig.1A). Based on the competition experiment, the apparent dissociation constant of HU to supercoiled DNA was estimated to be 450 nM under stringent conditions, (200 mM NaCl) (Fig. 1A). Supercoiled plasmid was used as an example of genomic DNA. The plasmid after linearization was used to estimate the affinity of HU for linear DNA, a value of $K_d=1300$ nM was obtained under the same high salt conditions. This apparent dissociation constant accounts for the cooperativity of HU binding and dissociation/association of HU monomers. Thus, HU protein binds supercoiled DNA three times as strongly as linear DNA of the same length. This is in agreement with previous studies with HU chemical nuclease, which cleaves supercoiled DNA 2.5 times faster than relaxed DNA (40). To investigate if RNA may serve as a potential intracellular HU target, we isolated total bacterial RNA and estimated the affinity of HU by the same technique (Fig. 1A). The apparent dissociation constant of 2500 nM was calculated as described in the Materials and Methods (Fig. 1). Likewise, HU’s affinity to bulk $E. coli$ tRNA was estimated to be 2200 nM. Thus, HU binds the major species of nucleic acids present in bacterial cells with a similar affinity.

Finally the graph of bound nicked DNA versus the concentration of the different competitors, (Fig.1B), shows that the concentration of the nucleic acids species required to reduce the bound nicked DNA correspond well with their $K_d$ (Fig. 1B).
HU binding to linear dsDNA, dsRNA and to DNA-RNA hybrids - Double-stranded RNA and DNA as well as a DNA-RNA hybrid of 40 bp, all of the same sequence, were constructed. Their binding to HU was studied using now the classical gel mobility shift assay. Figure 2 shows first that the gel mobilities of the duplexes of the three different types of nucleic acids (dsDNA, dsRNA and DNA-RNA) are rather different, probably reflecting the different conformation of the three nucleic acids. These conformations seem to be sensitive to the salt conditions. At high salt, the hybrid DNA-RNA migrates the fastest while in low salt, dsDNA is faster. Under both sets of conditions, duplex RNA is the most retarded matrix. In terms of their binding to HU, under high salt conditions (200mM NaCl) no complex was detectable with any duplex studied (Fig. 2A). This was expected for dsDNA, since under high salt conditions only a smear is visible due to the dissociation of non-specific HU-linear DNA complexes during their migration in the polyacrylamide gel (19). In contrast, a defined complex could be isolated under low salt conditions with dsDNA having an apparent dissociation constant $K_d$ of 450 nM for the first complex and a cooperativity of $\omega = K_{d1}/K_{d2} = 30$. One HU dimer occupies 9 bp, so that four HU complexes are formed with the 40-bp dsDNA (41 and Fig. 2B). Figure 2B also shows that four HU dimers can be similarly accommodated by 40-bp dsRNA and by the hybrid DNA-RNA. Thus, each HU dimer would cover 9-10 bp of dsRNA, in a manner similar to the HU binding on dsDNA. The apparent dissociation constant of the first HU-dsRNA complex is 1800 nM. It is 4-fold higher than for dsDNA, but the cooperativity of binding is 2.5 times higher, $\omega=70$. Dissociation constants for the second complexes $K_{d2}$ for dsDNA and RNA are 15 and 25 nM, respectively. The opposing differences in the dissociation constants for the first complexes and cooperativity suggest in fact that HU binds 1.6-fold stronger dsDNA than dsRNA. This result is in agreement with the ratio of affinities obtained in our competition studies performed under stringent salt conditions, which shown that HU binds linear DNA twice as strongly as total RNA (Fig.1). A 40-bp DNA-RNA hybrid of the same sequence was also checked for HU.
binding. Formation of four complexes with the 40 bp hybrid (and three complexes with 30 bp hybrid, data not shown) corresponds also to the binding model of one HU dimer to every 9-10 bp. The apparent dissociation constant of the first complex $K_d=450$ nM is the same as for dsDNA, the cooperativity being slightly higher, $\omega=40$.

Thus, HU is able to bind dsDNA, dsRNA and DNA-RNA hybrids with very similar affinities. The conformations of B-DNA and A-RNA are known to differ significantly, whilst DNA-RNA hybrids were shown to assume an intermediate A/B conformation (42). Nevertheless, HU seems to recognize both the A and B conformations of RNA and DNA duplexes. This wide spectrum of binding can be explained by the high flexibility of HU arms (42).

