Conformational transition of DNA bound to Hfq probed by infrared spectroscopy

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Received 5th July 2010, Accepted 11th October 2010
DOI: 10.1039/c0cp01084g

Hfq is a bacterial protein involved in RNA metabolism. Besides this, Hfq’s role in DNA restructuring has also been suggested. Since this mechanism remains unclear, we examined the DNA conformation upon Hfq binding by combining vibrational spectroscopy and neutron scattering. Our analysis reveals that Hfq, which preferentially interacts with deoxyadenosine rich sequences, induces partial opening of dA–dT sequences accompanied by sugar repuckering of the dA strand and hence results in a heteronomous A/B duplex. Sugar repuckering is probably correlated with a global dehydration of the complex. By taking into account Hfq’s preferential binding to A-tracts, which are commonly found in promoters, potential biological implications of Hfq binding to DNA are discussed.

Introduction

Hfq is a highly abundant and phylogenetically conserved bacterial protein. It was discovered as a factor required for the activity of bacteriophage RNA polymerase1 and has been proved later to be a major coordinator of bacterial metabolism. The pleiotropic effects of Hfq are illustrated by the fact that disruption of the Escherichia coli hfq gene affects cell viability in numerous ways, including higher osmo-sensitivity, higher sensitivity to oxidants and UV irradiation and decreased growth rates. These deleterious phenotypes are mainly linked to the role of Hfq in modifying the gene expression of bacteria when subjected to environmental stresses by using small non-coding RNA (sRNA). For instance, the most abundant outer membrane porin OmpA expression is regulated by sRNA: 2,3

As in Sm proteins, Hfq N-terminal region adopts an OB-like fold consisting of a five strongly-bent antiparallel β-sheet capped by an α-helix; individual strongly bent β-sheets of each monomer interact to drive the assembly of a toroidal hexameric structure. But in addition to this Sm N-terminal domain (about 65 amino acid residues), the protein also features a C-terminal domain of about 35 amino acid residues that appears mainly non-structured.4 Even if the function of the C-terminal domain is not completely understood, it is now established that the N-terminal Sm domain binds RNA in the absence of the C-terminal domain11,12 and that uridine-rich sequences are bound to the “proximal” face of the Sm tetra, while the poly(A) sequences bind to the “distal” face of Hfq.13

Investigations about Hfq cellular localization with respect to its influence on multiple phenotypic traits have shown that Hfq binds DNA in the cell;14 indeed about 20% of Hfq present in the cell is associated to DNA.15,16 For this reason, it has been suggested that Hfq could be a component of the nucleoid, jointly with a dozen of DNA-binding proteins that enable DNA to fit within the bacteria.15,17,18 While several nucleoid-proteins, such as H-NS (histone-like nucleoid structuring protein), HU (heat-unstable nucleoid protein), IHF (integration host factor), StpA (suppressor of td” phenotype A) and Dps (DNA-binding protein from starved cells) are uniformly distributed within the nucleoid, Hfq seems to be irregularly positioned.19 Furthermore, its association to the nucleoid was suggested to be dependent on the growth phase.17 Motivated by these properties of Hfq, we investigated the mechanisms behind its interaction with DNA. Fourier Transform Infrared Spectroscopy (FTIR) and small angle
neutron scattering measurements give us details on the DNA conformation changes induced by Hfq binding. These results enable us to propose a new mechanism underlying the possible role played by Hfq in vivo, when it is bound to DNA.

