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Increased sensitivity to γ irradiation in bacteria lacking protein HU

(bacterial chromatin/DNA damaging agent/repair mechanism/HMG1)

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ABSTRACT **The heterodimeric HU protein, isolated from *Escherichia coli*, is associated with the bacterial nucleoid and shares some properties with both histones and HMG proteins. It is the prototype of small bacterial DNA binding proteins with a pleiotropic role in the cell. HU participates in several biological processes like cell division, initiation of DNA replication, transposition, and other biochemical functions. We show here that bacteria lacking HU are extremely sensitive to γ irradiation. Expression of either one of the subunits of HU in the *hupAB* double mutant nearly restores the normal survival rate. This shows that the sensitivity is due to the absence of HU rather than being the result of a secondary mutation occurring in the *hupAB* cells or a modification of the SOS repair system, since SOS genes are induced normally in the absence of HU. Finally, *in vitro* studies give an indication of its potential role: HU protects DNA against cleavage by γ-rays.**

The HU protein was isolated from *Escherichia coli* (1) as a heterodimer composed of two homologous subunits HU_α and HU_β (2) encoded by the *hupA* and *hupB* genes (3). This abundant DNA binding protein shares with the eukaryotic histones the properties of introducing negative supercoils into relaxed, circular DNA in the presence of topoisomerase I (4), of being well conserved during evolution (5), and of having an amino acid composition similar to histones H1 and H2B (1). On the other hand, the protein HM that was initially isolated from yeast mitochondria as a functional homologue of HU (6) was recently shown to share sequence homology with HMG1 rather than with HU (7). Interestingly, however, HU can replace HM, indicating a strong homology of functions in the mitochondria (7). *In vitro* HU, like HMG1, was shown to bind strongly to cruciform DNA (8). Chemical footprinting of HU–cruciform DNA complexes shows that two HU dimers bind at two of the four angles of this four-way junction with high affinity and in a noncooperative fashion (9).

HU is a major constituent of the bacterial nucleoid (10) in the same way that histones and HMG proteins are the major chromosomal proteins of the eukaryotic cell. Besides the structural role of these proteins in compacting DNA, it can be asked whether histones, HMGs, or HU take part in regulating major processes of the cell by forming an essential link between structure and function. Single HU mutants *hupB* and *hupA* and *hupAB* double mutants have been constructed (11) and their phenotypes confirmed that HU indeed plays a pleiotropic role in bacteria. The absence of HU affects replication, transcription, recombination, transposition, and other biochemical functions. It was of interest to establish whether HU also affects one of the essential mechanisms that maintains the integrity of the cell by protecting its DNA against alterations caused by irradiation. To analyze the possible role of HU in DNA repair, we exposed wild-type or mutant strains, lacking HU partially or completely, to ionizing radiation. We show that strains lacking both HU subunits are very sensitive to γ irradiation.

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MATERIALS AND METHODS

Bacterial Strains, Plasmids, and Media. The bacterial strains and plasmids used are listed in Table 1. Standard P1 transductions, performed as described by Silhavy *et al.* (12), were used for construction of the strains. The fusion *recA*::*lacZ* and *sfiA*::*lacZ* carried, respectively, by λG214 (13) and λG172 (14) were introduced by lysogenization into MG1 Δ*lac*::Tn*10*, as described by Silhavy (12). After isolation of stable lysogens, the *hupA*::Cm and *hupB*::Km alleles (Cm, chloramphenicol; Km, kanamycin) were introduced by P1 transduction. The different *hup* mutants were constructed by P1 transduction as described (11). Bacteria were grown in Luria broth (LB) medium and in LB agar. Tetracycline, Cm, Km, and ampicillin were used at 10, 20, 80, and 40 μg/ml, respectively.