*Specific interaction of HU with DNA-RNA hybrid structures* - HU does not recognize any particular DNA sequence, but it binds, under stringent conditions, with high affinity to some altered DNA structures. The simplest DNA structure that HU binds strongly even in the presence of excess of dsDNA competitor, is either a DNA containing a single-stranded break (nicked DNA) or DNA 3’-overhang (19). The specificity of HU DNA binding was explained by the simultaneous interaction of the HU arms with the 5’double-stranded part of the molecule and the interaction of HU body with the flexible 3’ branch which can be either a double-stranded or a single-stranded DNA (19). Since we have seen that under low salt conditions HU binds an RNA-DNA hybrid with a similar affinity to which it binds DNA duplex (Fig.2), we then asked whether HU could also specifically recognize both nick and 3’-overhang structures where one of the DNA strands is replaced with RNA. Both structures are of particular interest being DNA replication intermediates (43). The DNA–RNA 3’-overhang is in addition involved in the priming repair of the double-stranded breaks and priA replication (44). Figure 3 shows that HU binds these structures under stringent conditions and forms, with each, a single complex. The apparent dissociation constants $K_d$ are respectively 10 nM and 16 nM for nicked RNA and 3’-overhang RNA, identical to the values found for
nicked DNA and DNA 3’-overhang and 100-times stronger than that for double-stranded nucleic acids. Thus, this binding is structure-specific.

Specific binding motif of HU to RNA - We have demonstrated recently that HU is able to bind \textit{rpoS} mRNA with a similar affinity to which it binds nicked DNA, one of its specific substrates (20). The folding of this RNA is not yet characterized in detail. Therefore, another RNA target with simple and well-characterized secondary structure was necessary to better understand the HU-RNA interaction. We investigated if HU could bind to the regulatory DsrA RNA of \textit{E. coli} which has both positive and negative action on global transcriptional regulators such as H-NS or RpoS, the σ factor controlling the nutrient starvation and stress responses (34, 35). DsrA was cloned under the control of T7 promoter, \textit{in vitro} transcribed and used for gel mobility shift assay with HU protein. The structure of this small RNA, which consists of three stem-loops (34, 38), is presented in Figure 4A. The configuration shown is derived from data based on ds or ss- specific nuclease susceptibility and phylogenetic data (38). Figure 4B shows that HU binds this DsrA RNA and forms three specific complexes under high salt conditions with an apparent dissociation constant of 20 nM for the first complex. To find the structural motifs recognized by HU within this small RNA, we truncated DsrA RNA from its 3’-end to two stem-loops connected by a single-stranded RNA of 12 nt. HU forms two complexes with this RNA, RNA-C, containing the stem-loops 1 and 2 (Fig. 4C). Further truncation results in the structure RNA-D, containing only one stem-loop connected to a ssRNA of 12 nt. This structure was still recognized by HU, forming one complex (Fig. 4D). As shown at the bottom of Figure 4, RNA-C can be considered as the superposition of two putative HU binding motifs, one RNA 3’ overhang and one 5’-overhang, while RNA-D has only the RNA 3’-overhang. We have recently shown that the corresponding DNA structure, the DNA 3’-overhang is HU-specific, in contrast to the 5’DNA overhang, a much less powerful target for HU (19). The fact that the RNA 3’overhang (RNA-D) as well as the DNA-RNA 3’-overhang (Fig. 3) were recognized with a similar high affinity to the DNA
3’overhang (Fig. 3), indicates that HU recognizes the same motif in both RNA and DNA molecules. To investigate the possibility that HU also specifically binds to RNA 5’overhangs, RNA-E, an RNA 5’- overhang was constructed by truncation of RNA-C from its 5’-end. Surprisingly, HU binds this structure with the formation of one complex as well as it binds the RNA 3’- overhang (Fig. 4-E). Finally, truncation of RNA-D from its 3’-end that leaves only the first stem-loop (RNA-F) abolishes HU binding. Thus there is one difference between the binding of HU to DNA and RNA. In contrast to its binding to DNA, HU recognizes RNA 3’ as well as 5’-overhangs.