**Results**

**Hfq preferentially binds single stranded A-rich DNA sequence**

Although the main function of Hfq is to control several aspects of RNA metabolism, Hfq binds DNA in vitro with a significant affinity: equilibrium dissociation constant ($K_d$) of Hfq-DNA complexes measured by fluorescence anisotropy experiments are $180 \pm 22 \text{ nM}$ for dA$_{20}$ (25 ± 7 nM for rA$_{20}$) and $250 \pm 14 \text{ nM}$ for dA$_{20}$-dT$_{20}$ duplexes (Fig. 1). The latter value is in accordance with that previously reported for dsDNA. While no significant $K_d$ can be measured by fluorescence anisotropy for dC$_{20}$ and dC$_{20}$-dG$_{20}$ duplex (not shown), a weak affinity is measured for single stranded dT$_{20}$ ($K_d = 2.5 \pm 0.5 \mu M$) and for a shorter dA$_7$ oligonucleotide ($K_d = 4.8 \pm 0.8 \mu M$). This latter value is close to the $K_d$ of 9.5 ± 2.8 μM measured by Link et al. for dA$_{8}$. Interestingly, the overall capacity of Hfq to efficiently bind to dsDNA was confirmed by transmission electron microscopy imaging, showing at the same time that its strong preference for A-rich sequences does not prevent it from binding to any DNA sequence, up to totally covering a plasmid in vitro (Fig. 2). However, its low affinity for dT sequences suggests that Hfq could bind double stranded deoxyribonucleic A-rich sequences rather on the A-rich strand, possibly by opening the double stranded dA–dT DNA. In order to test this hypothesis, we characterized the dA–dT:Hfq complex by FTIR spectroscopy.

**FTIR vibrational spectroscopy**

**Binding of Hfq on single stranded dA$_7$ and rA$_7$.** Fig. 3(left) presents FTIR spectra recorded in D$_2$O solutions in the region containing the in-plane base vibrations of dA$_7$ (solid line (a)) and rA$_7$ (solid line (b)) as well as the calculated spectra of dA$_7$ and rA$_7$ in the complexes with Hfq (dotted lines) obtained by subtraction of the Hfq spectrum from those of the complexes. The complexes were prepared with a DNA/protein ratio of 7 bases per Hfq hexamer. The spectrum of free dA$_7$ shows a strong absorption at 1626 cm$^{-1}$, assigned to a ND$_2$ bending vibration (the hydrogen atoms of the adenine NH$_2$ group are exchanged by deuterium) coupled to a ring vibration of the adenine. The corresponding adenine absorption band is shifted to lower wavenumbers (respectively by −7 and −3 cm$^{-1}$). This shift reflects an interaction of Hfq with both the DNA and the RNA sequences containing adenines at the N$_6$ amino-group of the bases, as it was observed in the crystal structure.13

Sugar conformations: evidence of structural changes. FTIR spectroscopy allowed us to characterize the sugar conformations by using absorption bands located in the 950–800 cm$^{-1}$ region that is sensitive to their geometries. Absorption observed around 840 cm$^{-1}$ is indicative of S-type sugars (mainly C2′-endo geometry with C1′-exo and C3′-exo, which are indistinguishable in solution because of thermal agitation) while a band around 867 cm$^{-1}$ reflects the existence of N-type sugars (C3′-endo and C2′-exo geometry).21 As expected, in this frequency domain the spectra of both free and complexed rA$_7$ present an absorption band at 873 cm$^{-1}$ indicative of N-type sugars (Fig. 3(right), (d)). Note that our results in solution differ from that observed in the crystal structure, where sugars are frozen in a S-type conformation.13
residue. However, by taking into account the contact between the N6 of speculative to formally attribute this vibration to a specific amino acid /C0 thymine band around 770 cm
eq 1 et al. adenine and the O left panel is likely due to a change in the environment of an amino acid side chain, probably the C
c
dA 20–dT 20 in the complex with Hfq obtained by subtracting the absorption at 841 cm
c
eq 1 (spectra recorded in D2O solution). The two insets show contribution of the protein from the spectrum of the complex (dotted line), ''absorbance'' is a ''/C0 complex (dotted line), ''absorbance'' resulting from the subtraction process. Note that the peak around 1600 cm
eq 1 in left panel is likely due to a change in the environment of an amino acid side chain, probably the C==O vibration of a Gln.36,39 It would be speculative to formally attribute this vibration to a specific amino acid residue. However, by taking into account the contact between the N6 of adenine and the Oe of Gln52 evidenced by the crystal structure of Link et al.,13 we propose that this band could correspond to the C==O vibration of Gln52.