UV-Irradiation Experiments. Bacteria were grown in M9 medium (1× M9 salts/0.4% Casamino acids/0.4% glucose/5 μg of thiamine per ml/10^{-3} M $MgSO_4$/10^{-4} M $CaCl_2$) until an A_{600} of 0.3 and were washed once with M9 buffer (1× M9 salts/10^{-3} M $MgSO_4$/10^{-4} M $CaCl_2$). For UV irradiation, cells were suspended in the same M9 buffer at 10^9 cells per ml and were irradiated in an open glass Petri dish at room temperature in the absence of direct illumination, with a UV germicide lamp (Gelman) at 253.7 nm (without filter), and set at a distance of 45 cm from the bacteria suspension. For assays of β-galactosidase, 0.8 ml of irradiated cells was incubated at 37°C with 5 ml of prewarmed M9 medium. At the indicated times, the cell density was measured at 600 nm and 100 μl of culture was removed and cooled. The assay was performed as indicated by Miller (15) after treatment with toluene; 100% of induction represents the control without irradiation.

γ Irradiation of Bacteria. To measure the number of surviving bacteria after irradiation, the different strains were grown in LB at 37°C to 2× 10^8 cells per ml. Before irradiation, cells were washed with M9 buffer supplemented with 170 mM NaCl (2 ml of culture, washed five times with 10 ml of buffer) and resuspended into the same buffer. Cell suspensions (2× 10^8 cells per ml) were irradiated with the indicated doses (Gy) of γ-rays with a ^{60}Co source at a dose rate of 17.3 Gy/min. Several appropriate dilutions of irradiated cells were plated in duplicate on LB agar. Plates were incubated at 37°C and colonies were counted after 30 h of incubation. Each experiment was repeated two or three times with each sampling in duplicate to calculate SD values, which are given as bars of errors on the survival curves.

γ Irradiation of DNA and Protein–DNA Complexes. HU protein was purified according to Rouviere-Yaniv and Kjeldgaard (2) followed by an additional step of carboxymethyl

Abbreviations: Cm, chloramphenicol; Km, kanamycin.

Table 1. Bacterial strains and plasmids

Strains and plasmids	Relevant genotype	Ref.
AB1157	*thr-1 ara-14 leuB6 Δ(gpt-proA)62 lacY1 tsx-33 supE44 galK2 hisG4 rpsL31 xyl-5 mtl-L argE3 thi-1*	B. J. Bachmann
JR1648	AB1157 *recA13*	R. Devoret
JR1713	AB1157 Δ*recA*	This work
MG1	*thr leuB6 pro lacY1 thi tonA21 supE44* rK^- mK^+	R. Davies
JR1855	MG1 Δ*lac*::λG272(*recA*::*lacZ*)	This work
JR1863	MG1 Δ*lac*::λG172 (*sfiA*::*lacZ*)	This work
pMW1	pBR322 with *hupB* gene	3
pMWKP	pBR322 with *hupB*::Km	12
pKO1	pUC9 with *hupA* gene	3
p387	pUC9 with *hupA*::Cm	12

cellulose chromatography under denaturing conditions. HMG1 protein was provided by F. Strauss and H-NS protein was provided by S. Rimsky and H. Buc. Plasmid pBR322 was purified by using a Qiagen kit. pBR322 DNA diluted in phosphate buffer (10 mM KH_2PO_4/50 mM NaCl, pH 7.4) at a concentration of 66 μg/ml was exposed to γ-rays (0–60 Gy) at 0°C in the presence or absence of the indicated proteins. In Fig. 5*D*, the irradiated DNA was deproteinized by phenol/chloroform extraction, precipitated by ethanol/sodium acetate, and redissolved in TE buffer before electrophoresis. DNA suspensions were then applied to an agarose slab electrophoresis gel (1% agarose in 40 mM Tris·HCl, pH 7.9/5 mM sodium acetate/1 mM EDTA), run overnight at 40 V, and stained with ethidium bromide at a concentration of 0.5 μg/ml for 30 min.