Since HU has high affinity for RNA 5’-overhangs in contrast to the corresponding DNA structure, we reconsidered the DNA-RNA hybrid analysis shown in Figure 3 and compared the binding of HU to a DNA-RNA-5’overhang to that of the DNA 5’-overhang. Using this approach, we confirmed that HU binds poorly to the DNA- 5’overhang (19) whereas it binds strongly to the DNA-RNA- 5’overhang (Fig. 5). Under high salt conditions HU forms one complex with the DNA–RNA 5’-overhang with an apparent dissociation constant $K_d$ of 16 nM. It is interesting to note that this DNA- 5’overhang structure is involved in DNA replication, as is its DNA-RNA 3’ -overhang counterpart.
DISCUSSION

We report here that the DNA binding protein HU is also an RNA-binding protein. First, we found that under physiological conditions HU affinity to total bacterial RNA is only 4.5-fold lower than that for supercoiled DNA which is representative of the bacterial chromosome DNA. Based upon these values and those for the DNA and RNA content of the bacterial cell, we suggest that the distribution of HU between DNA and RNA should be roughly two to one. In addition, using gel shift retardation assays which directly measure the affinity constants, we could show that the characteristics and patterns of HU binding to short DNA or RNA duplexes, as well as to DNA-RNA hybrids, are very similar with one dimer packed every 9 to 11 bp. This indicates that HU is able to adapt both the A and B conformations of nucleic acids, probably due to the high flexibility of its arms which have been shown to contact the minor groove of DNA (19, 31, 32). However a first difference is observed in the binding of HU to dsDNA and dsRNA, which has a higher cooperativity value than was found for HU binding to dsRNA (Fig. 2B). This probably reflects a more advantageous orientation of tandemly bound HU molecules along the A-helix for dimer-dimer interactions. It is also noteworthy that HU exhibits in its binding to DNA-RNA hybrids more resemblance to dsDNA than is seen for dsRNA, even though the DNA-RNA heteroduplex assumes the intermediate A/B conformation (42). Interestingly, the Zα domain of dsRNA adenosine deaminase (ADAR1) was demonstrated to bind specifically left-handed Z-RNA as well as Z-DNA and to stabilize the Z-conformation (45). The helix-turn-helix motif is used by Zα for conformation specific interaction with Z-DNA (46). Probably, Zα is able to bind to both nucleic acids since Z-DNA and Z-RNA adopt similar Z-conformations. It remains to be elucidated if HU with its rather different DNA-binding motif could possess a Z-conformation binding activity.

HU is well characterized as a protein which binds to DNA with low affinity and without sequence specificity but which recognizes on the other hand, with high specificity,
DNA structures such as nicked DNA or 3’ overhangs. Since HU binds DNA-RNA hybrids as strongly as it binds dsDNA it was of interest to see if it also interacts with nicked and 3’ overhang DNA-RNA hybrids. For this, we compared the binding of HU to short DNA and RNA duplexes and to DNA-RNA hybrids. Effectively HU does bind such DNA-RNA structures, under stringent conditions with an affinity similar to that for related DNA substrates. Interestingly these hybrid structures, the nicked RNA and the overhang RNA, appear as intermediates of DNA replication via the Okazaki fragments where the short RNA primers hybridize to the lagging DNA strand. Furthermore, the 3’ DNA-RNA overhang is also involved in priA replication and in priming repair of DNA ds breaks (44). Since the HU concentration in \textit{E. coli} was estimated to be around 30 000 copies/cell which corresponds roughly to 60µM, the concentration of free HU is therefore well above its dissociation constants ($K_d$) for these structures which are in the low nanomolar range. This suggests that this specific binding could occur \textit{in vivo}. This finding could explain the role of HU in DNA replication and repair (8, 9).

Although HU is the only bacterial protein which clearly exhibits a dual specific DNA and RNA binding activity, such proteins have been characterized in eukaryotes. Whereas most such proteins possess separate DNA and RNA-binding domains, some of them contain multifunctional domains. Although the RNA recognition motifs (RRMs) are commonly found in RNA and single-strand binding proteins, at least two transcription factors use an RRM for their preferential binding to double-stranded DNA (28, 29) and RRM of murine IPEB protein recognizes both ds-DNA and pre-mRNA (30). The Cys$_2$-His$_2$ zinc fingers represent the canonical DNA-binding motif which is able to interact specifically with RNA. The zinc finger domains of TFIIIA, PEP, MOK2 and WT-1 proteins mediate specific binding to both RNA and dsDNA (22-26). One of the best studied is TFIIIA transcription factor which contains zinc finger modules specialized for either DNA or RNA recognition. Some of these modules provide the majority of the protein binding affinity for DNA, while the others form specific
complexes with their RNA target (47-49). Thus, zinc fingers differ somewhat in their ability to bind tightly to DNA and RNA.