In contrast, the spectrum of the single strand with deoxyribose sugars, the free dA7, presents a unique absorption at 841 cm
eq 1 indicative of a S-type conformation (Fig. 3(right), (c) solid line). However, in the spectrum of the same dA7 oligonucleotide in the presence of Hfq (Fig. 3(right), (c) dotted line) we observe the existence of two bands, one located at 838 cm
eq 1 and the second one at 867 cm
eq 1, which indicates that part of the sugars have undergone an S- to N-type repuckering. Thus, dA7 in the presence of Hfq adopts at least partially a N-type geometry similar to that found in oligoribonucleotides.

Formation of the complex results in a partial opening of the dA20–dT20 duplex. Fig. 4(bottom) represents the spectrum of dA20–dT20 in the complex with Hfq obtained by subtracting the contribution of the protein from the spectrum of the complex (spectra recorded in D2O solution). The two insets show regions of interest, which are discussed in detail. The right inset presents the enlarged domain between 810 and 750 cm
eq 1 of the spectrum of dA20–dT20 in the complex (E) compared with the spectra of dA20–dT20 recorded at 4 °C (duplex, (D)) and at 80 °C (melted duplex, (F)). In this spectral region we expect an adenine band located around 795 cm
eq 1 and a thymine band around 770 cm
eq 1 assignable to out-of-plane vibrations of the bases.22 The shift of these bands upon melting of the double helix reflects the disruption of the A–T H-bonds. Thus, upon melting, adenine absorption is displaced from 793 to 798 cm
eq 1 (control observed at 798 cm
eq 1 in the single stranded dA15 spectrum, not shown) while the thymine absorption is displaced from 770 to 777 cm
eq 1 (control observed at 777 cm
eq 1 in the single stranded dT15 spectrum, not shown) (Table 1). The spectrum of the DNA in the complex (E) presents both sets of bands, reflecting the simultaneous existence of free adenines and thymines (bands at 798 cm
eq 1 and 777 cm
eq 1, respectively) and of adenines and thymines involved in H-bonding (absorptions located at 793 and 770 cm
eq 1, respectively). This result indicates a partial opening of the double stranded structure upon interaction with Hfq.

Partial sugar repuckering in dA20–dT20 induced by the interaction with Hfq. As discussed above, the sugar geometries can be determined using characteristic FTIR absorption bands. Table 1 (columns 1, 2) summarizes the results concerning the sugar conformations of the nucleic acids in the complexes with Hfq. The spectrum concerning the dA20–dT20:Hfq complex is presented in Fig. 4. The spectrum of free dA20–dT20, characterized by an absorption band located at 842 cm
eq 1, reflects the existence of S-type conformation (A). dTn:rA n heteromeric duplexes are known to harbor S-type conformation in the dT n strand, while those in the rA n strand are in a N-type conformation.23 These features are observed in the spectrum of free rA15–dT15 (C) which shows two bands at 865 and 838 cm
eq 1, respectively assigned to the N-type conformation of rA15 and to the S-type conformation of dT15. In the case of the dA20–dT20:Hfq complex (B), the existence of two absorption bands at
840 and 865 cm$^{-1}$ characteristic of both N- and S-type indicates that both conformations co-exist in the complex.

**Conformation of the deoxythymidine nucleotides.** The absorption spectra of DNA containing A–T base pairs are very sensitive to the deoxythymidine nucleotide conformation, as observed in the spectral domain between 1290 and 1270 cm$^{-1}$. Fig. 5 presents the spectra recorded in H$_2$O solutions of dA$_{20}$–dT$_{20}$ in the complex (A) along with the spectra of free dA$_{20}$–dT$_{20}$ (B) and of a hydrated film of d(A–T)$_n$ recorded at 100% relative humidity (C) and 58% relative humidity (D). The B to A conformational transition in d(A–T)$_n$ induced by dehydration, shifts the deoxythymidine band from 1281 to 1275 cm$^{-1}$. These two positions correspond to nucleotides in which thymines are associated, respectively, to S- and N-type conformations. We observe that the deoxythymidine absorption in the spectrum of the complex is located at 1281 cm$^{-1}$, i.e. the same position as for free double stranded dA$_{20}$–dT$_{20}$ and all structures containing deoxythymidines with S-type sugars. The absence of any absorption band around 1275 cm$^{-1}$ allows us to discard the existence of the complexes with deoxythymidines associated with N-type deoxyriboses. In the previous section, we have shown the presence of two sugar geometries (N- and S-type conformation) in the dA$_{20}$–dT$_{20}$ :Hfq complex. Therefore, we propose that the interaction of Hfq with the dA$_{20}$–dT$_{20}$ duplex induces a partial sugar repuckering of the dA$_{20}$ strand while the sugar geometry of the dT$_{20}$ strand remains unchanged.

