Lon-dependent regulation of the DNA binding protein HU in *Escherichia coli*

**Abbreviations:** Km, kanamycin; Cm, chloramphenicol; IFH, integration host factor.

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**Communicated by I. Robert Lehman, June 30, 1989**

**ABSTRACT**

HU, the major DNA binding protein of *Escherichia coli*, exists in solution as a heterodimer composed of two highly homologous subunits: HU1, encoded by hupB; HU2, encoded by hupA. The purification of the HU protein from *hupA*” or *hupB*” bacteria showed that the *hupB* mutant strains synthesize normal amounts of the HU2 subunit (which corresponds to 60% of the total HU present in wild-type cells). On the contrary, the amount of HU1 present in *hupA* mutant strains corresponds to only 6% of the total HU present in wild-type cells. We showed by fusions of the *hupB* and *hupA* promoters to the *malPQ* operon that the absence of one subunit has no major effect on the transcription rate of the gene encoding the other subunit. Analysis of the stability of the HU1 and HU2 subunits, using pulse-chase labeling experiments, showed that the HU1 subunit is degraded specifically in the absence of the HU2 subunit and, moreover, that this degradation is dependent on the presence of the Lon protease.

HU is the most abundant DNA binding protein of *Escherichia coli* with 30,000 dimeric molecules per cell. It is considered to be a histone-like protein (1), which binds both double- and single-stranded DNA and RNA (2). The protein is composed of two highly homologous (70% amino acid sequence identity) subunits—HU2 (HUA) and HU1 (HU1) of 90 amino acids each—which exist in solution mainly as an αβ heterodimer (3).

Various roles for HU have been suggested by *in vitro* studies. HU was shown to stimulate the replication of phages and plasmids bearing the *E. coli* origin of replication oriC (4), to enhance the transposition of Tn10 (5), and to be indispensable for the transposition of bacteriophage Mu (6). HU affects DNA structure: like eukaryotic histones, HU condenses DNA *in vitro* and can introduce negative supercoils into a relaxed circular DNA in the presence of topoisomerase I (7, 8). Recently, the first studies performed with *hup* mutants confirm the role of HU in transposition (9, 10). The double *hupA hupB* mutation was not lethal, but the resulting HU+ cells were very unhealthy and rapidly acquired compensatory mutations (10).

Among prokaryotic species, HU is a highly conserved protein, but in bacterial species other than *E. coli* or *Salmonella typhimurium* there is only one type of subunit, which exists as a homodimeric protein (for review, see ref. 1). In *E. coli* K-12 the subunits are encoded by two independent genes [the *hupB* gene located at 10 min of the bacterial map encodes the HU1 subunit (11) and the *hupA* gene at 90 min encodes the HU2 subunit (12)]. Almost equal amounts of the two subunits exist in the wild-type cell (3); this implies the existence of some mechanism of co-regulation to achieve the coordinate synthesis of the two subunits.

We have recently constructed by gene disruption *E. coli* K-12 mutants completely lacking one or the other subunit (10). In the present work, we examine the HU species present in the mutant strains and examine their rates of synthesis and degradation. The absence of one subunit has no major effect on the rate of synthesis of the other subunit. However, in the absence of HU2, the HU1 protein was present in a considerably reduced amount. This low amount of HU1 was the result of a markedly decreased half-life. Moreover, the use of a *lon*” mutant strain shows that this degradation was due to a Lon-dependent proteolysis.

**MATERIALS AND METHODS**

**Strains and Bacteria.** The bacterial strains used in this study are listed in Table 1. Bacteria were grown in LB and titrated on L agar, synthetic medium, or MacConkey maltose indicator agar as described by Miller (15). Minimal medium was supplemented with M9 with thiamine (10 µg/ml), amino acids (100 µg/ml), and 0.4% (wt/vol) sugar (maltose, lactose, or glucose). Kanamycin (Km, 40 µg/ml), chloramphenicol (Cm, 12.5 µg/ml), ampicillin (40 µg/ml), and tetracycline (10 µg/ml) were included when appropriate. The HU mutants used in this work were constructed by P1 transduction as described (10).