The HU protein provides an extension to this bifunctional DNA and RNA binding module. The structure of HU consists solely of the concave, positively charged surface made up of β-ribbon arms and α-helical hydrophobic core (31-33). Hence, HU RNA and DNA-binding domains should be equivalent. HU, thus, should bind dsRNA in a similar way as it binds dsDNA, namely, through its β-arms. Although DNA and RNA-binding domains are generally distinct, ribosomal protein S7, a primary 16S rRNA binding protein, possesses striking structural similarity with the HU family of DNA-binding proteins (50, 51). Both have a very similar β-hairpin architecture grasping the double-stranded nucleic acid and a pair of S7 α-helices which superimpose quite well onto those of the HU monomer. The structure of the β-hairpin bound to 16S rRNA via the minor groove (52) allows us to suggest the possibility that the HU arms contact dsRNA in a similar way. It has also been shown that TFIID, the TATA-box binding protein, shares homologies with IHF and HU (53). The highly symmetric structure of TFIID contains a DNA binding module resembling a molecular saddle that sits astride the DNA (54) very similar to the saddle formed by the flexible arms and the platform of the dimeric HU (31-32). It will be interesting to see if TFIID could also, as HU, interact with RNA or DNA-RNA hybrids.

We have demonstrated here that HU binds strongly to DsrA RNA, the secondary structure of which has been characterized as three stem-loops (34, 38). To localize the structural determinants of HU binding, we analyzed HU interaction with truncated DsrA RNA fragments. Two HU dimers could be bound onto two stem-loops connected together by a 12 nt ssRNA linker and one HU dimer was bound to one stem-loop with 12 nt ssRNA from 3’ end. These RNAs can be considered as the superposition of two RNA overhang motifs and a single RNA 3’-overhang, respectively. We have shown that relative DNA structure, that is, a DNA 3’-overhang, is HU-specific. It seems plausible that HU recognizes the same motif in
RNA molecules. We proposed recently a model for specific binding of HU to DNA 3’-overhangs (19). The specificity of binding was explained by the simultaneous interaction of HU flexible arms with 3’-dsDNA branch in the region of the discontinuous point and of HU body with the second ssDNA branch. Our finding that HU binds double-stranded DNA and RNA with the similar affinity as well as the capability of HU to bind analogous RNA structures allows us to propose a similar model for the interaction of HU with RNA 3’-overhangs. The interaction of HU arms with the double-stranded part of RNA and the interaction of HU body with single-stranded part of the molecule renders the binding specific and high-salt resistant. Interaction of HU arms with DNA minor groove prevents HU binding to DNA 5’-overhangs, as the 5’-ss branch must cross the negatively charged double helix to reach the HU body (19). Since HU is capable of forming complexes with both RNA 3’ and 5’-overhangs, we conclude that HU is able to interact not only with the minor groove as it does for DNA, but also with narrowed RNA major groove. Binding of both minor and major RNA grooves is an unusual feature among nucleic acid-binding proteins. We demonstrated that HU binds to DNA-RNA 5’-overhangs. This specific interaction is possible due to the intermediate A/B conformation of DNA-RNA hybrid of ds-part of the structure, which is more close to A-form RNA helix.