In summary, our FTIR study has shown that (i) Hfq interacts with deoxyadenosines, inducing a sugar repuckering of the dA$_{n}$ strand; (ii) a partial opening of the duplex dA$_{20}$–dT$_{20}$ exists in the complex with Hfq; (iii) deoxythymidine strand geometry remains unaffected with a S-type sugar conformation while the deoxyadenosine strand geometry changes and sugars adopt a N-type conformation.

**Sugar repuckering is correlated with dehydration of the nucleic acid complexed with Hfq.** We used small angle neutron scattering measurements to quantify the hydration level of Hfq and its evolution upon the formation of the complex with the oligonucleotide. This represents a key aspect to highlight the mechanism driving the structuring action of Hfq on DNA. In order to avoid self-assembly of proteins into fibers and/or protein precipitation, we used a shortened form of Hfq without its C-terminal region and unable to self-associate (referred as HfqS and corresponding to the first 72 amino acid residues of Hfq). We first confirmed that this truncated protein has an affinity for the dA strand similar to that of the full-length protein (Fig. 1), the $K_d$ of the truncated and full-length proteins were 240 ± 28 nM vs. 180 ± 22 nM for dA$_{20}$ and 6.2 ± 1.2 µM vs. 4.8 ± 0.75 µM for dA$_7$, respectively. This result agrees closely with that of Link et al. who measured a $K_d$ of 9.5 ± 2.8 µM for the complex dA$_6$:Hfq$^S$ (residues 2–69 in this case). Note that our result and that of Link et al. differs from the conclusion of Updegrove et al. indicating that the first 66 amino acid residues of the protein failed to bind DNA. Nevertheless, this difference is probably explained by (i) the use of ssDNA in this paper and that of Link et al. and (ii) the use of different methods to measure the affinities.

Then, solutions of Hfq$^S$, single stranded oligonucleotides dA$_7$, and dA$_7$:Hfq$^S$ complexes were prepared in a deuterated

<table>
<thead>
<tr>
<th>Sugar geometry</th>
<th>Base assignments</th>
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<tr>
<td>N type</td>
<td>S type</td>
</tr>
<tr>
<td>dA$<em>{20}$–dT$</em>{20}$ 4 °C duplex</td>
<td>—</td>
</tr>
<tr>
<td>dA$<em>{20}$–dT$</em>{20}$ 80 °C melted duplex</td>
<td>—</td>
</tr>
<tr>
<td>dA$<em>{20}$–dT$</em>{20}$ in the complex</td>
<td>865</td>
</tr>
<tr>
<td>dA$_7$</td>
<td>—</td>
</tr>
<tr>
<td>dA$_7$ in the complex</td>
<td>867</td>
</tr>
<tr>
<td>rA$_1$</td>
<td>—</td>
</tr>
<tr>
<td>rA$_1$ in the complex</td>
<td>873</td>
</tr>
<tr>
<td>rA$<em>{15}$–dT$</em>{15}$</td>
<td>865</td>
</tr>
<tr>
<td>rA$_{15}$</td>
<td>867</td>
</tr>
<tr>
<td>dT$_{15}$</td>
<td>—</td>
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### Table 1 Assignment of sugar geometries in the complexes (left) and evidence of partial dA$_{20}$–dT$_{20}$ duplex opening induced by Hfq (right)

<table>
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<tr>
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<tr>
<td>rA$_{15}$</td>
<td>867</td>
</tr>
<tr>
<td>dT$_{15}$</td>
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### Conformation of the deoxythymidine nucleotides.