RESULTS

Sensitivity of *hupAB* Double Mutants to γ Irradiation. Although HU is not essential to *E. coli*, cells totally lacking HU grow slower than normal. The doubling time in LB at 37°C of a *hupAB* mutant (90 min) is much longer than that of either single mutants (30 min) or the wild-type strain (25 min). To estimate the possible effect of ionizing irradiation, we measured the survival of the different *hup* mutants after their irradiation with increasing doses of γ-rays. The survival curves were compared to those of the isogenic hup^+ cells of a *recA13* mutant. It is well established that mutations in the *recA* gene (*recA13* or Δ*recA*) strongly sensitize cells to killing by ionizing radiation, indicating that a powerful RecA-dependent repair system is crucial for the survival of damaged cells (16).

As shown in Fig. 1, survival of the *hupAB* double mutant decreased dramatically with the increase in Gy compared to control AB1157 hup^+ cells. This isogenic *hup* wild-type strain exhibits, as expected, a significant resistance to irradiation and its survival curve is characterized by an important shoulder. In comparison, the curve of the strain lacking RecA, an essential protein for DNA repair, is sigmoidal and the lethal dose leaving 50% survivors (LD_{50}) is ≈14 Gy. This LD is 54 Gy for the *hupAB* mutant. These values represent a 12-fold increase in the sensitivity of the *recA* strain and a 5-fold increase for the *hupAB* strain compared to the wild-type strain ($recA^+hup^+$). Several independently constructed *hupAB* double mutants gave very similar survival curves (results not shown). At first view, the survival curve of the *hupAB* strain also seems sigmoidal. Clearly, at high doses of irradiation the curve parallels the sigmoidal curve of the *recA* strain; however, at low doses of irradiation the difference between the two curves is much more pronounced. To explore a possible duality in the response of the *hupAB* mutant to ionizing irradiation, the LD of *hupAB* was compared to the LD of the *recA* strain, both measured at different points of their survival. Fig. 2 shows the ratios of the LDs of the *hupAB* mutant compared to that of the *recA* mutant. This ratio is not constant but decreases much faster when the radiation increased, demonstrating the duality of the response of *hupAB* survival.

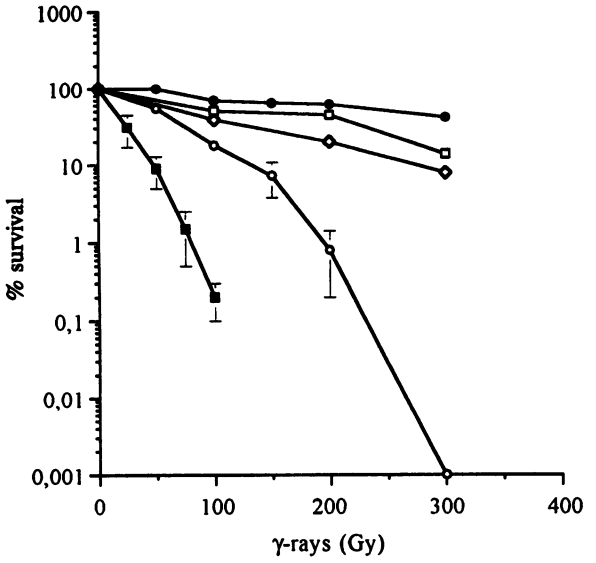
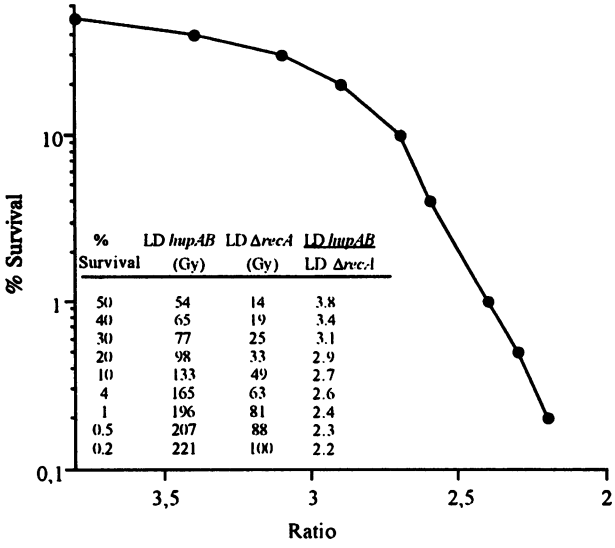


FIG. 1. Response of AB1157 *hup* mutants to ^{60}Co irradiation. Strains were irradiated with the indicated doses of damaging agent and survival was measured as described. ●, Wild type; □, *hupB*; ◇, *hupA*; ○, *hupAB*; ■, *recA13*. For the three upper curves the SEM was too small to be visible.