HBsu protein, the HU in *Bacillus subtilis*, has been identified as a component of SRP-like particle that interacts specifically with *Alu* domain of small cytoplasmic RNA (scRNA) (20). The *Alu* domain of scRNA is composed of two stem-loops followed by extended double-stranded region. It appears likely that the three-way junction is an element required for HBsu binding to *Alu* domain. We have shown that HU has a high affinity to the corresponding DNA structure, namely, the three-way DNA-junction (19). This is further evidence that HU applies the same strategy for structure-specific recognition with its RNA and DNA targets and the universal DNA/RNA motif for HU binding consists of two double-stranded or double-stranded and single-stranded modules apt to be inclined. RNA adopts many complex tertiary
structures and it is conceivable that HU also recognizes this rather widespread structural motif within a great number of RNAs. One example of such an RNA is DsrA, a member of the sRNA (small RNAs) family, considered as mediators of cellular processes in bacteria. DsrA was first characterized as an untranslated small RNA which, when overproduced, activates transcription of RcsA by counteracting H-NS silencing (34). RcsA is a positive transcription regulator of \( cps \) genes necessary for capsid synthesis. It is interesting to recall that overproduction of HU also stimulates RcsA synthesis (55). In addition, HU like DsrA stimulates RpoS translation (20, 35-38). By binding strongly to the mRNA encoding RpoS (20) and to DsrA (this work), HU could regulate major programs in the bacterial cell, including the starvation phase, the stress response and the bacterial envelope synthesis.
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REFERENCES