**Pulse–Chase Labeling of the HU Protein.** *E. coli* JRY1 and its derivatives were grown in M9 medium supplemented with 0.4% glucose and all amino acids (100 µg/ml each) except methionine to an OD_{600} of ~0.3. The cells were centrifuged at 4°C and the pellets were resuspended in 1/4 vol of the same medium. The cells were incubated for 3 min at 37°C or 42°C prior to pulse labeling, which was carried out for 45 sec with [35S]methionine (800 Ci/mmol; 1 Ci = 37 GBq). An excess of nonlabeled methionine was added and immediately a 1.5-ml sample was removed. The incubation was continued at either 37°C or 42°C and 1.5-ml samples were removed at the times indicated in the corresponding figures. After removal, the samples were cooled, centrifuged, washed, and frozen at −20°C. Since HU is a heat-stable protein, it was extracted from each sample by heating the samples at 100°C for 10 min in 250 µl of 20 mM Tris-HCl, pH 7.5/0.4 M NaCl/1 mM EDTA buffer. After centrifugation at 10,000 × g for 20 min, the HU protein present in the supernatant was immunoprecipitated and submitted to gel electrophoresis analysis in NaDodSO4/polyacrylamide gels or acid/urea/Triton X-100/polyacrylamide gels.

**Construction of hupB–malPQ and hupA–malPQ Fusion.** The *hupB–malPQ* fusion was constructed by insertion of the 1269-base-pair BamHI/Pst I fragment from pMW1 (11) carrying the 3' end of the *lon* gene, the *hupB* promoter, and the region of the *hupB* gene encoding the first 12 amino acids of the HU1 subunit into pSB118. This plasmid is a derivative of pUC18 (16) with EcoRI sites at both ends of the polylinker.

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It permitted the conversion of the BamHI/PstI fragment, to an EcoRI fragment that was cloned into the EcoRI site of pOM41 (14) and transferred onto the chromosome in front of the malPQ operon of the mal− strain pop2150 (ΔmalAS10) as described by Vidal-Ingiigliardi and Raibaud (14). The hupA−malPQ fusion was similarly constructed by inserting the 600-base-pair Kpn I/PstI fragment of pKO1 (12) carrying the hupA promoter region and the region of the hupA gene encoding the first 50 amino acids of the HU2 subunit, which was also cloned into pOM41 (after its conversion to an EcoRI fragment via pSB118).

**RESULTS**

**Characterization of HU from hupB and hupA Mutants.** We purified HU protein from the wild-type strain JRY1 as well as from insertion hupB mutant (hupB::Km; strain OHP96) and insertion hupA mutant (hupA::CM; strain OHP109). The standard purification procedure described in Table 2 was applied to extracts of all three strains. This procedure is reproducible and quantitative. In addition, we took care to follow all steps of the purification and column fractions by NaDodSO4/polyacrylamide gel electrophoresis and Ouchterlony immunoprecipitation to verify that no HU or HU protein fragments were lost.

**DNA binding properties of HU1 and HU2 are not affected by the lack of one subunit.** The HU2 subunit from hupB mutant (strain OHP96) as well as the HU1 subunit from hupA mutant (strain OHP109) eluted at the same position (0.24 M NaCl) as did the wild-type HU heterodimer from JRY1 (wild-type) strain. This seems to indicate that for nonspecific DNA interaction as monitored by binding to the DNA-cellulose column matrix, the affinity of HU toward DNA is the same for the α or β subunit as for the αβ heterodimer.

**Behavior of the integration host factor protein (IHF) is different in mutant cells as compared to wild-type cells.** The overall proteins that eluted from the different DNA-cellulose columns (including the proteins that coeluted with HU) were analyzed by NaDodSO4/polyacrylamide gel electrophoresis (data not shown). We were unable to detect differences concerning either the presence or the intensities of the bands observed in the gels except for the proteins present in the fractions that eluted from the JRY1 DNA-cellulose column at 0.3 M NaCl. No equivalent fractions were eluted from the DNA-cellulose column of the hupB mutant but some protein eluted from the DNA-cellulose column of the hupA mutant, although at a lower salt concentration (0.25 M NaCl). This protein fraction from the wild-type JRY1 strain is composed of a nonidentified 25-kDa protein plus a doublet polypeptide that migrated in the same position as the integration host factor of purified E. coli and cross-reacted with IHF antiserum. The protein fraction eluting at 0.25 M NaCl in the hupA mutant strain appeared to be composed mainly of the nonidentified 25-kDa protein, a trace of HU but no IHF. We could, however, show that normal amounts of IHF were present in the crude extracts of both the hupA and hupB mutant cells, as determined by Western blotting with an IHF antiserum (data not shown). This point is further considered in the Discussion.