Conformation of the deoxythymidine nucleotides. FTIR spectra recorded in H$_2$O of: (A) dA$_{20}$–dT$_{20}$ in the dA$_{20}$–dT$_{20}$:Hfq complex, (B) free dA$_{20}$–dT$_{20}$, (C) hydrated film of d(A–T)$_n$ at 100% relative humidity, (D) hydrated film of d(A–T)$_n$ at 58% relative humidity. Absorbance of (A) is a “A absorbance” resulting from the subtraction process.
buffer containing 10 mM Tris HCl pH 7.5 and 50 mM NaCl. Hfq\(^6\) concentration was 1.25 mM (corresponding to \(7.4 \times 10^{13}\) Hfq cm\(^{-3}\)) and the dA\(_7\) concentration was adjusted to have a stoichiometry of 1 Hfq hexamer per 1 oligonucleotide. The solutions were dialyzed overnight against deuterated buffer in order to exchange labile hydrogen with deuterium atoms. Following the procedure described below (see experimental procedure section), we estimated the H atoms content of each sample. The data are summarized in Table 2. Hydrogen atoms include both the non-labile H of the protein and the H belonging to the water molecules that are not discarded by extensive dialysis, being entrapped in internal cavities of the solute. We note that the number of protons per molecular unit reduces to about half passing from pure Hfq to Hfq:dA\(_7\) complex, while in the case of dA\(_7\), no measurable content of H atoms has been detected.

Assuming that about 75% of the protons belonging to the chemical structure of Hfq\(^6\) (i.e. \(\sim 2500-3000\) protons per hexamer) are not labile, we estimate \(\sim 1300\) entrapped water molecules per protein. In this context, the presence of strongly bounded water molecules was confirmed by crystallographic studies, which reports the existence of 136 water molecules in the crystallographic structure of Hfq (PDB entry 1HK9). Moreover, since the H atoms belonging to the chemical structure of dA\(_7\) are negligible with respect to those of the Hfq and assuming that the number of not labile H in Hfq remains constant upon association to A\(_7\), we conclude that the number of water molecules after the complex formation lies in the range which is smaller than the accuracy of our estimation and therefore that the complex formation is accompanied by a substantial global dehydration. We assume that the effect observed on the nucleic acid structure (sugar pucker) is linked to a partial dehydration in close proximity of the bound DNA.

### Discussion

In addition to regulating RNA metabolism, our data confirm that Hfq binds DNA strongly and affects its structure. Even if the protein seems globally non-specific (at least at high concentration), our results address the question of how Hfq may bind specific regions of the chromosome \textit{in vivo} and influence the nucleoid structure. Indeed, Hfq was previously shown to have a higher affinity for curved DNA\(^1\) and here we confirm its preference for A-rich sequences. These sequences, referred as A-tracts, indeed induce a pronounced DNA curvature with a local bend of \(\sim 40-60^\circ\) in the DNA helix.\(^\text{26}\)

Table 2  Hydration properties of Hfq upon complex formation by SANS measurements. The content of hydrogen atoms in each sample is shown in the first and second column of the table. These hydrogen atoms include both the nonlabile H of the solute, and the H belonging to the water molecules that are not removed by extensive dialysis, being entrapped in internal cavities of the solute. Assuming that about \(\sim 2500-3000\) protons per hexamer are not labile, we estimate \(\sim 1300\) entrapped water molecules per protein. Since the H atoms belonging to the chemical structure of A\(_7\) are negligible with respect to those of the Hfq and assuming that the number of not labile H in Hfq remains constant upon association to A\(_7\), the complex formation is accompanied by a substantial dehydration. The data are summarized in the third column. In the table, \textit{molecular unit} stands for 1 Hfq hexamer in the protein solution, 1 Hfq:A\(_7\) in the complex solution and 1 dA\(_7\) in the oligonucleotide solution.