1. Rouviere-Yaniv, J. and Gros, F. (1975) Proc. Natl Acad. Sci. USA, 72, 3428-3432
2. Rouviere-Yaniv, J. (1978) Cold Spring Harbor Symp Quant Biol 42, 439-447
3. Wery, M., Woldringh, C. and Rouviere-Yaniv, J. (2001) Biochimie 83, 193-20012.
4. Rouviere-Yaniv, J., Yaniv, M. and Germond, J.E. (1979) Cell 17, 265-274
5. Haselkorn, R. & Rouviere-Yaniv, J. (1976) Proc. Natl. Acad. Sci. USA 73, 1917-1920
6. Grove, A., Galeone , A., Maryol, L. and Geiduschek, E. P. (1996) J Mol. Biol. 260, 196-206
7. Oberto, J. and Rouviere-Yaniv, J. (1996) J. Bacteriol. 178, 293-297
8. Bramhill, D. and Kornberg, A. (1988) Cell 54, 915-918
9. Boubrik, F. and Rouviere-Yaniv, J. (1995) Proc. Natl Acad. Sci. USA 92, 3958-3962
10. Li, S. and Waters, R. (1998) J. Bact. 180, 3750-3756
11. Lavoie, B. D., Shaw, G. S., Millner, A. and Chaconas, G. (1996) Cell 85, 761-771
12. Aki, T. and Adhya, S. (1997) EMBO J. 16, 3666-3674
13. Claret, L. and Rouviere-Yaniv, J. (1997) J. Mol. Biol. 273, 93-104
14. Pontiggia, A., Negri, A., Beltrame, M. and Bianchi, M.E. (1993) Mol. Microbiol. 7, 343-350
15. Bonnefoy, E., Takahashi, M. and Rouviere-Yaniv, J. (1994) J. Mol. Biol. 242, 116-129
16. Castaing, B., Zelwer, C., Laval, J. and Boiteux, S. (1995) J. Biol. Chem. 270, 10291-10296
17. Kamashev, D., Balandina, A. and Rouviere-Yaniv, J. (1999) EMBO J. 18, 5434-5444
18. Pinson, V., Takahashi, M. and Rouviere-Yaniv, J. (1999) J. Mol. Biol. 287, 485-497
19. Kamashev, D. and Rouviere-Yaniv, J. (2000) EMBO J. 19, 6527-6535
20. Balandina, A., Claret, L., Hengge-Aronis, R. and Rouviere-Yaniv, J. (2001) Mol. Microbiol. 39, 1069-1079
21. Nakamura, K., Yahagi, S., Yamazaki, T. and Yamane, K. (1999) J. Biol. Chem. 274, 13569-13576
22. Engelke, D. R., Ng, S. Y., Shastry, B. S. and Roeder, R. G. (1980) Cell 19, 717-728
23. Pelham, H. R. and Brown, D.D. (1980) Proc. Natl Acad. Sci. USA 77, 4170-4174
24. Honda, B. M. and Roeder, R. G. (1980) Cell 22, 119-126
25. Caricasole, A., Duarte, A., Larsson, S. H., Hastie, N. D., Little, M., Holmes, G., Todorov, I. and Ward, A. (1996) Proc. Natl Acad. Sci. USA 93, 7562-7566
26. Arranz, V., Harper, F., Florentin, Y., Puvion, E., Kress, M. and Ernoult-Lange, M. (1997) Mol. Cell. Biol. 17, 2116-2126
27. Hamann, S. and Stratling, W. (1998) Nucleic Acids Res. 26, 4108-4115
28. DeAngelo, D.J., DeFalco, J., Rybacki, L. and Childs, G. (1995) Mol. Cel. Biol. 15, 1254-1264
29. Basu, A., Dong, B., Krainer, A.R. and Howe, C.C. (1997) Mol. Cel. Biol. 17, 677-686
30. Newberry, E.P., Latifi, T. and Towler, D.A. (1999) Biochemistry 38, 10678-10690
31. Tanaka, I., Appelt, K., Dijk, J., White, S.W. and Wilson, K.S. (1984) Nature 310, 376-381
32. White, S.W., Wilson, K.S., Appelt, K. and Tanaka, I. (1999) Acta Crystallogr. D 55, 801-809
33. Vis, H., Mariani, M., Vorgias, C.E., Wilson, K.S., Kaptein, R. and Boelens, R. (1995) J. Mol. Biol. 254, 692-703
34. Sledjeski, D. and Gottesman, S. (1995) Proc. Natl Acad. Sci. USA 92, 2003-2007
35. Sledjeski, D., Gupta, A. and Gottesman, S. (1996) EMBO J. 15, 3993-4000
36. Majdalani, N., Cunning, C., Sledjeski, D., Elliott, T and Gottesman, S. (1998) Proc. Natl Acad. Sci. USA 95, 12462-12467
37. Lease, R. A., Cusick, M. E. and Belfort, M. (1998) Proc. Natl Acad. Sci. USA 95, 12456-12461
38. Lease, R. A. and Belfort, M. (2000) Proc. Natl Acad. Sci. USA 97, 9919-9924
39. McGhee, J. D. and von Hippel, P. H. (1974) *J. Mol. Biol.* **86**, 469-89.
40. Kobryn, K., Lavoie, B. and Chaconas, G. (1999) *J. Mol. Biol.* **289**, 777-78
41. Bonnefoy, E. and Rouviere-Yaniv, J. (1991) *EMBO J.* **10**, 687-696
42. Salazar, M., Federoff, O.Y., Miller, J. M., Ribeiro, S. N. and Reid, B. R. (1993) *Biochemistry* **32**, 4207-4215
43. Kornberg, A. and Baker, T. (1992) in *DNA Replication*, eds. Freeman, W. (New York), pp. 475-491
44. Kuzminov, A. (1999) *Microbiol. Mol. Biol. Rev.* **63**, 751-813
45. Brown, B. A., Lowenhaupt, K., Wilbert, C., Hanlon, E. B. and Rich, A. (2000) *Proc. Natl Acad. Sci. USA* **97**, 13532-13536
46. Schwartz, T., Rould, M.A., Lowenhaupt, K., Herbert, A. & Rich, A. (1999) *Science* **284**, 1841-1845
47. Theunissen, O., Rudt, F., Guddat, U., Mentzel, H. and Pieler, T. (1992) *Cell* **71**, 679-690
48. Clemens, K. R., Wolf, V., McBryant, S. J., Zhang, P., Liao, X., Wright, P. E., Gottesfeld, J. M. (1993) *Science* **260**, 530-533
49. Searles, M. A., Lu, D., Klug, A. (2000) *J. Mol. Biol.* **301**, 47-60
50. Wimberly, B. T., White, S. W. and Ramakrishnan, V. (1997) *Structure* **5**, 1187-1198
51. Hosaka, H., Nakagawa, A., Tanaka, I., Harada, N., Sano, K., Kimura, M., Yao, M. and Wakatsuki, S. (1997) *Structure* **5**, 1199-1208
52. Schluenzen, F., Tocilj, A., Zarivach, R., Harms, J., Gluehmann, M., Janell, D., Bashan, A., Bartels, H., Agmon, I., Franceschi, F. and Yonath, A. (2000) *Cell* **102**, 615-623
53. Nash, H.A., Granston, A.E. (1991) *Cell* **67**, 1037-1038
54. Nikolov, D.B., Hu, S.H., Lin, J., Gasch, A., Hoffmann, A., Horikoshi, M., Chua, N.H., Roeder, R.G. and Burley, S.K. (1992) *Nature* **360**, 40-46
55. Painbeni, E., Mouray, E., Gottesman, S. and Rouviere-Yaniv, J. (1993) *J Mol. Biol.* **34**, 1021-1037
LEGENDS TO FIGURES