Fig. 1 also shows the survival curves of the single *hupA* and *hupB* mutants. Single *hup* mutants lacking either the α or β subunit of HU are not significantly affected by the ionizing radiation, at least up to a dose of 100–150 Gy. At higher doses, the *hupA* mutant exhibits a slightly higher sensitivity than the *hupB* mutant, which in turn is more sensitive than the wild-type strain. The sensitivity of the double mutant is clearly higher than that of the most sensitive of the single mutants and can be considered as a nonepistatic interaction of the two mutations. In fact, the increased sensitivity of *hup* mutants seems inversely proportional to the quantity of HU still present in the



% Survival	LD *hupAB* (Gy)	LD Δ*recA* (Gy)	LD *hupAB* / LD Δ*recA*
50	54	14	3.8
40	65	19	3.4
30	77	25	3.1
20	98	33	2.9
10	133	49	2.7
4	165	63	2.6
1	196	81	2.4
0.5	207	88	2.3
0.2	221	100	2.2

FIG. 2. Ratio of LDs of *hupAB* mutant compared to that of the Δ*recA* mutant.

cells since we have shown that a *hupB* mutant contains as α2 homodimers 50% of the $HU_{\alpha\beta}$ present in wild-type strains, while *hupA* mutants contain only 10% of HU, present as β2 dimers. The β2 homodimers were shown to be degraded in the absence of the α subunit by a Lon-dependent process (17).

Effect of the Overproduction of α2 or β2 Homodimers on the Sensitivity to γ-Rays. The severe defect in DNA repair observed in the *hupAB* mutants certainly could be due to the absence of HU, but it could also be the consequence of secondary mutations, which we know accumulate in the *hup* double mutants to compensate for the absence of HU (11). The fact that either of the *hup* single mutants behaved almost like a wild-type strain could indicate that HU homodimers $HU_{\alpha 2}$ and $HU_{\beta 2}$ are equivalent for cell survival to HU heterodimers $HU_{\alpha\beta}$.

To test this proposal and to exclude the possibility that the defect in repairing ionizing radiation damage observed in the *hupAB* mutants was due to compensatory mutations, we introduced into *hupAB* mutants a plasmid carrying the gene coding for either HU_{α} or HU_{β}. Overproduction of the α subunit is deleterious for the cell even in a bacteria completely lacking HU, where a large lag is observed upon dilution from an overnight culture before the cells started to divide. Contrary to that, the presence of extra *hupB* genes is sufficient to render the *hupAB* mutant healthier. This difference may be related to the observation that α2 homodimers accumulated to higher concentrations than the β2 homodimers (data not shown). In any case, the production of either α2 or β2 homodimers in the *hupAB* mutant increased the resistance of *hupAB* mutants to γ irradiation to nearly the level of the control cells (Fig. 3). They behave in fact as the single *hup* mutants (see Fig. 1). To disregard the possibility that the plasmid by itself was responsible for the resistance observed in transformed cells, we used as controls these plasmids carrying the disrupted derivative genes in place of the intact genes. Transformation with plasmids carrying either the *hupA* or the *hupB* interrupted genes did not affect the sensitivity of the *hupAB* mutant (Fig. 3). These experiments show that a normal repair mechanism after γ irradiation by ^{60}Co is restored in the *hupAB* double mutant because of the presence of either α2 or β2 homodimer encoded by plasmids carrying one or the other of the *hup* genes.