**HU1 and HU2 are present as homodimers in mutant cells.** The fractions containing HU from the three different DNA-cellulose columns were subjected to phosphocellulose chromatography. The two homodimers and the heterodimer eluted at different ionic strength, showing that the three forms of HU (α, β, and αβ) have different phosphate binding/recognition properties. Since no difference was observed in the elution pattern of the three forms of HU from DNA-cellulose, we think that factors other than interaction with phosphate groups affect the affinity of HU to DNA.

At this stage, the proteins were pure as judged by NaDodSO4/polyacrylamide gel electrophoresis. We also analyzed the purified HUs by electrophoresis in acid/urea/Triton X-100/polyacrylamide gel. Fig. 1 shows the presence of both subunits in the wild-type strain (lane A) and confirms the total absence of HU1 from the hupB mutant (lane B) and of HU2 from the hupA mutant (lane C).
The wild-type HU protein purified under similar conditions exists in solution as a dimer (3). To confirm that the mutant proteins also were present in solution as dimers, the purified HUs were submitted to a high-performance gel filtration chromatography using a Pharmacia Superose 12 column. The HU protein from hupB and hupA mutants had the same retention time, hence the same size, as the wild-type HU, indicating that, under the conditions used for the purification, the HU protein from the mutants exists in solution predominantly as a dimer (data not shown).

Quantification of HU Present in hupB and hupA Mutants. Table 2 represents the amount of total HU obtained from each purification. If we consider as 100% the amount of HU obtained from the wild-type JRY1 strain, the equivalent of 60% of HU is obtained from the hupB mutant (strain OHP96 only producing the HU2 subunit) but only 6% of HU from the hupA mutant (strain OHP109 only producing the HU1 subunit). The 60% of HU obtained from the hupB mutant is a quite reasonable value, since in wild-type cells there is a slight excess of HU2 compared to HU1 (Table 2, strain JRY1). On the contrary, the low amount of HU1 (6%) obtained from the hupA mutant is very surprising; it is 8 times less than the amount of HU1 normally present in wild-type cells. This deficiency in the total amount of HU present in hupA mutants could mean that the presence of the HU2 subunit is necessary for a normal synthesis and/or stability of the HU1 subunit.

Comparative Efficiencies of hupA and hupB Promoters in Wild-Type Cells and in hupA and hupB Mutant Cells. Operon fusions between hupBp and hupAp and the malIPQ genes were used to search for any possible effect of the HU proteins on their own or each other’s expression.

The fusions of the hupB or hupA promoters to the malIPQ operon were first constructed on plasmids and then transferred, by homologous recombination, into the chromosome as described in Materials and Methods to give JRY624 (malPpΔ534::hupBp) and JRY717 (malPpΔ534::hupAp). The interrupted HU genes from strains OHP96 and OHP109 were subsequently introduced by P1 transduction into the chromosomes of strains JRY624 and JRY717.

The results of the amylomaltase (product of the malQ gene) activity tests are summarized in Table 3. First, we considered the efficiencies of hupBp and hupAp in a pop3 wild-type HU background. The hupA promoter (strain JRY717) appears a little stronger than the hupB promoter (strain JRY624). Moreover, both promoters seem to be slightly stronger than the wild-type-induced malPQ promoter (strain pop3), which is considered moderately strong—e.g., the same order of magnitude as the lacZp (14), a strong promoter in E. coli. This is not surprising in view of the abundance of the HU protein in the cell.