<table>
<thead>
<tr>
<th></th>
<th>H/cm(^3) buffer excluded</th>
<th>H/molecular unit</th>
<th>H(_2)O/molecular unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer + Hfq</td>
<td>((4 \pm 0.3) \times 10^3)</td>
<td>5400 (\pm) 400</td>
<td>1300 (\pm) 300</td>
</tr>
<tr>
<td>Buffer + Hfq + A(_7)</td>
<td>((2 \pm 0.3) \times 10^3)</td>
<td>2700 (\pm) 400</td>
<td>(&lt;300)</td>
</tr>
<tr>
<td>Buffer + A(_7)</td>
<td>Not significant</td>
<td>Not significant</td>
<td>Not significant</td>
</tr>
</tbody>
</table>

To understand the mechanism of Hfq-based conformational transformation, we hence characterized the effect of Hfq on DNA structure by spectroscopic methods. We show that Hfq binds double-stranded A–T sequence with an affinity of 250 nM. It opens the double-stranded A–T sequence, binds to the dA strand only resulting in a conformational change of this strand. By taking into account the recent crystal structure of \(E.\ coli\) Hfq with a poly(rA) and the results of Updegrove \textit{et al.} showing that DNA interacts with Hfq’s distal surface, it is likely that Hfq binds the dA-rich strand on the distal face of the protein.\(^\text{13,14}\) We thus tried to replace the C2'-endo pucker of the crystal structure by a C3'-endo pucker DNA into the distal binding site. As shown in Fig. 6, successive R pockets can be occupied in the Hfq-dA complex structure if all sugar puckers are C3'-endo, similarly to that observed in Hfq-rA15 complex structure.

Hfq binding results in a structure where the form of unbound sugar within a thymidine is in S-conformation and that of bound deoxyribose within adenine is in N-conformation. Thus, Hfq binding results in a nonhomogeneous conformation, referred as heteronomous conformation with coexisting N- and S-type furanose rings. Note that since Raman and infrared spectroscopy studies have confirmed the coexistence of

![Fig. 6 Model structure of Hfq bound to a poly(dA). The molecular surface of Hfq is represented by grey surface and poly(dA) in Stick model. A, R and E refer to the tripartite RNA binding motif described by Link \textit{et al.}\(^\text{23}\) R is at the interface of 2 Hfq monomers. The Gin 52 and Tyr 25 amino acid residues of the R pocket are colored in dark grey and localised by their labels. Analysis by the prosit server (http://cactus.nci.nih.gov/prosit/) after the model refinement confirms that all sugars are N-type puckered.\(^\text{40}\)](http://cactus.nci.nih.gov/prosit/)
these sugar conformations in AT sequences of free DNA,27,28 we hypothesized that Hfq stabilizes the N-type conformation found naturally within A-tracts rather than catalytically, changing the conformation of the sugar when it is bound to the A-strand.

Our SANS analysis furthermore indicates that Hfq stabilizes the N-type sugar conformation probably because it induces the dehydration of the nucleic acid within the protein. In this context, note that the ligand usually binds to proteins by displacing water from their binding site.29 Our SANS results are thus qualitatively compatible with the FTIR data. The latter show that nucleic acid binding to Hfq is accompanied by a transition in the furanose ring resulting from the dehydration of the nucleic acid–protein complex.30

**Experimental**

**Protein purification**

Full-length *E. coli* Hfq (102 amino acid residues) was overexpressed and purified as described previously.25 The truncated form of Hfq (Hfq<sup>S</sup>) corresponding to the N-terminal part of Hfq protein truncated after Ser 72 corresponds to the *E. coli* protein, whose structure is known.9,13 It was purified in a way identical to the full length Hfq.