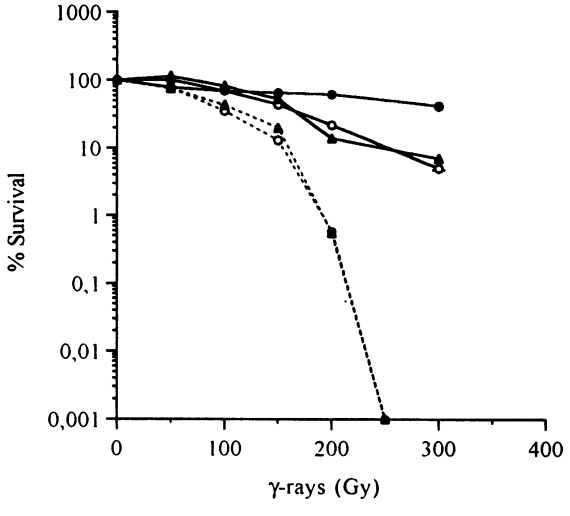


FIG. 3. Effect of overproduction of HU homodimers (α2 or β2) on sensitivity to ^{60}Co irradiation. The AB1157 *hupAB* mutant transformed with either pMW1 (*hupB* gene) or pKO1 (*hupA* gene) were grown in the presence of ampicillin (20 μg/ml) and irradiated with the indicated doses of ^{60}Co. Plasmids carrying the interrupted genes (*hupB*::Km or *hupA*::Cm) were used as controls. ●, Wild type; ▲–▲, *hupAB*/phupB; ▲- - -▲, *hupAB*/phupB::Km; ○–○, *hupAB*/phupA; ○- - -○, *hupAB*/phusA::Cm. For this experiment, the SEM was too small to be visible.

Normal SOS Response in *hupAB* Strains. Since it was shown that the presence of an active RecA protein is required for induction of the repair system responding to genotoxic aggression (ref. 16; see Fig. 1), it was essential to check whether the RecA protein and the SOS response function normally in a *hupAB* mutant. To explore this eventuality, strains carrying a *recA*::*lacZ* fusion or a *sfiA*::*lacZ* fusion in a *hup*$^+$ or *hupAB* background were constructed as described. The SOS response was induced in exponentially growing cells by UV irradiation for 20, 50, or 70 J/m^2 and the β-galactosidase activities were measured. Fig. 4 shows that the activity of both fusions is increased after UV irradiation nearly independently of the presence of HU. Fig. 4*A* shows that the induction of the *recA*::*lacZ* fusion after UV irradiation has the same profile and the same maxima in *hup*$^+$ and *hupAB* strains. Even though the induction of *sfiA* is slightly inhibited in the absence of HU early in the induction (20 J/m^2), this inhibition is not observed at 50 J/m^2 (data not shown) and at 70 J/m^2 (Fig. 4*B*), implicating a normal activation of the RecA protein itself. Similarly, γ irradiation induced both *lacZ* fusions to a similar extent in *hup*$^+$ and *hupAB* strains (data not shown). These results imply that the absence of HU does not curtail rapid induction of the SOS machinery.

Construction of a Triple Mutant *hupAB* Δ*recA*. Since the experiments described above indicated clearly that the deficiency in repair observed in cells lacking HU is not due to a direct effect on RecA protein, it was of interest to determine whether the effect of HU is additive to that of RecA. For this purpose, we attempted to introduce the deletion of the *recA* gene into the *hupAB* double mutant in the AB1157 background. The frequency of transduction of a Δ*recA* (Tn*10*) mutation in a *hupAB* host was lower than that obtained with an isogenic *hup*$^+$ strain (5×10^{-2}). In addition, the tetracycline-resistant colonies that were obtained grew very poorly, even compared to the *hupAB* mutant. Even if their doubling time in liquid medium was longer than that of the *hupAB* double mutant, their sensitivity to killing by ^{60}Co was roughly equal to that of Δ*recA* strains (data not shown). These results indicate that HU does not contribute, at least to a clearly detectable level, to the action directed by RecA protein in the DNA repair mechanisms.