In the HU mutant backgrounds, we observed that the hupB promoter was not affected by the absence of the HU1 subunit (strain JRY772) but it was derepressed by a factor of 1.4 in the absence of the HU2 subunit (strain JRY782). Equivalent results were obtained with the hupA promoter, which also was not significantly affected by the absence of the HU1 subunit (strain JRY768) and was derepressed by a factor of 1.4 in the absence of its own encoded protein subunit HU2 (strain JRY810). We conclude that the HU1 subunit has no evident regulatory effect on the transcription of either the hupB gene or the hupA gene. On the contrary, the absence of the HU2 subunit derepresses by a factor of 1.4 both the hupB and the hupA genes.

HU1 Is Degraded in the Absence of its Homologous HU2 Subunit. Since a small derepression rather than a repression of the hupB gene in the absence of the HU2 subunit was observed with the promoter fusion experiments, we considered the possibility that the low amount of HU1 present in hupA- cells could be caused by a degradation of the HU1 subunit in the absence of its homologous HU2 subunit. The stability of the HU1 subunit was assayed by pulse-chase labeling (as described in Materials and Methods) of the proteins in hupA- cells and compared to the wild-type JRY1 cells. As shown in Fig. 2A, no significant degradation of the wild-type heterodimer was observed at 37°C or at 42°C, even after a 60-min chase. On the contrary, the HU1 subunit present in hupA- cells was degraded at 37°C with a half-life of ~60 min (Fig. 2B, lanes 1, 3, and 5; Fig. 3). This instability was enhanced at 42°C, reducing the half-life of HU1 to the order of 35 min (Fig. 2B, lanes 2, 4, and 6; Fig. 3).

We also analyzed the stability of the HU2 subunit in a hupB- background at 37°C and at 42°C by the same technique. As shown in Fig. 2C, no degradation of the HU2 subunit in the absence of the HU1 subunit was observed after a 60-min chase at 37°C or at 42°C. This result clearly shows that the degradation previously observed is specific for the HU1 subunit in a hupA- background.

Table 3. Efficiency of the hupA and hupB promoters as measured by amylomaltase activity

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>HU genotype</th>
<th>Amylomaltase activity, nM glucose per min per mg of protein</th>
<th>Factor of induction</th>
</tr>
</thead>
<tbody>
<tr>
<td>JRY624</td>
<td>malPpΔ534::hupBp</td>
<td>HU wt</td>
<td>250</td>
<td>1</td>
</tr>
<tr>
<td>JRY772</td>
<td>malPpΔ534::hupBp</td>
<td>hupB-</td>
<td>250</td>
<td>1</td>
</tr>
<tr>
<td>JRY782</td>
<td>malPpΔ534::hupAp</td>
<td>HU wt</td>
<td>350</td>
<td>1.4</td>
</tr>
<tr>
<td>JRY717</td>
<td>malPpΔ534::hupAp</td>
<td>HU wt</td>
<td>390</td>
<td>1.2</td>
</tr>
<tr>
<td>JRY768</td>
<td>malPpΔ534::hupAp</td>
<td>hupB-</td>
<td>470</td>
<td>1.4</td>
</tr>
<tr>
<td>JRY820</td>
<td>malPpΔ534::hupAp</td>
<td>HU-</td>
<td>200</td>
<td></td>
</tr>
</tbody>
</table>

All strains are derived from strain MC4100 (pop3). All strains were grown at 37°C, unless marked otherwise, in M9 medium supplemented with 0.4% glucose (except pop3, which was supplemented with 0.4% maltose) and all amino acids (100 µg/ml each) except methionine. Amylomaltase was assayed as described (14). Protein concentration was determined by the Lowry method (19) with a bovine serum albumin standard. All values are the average obtained from at least three independent cultures. wt, Wild type.

*Strains grown at 42°C.

Fig. 2. Pulse–chase labeling of HU in JRY1 wild-type (wt) cells (A), OHP109 (hupA-) cells (B), OHP96 (hupB-) cells (C), and OHP187 (hupA- lon-) cells (D). For this experiment, the hupA- lon- strain OHP187 was constructed by P1 transduction of the lon mutation carried by strain SG1095 (lon-146::Stn10) into the hupA- strain OHP109. Cells were pulse-labeled with [35S]methionine for 45 sec at either 37°C (lanes 1) or 42°C (lanes 2) and chased with an excess of unlabeled methionine. Cells were chased for 35 min at either 37°C (lanes 3) or 42°C (lanes 4) and for 60 min at either 37°C (lanes 5) or 42°C (lanes 6). After the extraction of the heat-stable proteins and immunoprecipitation, the HU protein was submitted to a 10–25% linear gradient NaDodSO4/polyacrylamide gel electrophoresis. The gel was stained with Coomassie blue, dried, and autoradiographed on Kodak X-Omat film. The autoradiograph was scanned to determine the half-life of HU1 at 37°C and at 42°C.
7694 Biochemistry: Bonnefoy et al.