**Fluorescence anisotropy measurements**

Fluorescence anisotropy measurements were collected with a Varian Eclipse fluorospectrophotometer. Samples were excited at 490 nm and emission was measured at 520 nm. 5'-fluoresceinated oligonucleotides were purchased from Dharamacon. The binding buffer used for measurements contained 20 mM Tris–HCl pH 7.0 and 100 mM NaCl. 1 nM of 5'-fluoresceinated oligonucleotide was added to the cuvette (1 ml) and titrated by Hfq. The measurements were performed at 298 K. Samples were incubated 60 s prior to each measurement, ensuring equilibrium binding. The normalization of fluorescence anisotropy was carried out after determination of the A<sub>max</sub> value, which was obtained at saturating Hfq concentrations. A/A<sub>max</sub> ratios were determined and plotted versus the Hfq concentrations. The data were plotted using Kaleidagraph software and the curves were fitted by the nonlinear least-squares regression method, assuming a bimolecular model with Hfq hexamer as protein unit (the biological form of the protein). Binding affinities were measured for 3 different experiments and different preparations of Hfq were tested. Note that Fig. 1 shows only one of these measurements but the standard deviation given in the text takes into account the error associated to the curve fitting procedure and the variance of the 3 different experiments.

**FTIR spectroscopy**

**Preparation of the complexes for FTIR spectroscopy.** The Hfq protein was analyzed in 50 mM Tris–HCl pH 7 containing 50 mM NaCl and 0.005% dodecyl-β-D-maltoside. Protein concentration was estimated by UV absorbance spectroscopy measurements. Oligonucleotide concentrations were also determined by UV spectroscopy at 260 nm. Complexes between Hfq and single stranded oligonucleotides were prepared with a stoichiometry of 1 Hfq hexamer per 7 nucleotides. For the dA<sub>20</sub>–dT<sub>20</sub>–Hfq complex the stoichiometry was 1 Hfq hexamer per 7 A–T base pairs. The solutions were lyophilized and redissolved in D<sub>2</sub>O (> 99.8% purity, Euriso-Top CEA) at the desired dilution, depending on if measurements were made by transmission or ATR; the ATR procedure allows the measurements to be carried out in more diluted conditions. 20 or 2 μl were used for ATR or transmission experiments, respectively.

FTIR spectra were recorded using a Perkin-Elmer 2000 spectrophotometer. For transmission experiments, samples were deposited between two ZnSe windows without spacer at a concentration of about 30 mM oligonucleotide strand. 10 to 30 scans were usually accumulated under continuous dry air purge, with a resolution of 1 cm<sup>-1</sup>. For reflection experiments the samples were deposited on a ZnSe crystal of the ATR device (12 reflections) at a concentration of about 3 mM oligonucleotide strand. Usually 25 scans were accumulated using a resolution of 2 cm<sup>-1</sup>. The physiological concentration of the protein in the cell is about 20 μM. However, it has to be taken into account that this cellular concentration refers to a measurement over the whole cell, while Hfq is concentrated in specific zones of the cell (*i.e.* the nucleoid). Thus the local concentration of Hfq is of the same order of magnitude than that used for FTIR analysis. Both ATR and transmission techniques of different samples lead to comparable results, showing the low variability between samples. Data treatment was performed using the Perkin-Elmer Spectrum program and consisted of multiple point base line correction and spectral subtraction after normalization. In order to prove the robustness of the subtraction process, various levels of the reference spectrum (protein) were subtracted from the complex spectrum. No significant variation in the spectra was observed, indicating that the changes observed are not due to the subtraction process (not shown).

**Small angle neutron scattering measurements**

SANS measurements were performed on the spectrometer PAXE at Laboratoire Léon Brillouin (Saclay, France). Samples were measured at 25°C in 2 mm path length quartz cells using an incident neutron wavelength λ = 6 Å and a sample-to-detector distance of 5 m. The wavelength spread was Δλ/λ = 0.10. The scattered neutrons were detected by a two-dimensional array detector (64 × 64 cm<sup>2</sup>) consisting of 4000 cells (1 × 1 cm<sup>2</sup>). The investigated momentum transfer Q ranges between 8 × 10<sup>-3</sup> and 8 × 10<sup>-2</sup> Å<sup>-1</sup>. The acquisition time for each sample was ~4 h. Radially averaged scattered intensities were corrected for detector sensitivity, empty cell and solvent contribution, and then reduced to absolute units (cm<sup>-1</sup>), thus obtaining the so-called macroscopic differential cross sections.