Fig. 1. Comparative affinity of HU protein to linear DNA, supercoiled DNA, total cellular RNA and tRNA. (A) HU protein at a final concentration of 13nM was mixed with 2 fmol of [32P]-labeled nicked DNA and non-labeled linear DNA, supercoiled DNA, total cellular RNA or tRNA were added at concentrations indicated in _M. The complexes were analyzed in high salt conditions (200mM NaCl). The pNB1 plasmid (Biolabs) was used as supercoiled DNA, linearized pNB1 plasmid as linear DNA and total cellular RNA was obtained by hot phenol extraction (see Experimental Procedures). (B) Graph of bound nicked DNA versus concentration of the competitors: total RNA.

Fig. 2. HU binding to DNA, RNA and DNA-RNA duplexes. HU protein at the concentrations indicated in nM was mixed with 5 fmol of labeled DNA and RNA duplexes of 40 bp and DNA-RNA duplex of 30 bp (A) and 40 bp (B). In A, the reaction was performed in stringent conditions (high salt), in B, in low salt conditions and analyzed on the PAAG.

Fig. 3. HU binds specifically to DNA replication intermediates. HU protein at concentrations indicated in nM was mixed with 2 fmol of labeled nicked DNA and nicked DNA-RNA (A), with labeled DNA 3’-overhang and DNA-RNA 3’-overhang (B).

Fig. 4. HU binding to DsrA RNA: HU binding motifs. (A) Secondary structure of DsrA RNA as proposed by (38). (B-F) Gel mobility shift assay of HU binding to DsrA RNA and truncated DsrA. The [32P]-labeled RNAs were incubated with increasing concentrations of HU in high salt buffer. Protein concentrations (nM) as well as putative binding motifs are indicated below. (B) DsrA RNA (C, D, E and F) truncated RNAs.

Figure 5. HU binding to DNA-RNA 5’-overhang. HU protein at concentrations indicated in nM was mixed with 2 fmol of labeled DNA 5’-overhang or with DNA-RNA 5’-overhang, and analyzed on the PAAG.
Figure 1

A

|          | linear DNA         | sc DNA           |
|----------|--------------------|------------------|
| Concentration, µM | [Image] | [Image] |
| 0        | ![Image]           | ![Image]         |
| 0.13     | ![Image]           | ![Image]         |
| 0.25     | ![Image]           | ![Image]         |
| 0.5      | ![Image]           | ![Image]         |
| 1        | ![Image]           | ![Image]         |
| 2        | ![Image]           | ![Image]         |
| 2.5      | ![Image]           | ![Image]         |
| 3        | ![Image]           | ![Image]         |
| 3.8      | ![Image]           | ![Image]         |
| 5        | ![Image]           | ![Image]         |
| 8        | ![Image]           | ![Image]         |
| 10       | ![Image]           | ![Image]         |

|          | tRNA              | total RNA        |
|----------|-------------------|------------------|
| Concentration, µM | [Image] | [Image] |
| 0        | ![Image]           | ![Image]         |
| 0.13     | ![Image]           | ![Image]         |
| 0.25     | ![Image]           | ![Image]         |
| 0.5      | ![Image]           | ![Image]         |
| 1        | ![Image]           | ![Image]         |
| 2        | ![Image]           | ![Image]         |
| 2.5      | ![Image]           | ![Image]         |
| 3        | ![Image]           | ![Image]         |
| 3.8      | ![Image]           | ![Image]         |
| 5        | ![Image]           | ![Image]         |
| 10       | ![Image]           | ![Image]         |

|          | nicked DNA        |
|----------|-------------------|
| Concentration, nM | [Image] |
| 0        | ![Image]         |
| 0.16     | ![Image]         |
| 0.40     | ![Image]         |
| 1.00     | ![Image]         |
| 2.50     | ![Image]         |
| 5.00     | ![Image]         |
| 10.00    | ![Image]         |

B

% of bound nicked DNA vs. Concentration of competitor, µM

- ▲ tRNA
- □ total RNA
- ○ supercoiled
- △ linear DNA

Figure 1
A  high salt conditions

B  low salt conditions

Figure 2
Figure 3
Figure 4
Figure 4_1
Figure 5
The bacterial histone-like protein HU specifically recognizes similar structures in all nucleic acids: DNA, RNA and their hybrids
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