**Fig. 3.** Stability of HU in JRY1 (wild type) cells and OHP109 (hupA-) cells. The pulse-chase-labeled bands of the HU protein from the autoradiograph of Fig. 2A and B were scanned. The percentage of the remaining radioactivity was determined relative to the radioactivity found at 0 min of chase (corresponding to 100% of radioactivity). ○, Pulse-chase-labeled bands from JRY1 (wild type) cells at 37°C; ●, pulse-chase-labeled bands from JRY1 (wild type) cells at 42°C; □, pulse-chase-labeled bands from OHP109 (hupA-) cells at 37°C; ※, pulse-chase-labeled bands from OHP109 (hupA-) cells at 42°C.

Scanning of the short pulse-labeled samples in the autoradiograph showed that the synthesis of the wild-type heterodimer in JRY1 cells is roughly the same at 37°C and at 42°C (Fig. 2A, lanes 1 and 2). This was not the case for the synthesis of the HU1 subunit in hupA- cells. Only half the amount of HU1 was synthesized at 42°C compared to that synthesized at 37°C (Fig. 2B, lanes 1 and 2). Since the pulse time (45 sec) was much shorter than the half-life of HU1 (35 min at 42°C), the difference cannot be due to degradation during the labeling period. As no repression of the transcription was detected with the malPQ fusions at 42°C (see Table 3, *), we are tempted to postulate that the difference concerning the synthesis observed between 37°C and 42°C for the HU1 subunit in hupA- cells is probably due to a translational event. An equivalent difference between 37°C and 42°C was not observed for the synthesis of HU2 in hupB- cells (Fig. 2C, lanes 1 and 2). It is perhaps worth noting here that the hupB initiation codon is GTG and not ATG, which is present at the beginning of the hupA gene.

**HU2 Subunit Prevents HU1 Degradation.** The absence of degradation of HU1 observed in wild-type cells suggests that the HU2 subunit protects the HU1 subunit from degradation. To confirm this result, the hupA- strain was transformed with plasmid pKOI carrying the hupA gene encoding the HU2 subunit and the degradation of HU1 was analyzed under the same conditions as the ones described before. We observed that after the introduction of pKOI into the hupA- strain, an excess of the HU2 subunit was synthesized but neither the HU1 subunit nor the HU2 subunit was degraded at 37°C or at 42°C. Moreover, we did not observe a difference in the amount of HU1 synthesized during the pulse at 37°C or 42°C (data not shown).

**HU1 Degradation Is Dependent on Lon Protease.** Several observations made us suspect that Lon was responsible for the specific degradation of HU1: the increase in the rate of degradation of the HU1 subunit in hupA- cells at 42°C and the observation in our laboratory that a mutation in the lon gene [encoding Lon, an ATP-dependent (20) heat shock protease (21)] could partly compensate for the growth defects in the hupA- strains (data not shown). We analyzed, using the same pulse-chase labeling technique, the degradation of the HU1 subunit in a hupA- lon- strain at 37°C and at 42°C. Fig. 2D shows that the HU1 subunit of the hupA- lon- strain OHP187 was not degraded after a 60-min chase at 37°C or at 42°C. To confirm this result, we also purified the HU1 subunit from the hupA- lon- strain OHP187 under the same conditions as described in Table 2. A normal amount of HU1 was obtained, equivalent to 8 times the amount of HU1 obtained from the hupA- lon- strain OHP109 and approximately the same amount of HU1 as present in wild-type cells (see Table 2). It is also interesting to note that a protein fraction was eluted again at 0.3 M NaCl from the DNA-cellulose column of strain OHP187. Using gel electrophoresis, we observe that IHF was present in this fraction (data not shown).