HU Protein Protects DNA from Radiation Damage. Since we isolated HU as a histone-like protein, which can *in vitro* condense DNA as much as the four histones and therefore renders DNA molecules extremely compact (4), we investigated whether HU *in vitro* could protect DNA molecules against γ-rays. DNA–HU complexes prepared at two different concentrations of HU were irradiated and then analyzed on agarose gels. Fig. 5*A* shows that in the absence of HU most of

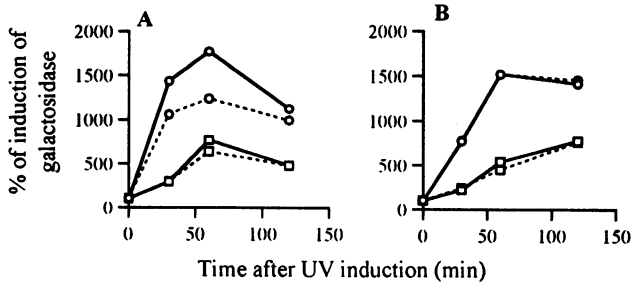


FIG. 4. Induction of SOS response after irradiation with UV light. MG1 wild-type and MG1 *hupAB* strains carrying the *recA*::*lacZ* or *sfiA*::*lacZ* fusions were irradiated with 20 (*A*) and 70 (*B*) J/m^2 and β-galactosidase activities were measured after 30, 60, and 90 min of incubation; □, *recA*::*lacZ* fusion; ○, *sfiA*::*lacZ* fusion; ——, *hup*$^+$; - - -, *hupAB* mutant.