SANS is a technique particularly well adapted to structural studies in biology because of the possibilities of enhanced contrast between biomolecules and a deuterated solvent. Also, in contrast with X-ray scattering, the extremely low energy of the neutrons of the incident beam cannot damage the integrity of the molecules. In our case, the energy corresponding to λ = 6 Å is 2.3 meV, two orders of magnitude lower than the typical energy of a hydrogen bond.
**Data analysis.** The content of hydrogen atoms in the samples has been estimated by the flat background of the corresponding macroscopic differential cross sections, as determined at high Q values after solvent subtraction. In this Q-region, the spectrum is indeed dominated by the incoherent scattering of the sample, which provides a Q-independent contribution. Due to the high incoherent cross section of hydrogen atoms, which is by far higher than that of any other element, this background arises almost exclusively from the H scattering and its intensity is proportional to the H number density. Using the spectrum of pure water as a reference, the content of H atoms in the samples was estimated.

**TEM imaging**

200 ng of pBR322 supercoiled plasmids (4361 bp) were incubated for 5 min with 200 to 400 ng of Hfq in TE. (Note that different incubation buffers comprising various amounts of monovalent and divalent salts were also used, but this apparently had no effect on the final product as subsequently observed by TEM). The reaction was then diluted to 1/30 in TE, and 5 μl were deposited onto a 600-mesh copper grid covered with a thin carbon film and activated by glow-discharge in the presence of pentylamine. Grids were washed with aqueous 2% (w/v) uranyl acetate, dried and observed in the annular darkfield mode using a Zeiss 902 electron microscope. Images were captured with a MegaViewIII camera CCD controlled by iTEM software (Olympus Soft Imaging).

**Molecular modeling of the complex with DNA**

The model of Hfq in complex with poly(dA) has been built using the structure of *E. coli* Hfq in complex with a poly(rA). Single C3'-endo puckered dA nucleotides were superimposed on the rA nucleotides. Stereochemistry of the sugar–phosphate backbone was idealized using Coot, with the position of the bases being restrained. The protein–DNA complex was then energy minimized using Refmac5. Conformation of the sugars was analyzed using the PROSIT website (http://cactus.nci.nih.gov/prosit/).

**Conclusion**

Our analysis reveals that the Hfq protein preferentially interacts with DNA A-rich sequences and that it induces a partial opening of dA–dT duplexes accompanied by sugar repuckering of the dA strand. In view of the important function of A-tracts in the stimulation of transcription, our results suggest that Hfq could be involved in the regulation of genetic processes at the transcriptional level by directly affecting the structure of several promoters. Our data thus support the conclusion of Le Derout et al., indicating that Hfq could influence transcription efficiency in vivo. Moreover, our study raises the question about the general effect of Hfq on transcription regulation, particularly in light of its interaction with *E. coli* RNA polymerase and suggests a possible function of Hfq at the interface between transcription and translation, two processes that occur simultaneously in bacteria.

**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ATR</td>
<td>Attenuated Total Reflectance</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier Transform Infrared Spectroscopy</td>
</tr>
<tr>
<td>sRNA</td>
<td>Small noncoding RNA</td>
</tr>
<tr>
<td>SANS</td>
<td>Small Angle Neutron Scattering</td>
</tr>
<tr>
<td>ss/ds</td>
<td>Single/double strand</td>
</tr>
<tr>
<td>S-type</td>
<td>South-type</td>
</tr>
<tr>
<td>N-type</td>
<td>North-type</td>
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<tr>
<td>RNAP</td>
<td>RNA Polymerase</td>
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<tr>
<td>TEM</td>
<td>Transmission Electron Microscopy</td>
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**Acknowledgements**

This research was supported by University Paris Diderot, CNRS and CEA. VC acknowledges financial support by the Agence Nationale de la Recherche (contract no. ANR-06-CIS6-012-01). We are indebted to M. Romero Guzman who contributed to this work at early stage. We are also grateful to Eric Le Cam (Institut Gustave Roussy, Villejuif) for the use of TEM facilities, to Florent Busi for his help in preparing figures, to Brigitte Hartmann (INTS, Paris) for many fruitful discussions and to Rahul Roy (Harvard, Cambridge, MA, USA) for critical reading of the manuscript.

**References**