The low amount of HU1 subunit synthesized at 42°C as compared to 37°C observed in the hupA- lon- strain is no longer observed in the hupA- lon- strain (Fig. 2D, lanes 1 and 2), thus suggesting that the translational event responsible for this phenomenon could also be lon dependent.

**DISCUSSION**

The purification of HU from the hup mutant strains revealed a surprising result—namely, the small amount of total HU present in the hupA- strain. In fact, in this mutant, which lacks the HU2 subunit, only 1/8 of the amount of HU1 is present, which corresponds to 1/16 of the total HU present in the wild-type strain. This very low level of HU, observed in the absence of the HU2 subunit, is not due to a transcriptional regulation, since we observed in the hupA- strains a small derepression of the activities of the hupA and hupB promoters rather than any repression effect. Nor does it seem to be due to a difference in the turnover of the corresponding mRNAs, since Wada et al. (9) have recently reported detecting normal amounts of HU1 mRNA in hupB mutants, but rather to a degradation of the HU1 subunit in the hupA mutant.

The experiments described here show that the HU1 subunit is degraded in vivo when it is not protected by the homologous HU2 subunit. The reciprocal effect is not observed for the HU2 subunit, which is normally stable in the absence of the HU1 subunit. This fact is surprising since the two subunits are highly homologous and both exist in the mutant cells, as shown here by gel-filtration experiments, as homodimers. It should be remembered that, in wild-type cells, HU exists primarily as heterodimers, with <5% being in the homodimeric forms.

The hupA mutant carries a Cm cassette, which interrupts the hupA gene 150 base pairs after its initiation codon, while the Km cassette is inserted in the hupB gene only 36 base pairs after its initiation codon. It is possible that a truncated HU2 subunit composed of the first 50 amino acids is being synthesized in hupA mutant cells and that this moiety of HU2 could form a pseudo-dimer with the HU1 subunit. This aberrant structure might then be a substrate for a proteolytic enzyme. However, analysis of the total proteins present in hupA- cells by gel electrophoresis failed to detect any truncated form of the HU2 subunit. Moreover, when the HU1 subunit is overproduced after the transformation of a wild-type strain with plasmid pMW1 carrying the hupB gene, a degradation of the HU1 subunit was also observed (data not shown). In this case, the degradation cannot be due to the presence of a pseudo-dimer since the hupA gene is not interrupted and, hence, no truncated HU2 subunit could be synthesized. Nevertheless, the construction of a complete hupA deletion will eliminate this potential problem.

Assuming that the degradation of the HU1 subunit is not due to an aberrant dimeric construction, the next question is why the HU1 dimer is degraded and not the HU2 dimer. One possibility is that the interaction of the HU1 subunit with DNA is different from that of the HU2 subunit on the wild-type heterodimer. This “abnormal” DNA–HU1 complex could induce or allow the degradation of the HU1 subunit. Some preliminary data, using a gel-retardation tech-


A puzzling observation was that IHF synthesized in the hupB and hupA mutants did not bind to the DNA-cellulose column, whereas IHF from the wild-type cells bound strongly. Since during DNA-cellulose chromatography of hupA-lon cells IHF did bind to the DNA column, it appears that under the conditions used for these purifications the binding of IHF to DNA-cellulose does not depend on the total amount of HU (almost the same amount of total HU is present in hupB cells and hupA-lon cells) but rather on a critical amount of the HU1 subunit. Even though the significance of this observation is not yet known, it is interesting to recall that HU and IHF have emerged from a common ancestor (1) and that similar observations have been made with IHF, showing that the overproduction of one subunit without the other leads to degradation in one case and insolubility in the other. In the case of degradation, the role of lon has not yet been demonstrated (27).

We would like to thank Jacqueline Plumbridge for her constructive criticism of this manuscript. We are grateful to Olivier Raibaud for his generous gift of strains and plasmids as well as for valuable discussions. The contributions of Susan Gottesman (for the gift of strains) and Howard Nash (for the gift of IHF antisemur) are gratefully acknowledged. This work was supported by the Centre de la Recherche Scientifique (UA1139) and the Association de la Recherche sur le Cancer.