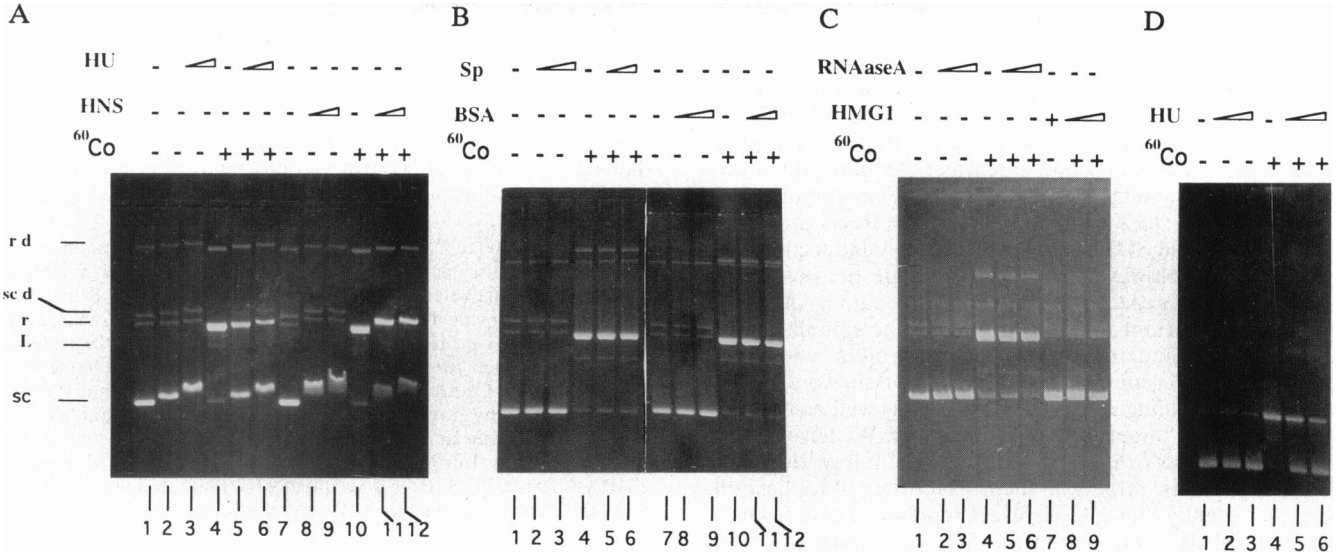


FIG. 5. Profile of damage induced in pBR322 DNA by γ-rays. DNA was irradiated (60 Gy) with or without the indicated proteins, and samples (1 μg of DNA) were analyzed on agarose gels. Two amounts of the indicated protein, corresponding to protein/DNA ratios of 1 and 2, were used. In *D*, the samples were deproteinized before electrophoresis. (*A*) Complexes pBR322–HU and pBR322–H-NS. (*B*) Complexes pBR322–BSA and pBR322–spermidine (Sp). (*C*) Complexes pBR322–RNase and pBR322–HMG1. (*D*) pBR322–HU complexes after deproteinization. SC, supercoiled DNA; L, linear DNA; r, relaxed nicked DNA; sc d, supercoiled dimers; r d, relaxed dimers.

the supercoiled pBR322 is converted into nicked and linear DNA by γ-rays (lane 1 vs. lane 4), while in its presence it is highly protected. It should be noted that the migration of the supercoiled DNA–HU complex is partially retarded (lanes 2 and 3 compared to lane 1). This retardation is due to a change in the structure of the supercoiled molecule of plasmid DNA since complete deproteinization of the complex prior to electrophoresis shows its integrity as illustrated in Fig. 5*D* (lanes 2 and 3 compared to lane 1).

Quantification of the data obtained after scanning the gels is given in Table 2. The comparison between lanes 2 and 3 vs. lanes 5 and 6 of Fig. 5*A* shows this protection, which seems to be proportional to the quantity of HU present in the complexes. Several controls indicate that protection is specific. Spermidine, which is known to counterbalance the negative charges of the DNA and to act on DNA structure, does not have this effect (Fig. 5*B*). H-NS, another bacterial protein that, like HU, is associated with the nucleoid, has, if any, only a very mild effect (Fig. 5*A*). However, similar to HU it also slows migration of the DNA in the gel (compare lane 7 to lanes 8 and 9), which proves its binding to DNA. Bovine serum albumin (BSA) neither shows this retardation effect on supercoiled DNA nor assures any protection against irradiation (Fig. 5*B*). Supercoiled pBR322 dimers present as a minority in this DNA preparation are extremely sensitive to γ-irradiation. Effectively, these supercoiled dimers, which are visible in nonirradiated samples, are completely absent after irradiation of naked DNA, while they are totally protected by HU. Contrary to this, none of them is protected in the H-NS–DNA complexes (Fig. 5*A*), which provides an additional argument in favor of the specificity of the action of HU.

Since a similarity of function between HU and HMG1 was emphasized by a number of recent studies (7–9, 18, 19), it was of interest to see if this specific action of HU was also shared by HMG1. Fig. 5*C* shows that effectively HMG1, similar to HU, can protect supercoiled DNA against cleavage.

Table 2. Distribution of DNA forms (percent) γ irradiated in the presence of HU

DNA form	pBR322	HU/pBR322 = 1	HU/pBR322 = 2
SC	14	53	82
r	74	42	14
l	12		
sc d		5	4

Data are from scan of gel of Fig. 5. Photographs were taken of gels stained with ethidium bromide using Polaroid 55 film, and negatives were scanned with a Gel Scanner (model 1312) C-R3A chromatopac (Shimadzu). Sc, supercoiled; r, relaxed nicked DNA; l, linear DNA; sc d, supercoiled dimer.

DISCUSSION

Ionizing radiation (x-rays and γ-rays) and UV light are known to have mutagenic and cytotoxic effects in different organisms as a result of various kinds of DNA damage—namely, single- and double-strand breaks, modification and damage of bases, and formation of apurinic/apyrimidinic sites (20, 21). Among these, the DNA double-strand break has been identified as the lesion most likely to be the cause of the lethal effects of ionizing radiation. We show here that HU plays a role in the resistance of *E. coli* to ionizing radiation. In the *hupAB* double mutant, which lacks HU, the mechanisms that maintain the integrity of the DNA by preventing or repairing the damage caused by the γ-rays are greatly altered. In contrast, these systems are not significantly modified in the single *hup* mutants compared to the *hup* wild-type strain, at least at low doses of irradiation. This means that for this special function, homomeric forms of HU, $HU_{\alpha 2}$ and $HU_{\beta 2}$, perform the same role as $\alpha\beta$ heterodimers usually present in the cell (2). This is not always the case since *in vitro* HU_{α}, and not HU_{β}, is able to introduce negative supercoiling into relaxed DNA, as does $HU_{\alpha\beta}$ (L. Claret and J.R.-Y., unpublished data). However, at high doses of γ-rays, the *hupA* mutant is more sensitive than the *hupB* mutant; this probably reflects the fact that it contains less HU than its *hupB* counterpart due to the instability of the remaining β2 homodimers (17).

We have shown that if HU is not essential for *E. coli*, growth and cell division are greatly perturbed in *hupAB* double mutants and the cells acquired compensatory mutations (11). The regaining of survival comparable to that of wild-type strain by the *hupAB* mutant transformed by a plasmid carrying either the *hupA* or the *hupB* gene eliminates the possibility that the role of HU described here was due to a secondary effect and not to the direct absence of HU.

Several hypotheses could explain this role of HU protein. It might protect the structure of the DNA against damaging agents, it might act on the DNA repair process itself, or it might do both. The repair of DNA double-strand breaks in *E. coli* requires the SOS response and, consequently, an active RecA protein. From the data on the behavior of the *recA::lacZ* fusion, it appears that HU does not affect the induction of the *recA*-dependent system for DNA repair. Furthermore, the induction the *sfiA::lacZ* fusion shows that the RecA protein is active and that the SOS response is normally induced in the absence of HU. However, we cannot exclude the possibility that HU increases the efficiency or the accuracy of DNA repair. One important role of HU may be the stimulation or inhibition of the interaction of regulatory proteins with their specific targets. Flashner and Gralla (22) have shown that HU stimulates the binding of the CRP protein as well as the Lac repressor to their targets on the *lac* operator. We have shown that HU stimulates or inhibits the binding of integration host factor to its specific targets on the *E. coli* origin of replication and more recently that HU displaces repressor LexA from at least three of its operators on the SOS promotors (23, 24). Similarly, HU could act by facilitating the action of other DNA binding proteins, particularly those involved in DNA repair. HU could also participate in DNA repair by being present in the vicinity of the damage to signal the target to the repair system, reacting or not, with the DNA radicals (or with chemical radioprotectors) causing reconstitution of the original molecule. In the absence of HU, these radicals would react with oxygen, causing fixation of the damage. Furthermore, we cannot exclude that HU introduces structural changes in the DNA molecules, making them more resistant to ionizing damages. Changes in the DNA UV photochemistry were observed recently when the small, acid-soluble spore proteins (SASPs) found in dormant spores of *Bacillus subtilis* bind DNA (25). These alterations of the chemical reactions affecting DNA after UV irradiation by the SASPs could be explained by the transition from B to A-like configuration of the DNA molecules. Since the spore proteins can cover the entire DNA molecule while HU, which is less abundant, can cover $\approx 1/5$th of the DNA, it is difficult to believe that HU could perform a similar action *in vivo*.

Our *in vitro* data clearly demonstrate that HU functions, at least partially, by protecting DNA against radiation-induced cleavage. HU, like HMG1, protects DNA against γ-ray-induced breaks *in vitro*. We believe this can be explained by the compaction of the DNA induced by HU. Consequently, the absence of HU in the cell could considerably increase the number of nicks and double-strand breaks and by exhausting the repair machinery could impair their reparation. The nonhomogeneous survival curve of the double mutant as a function of irradiation (Fig. 3) could, in fact, support this hypothesis.

In addition to an active RecA protein, the repair of DNA double-strand breaks in *E. coli* also requires the presence of another DNA duplex that has the same base sequence as the broken DNA (26). HU could affect this second requirement, which is to furnish an intact copy of the damaged chromosome. Certainly, as indicated by the irradiation of pBR322 DNA, the absence of HU could result in a large increase in breaks, thereby diminishing the probability of having an intact nucleoid. But a more direct effect could arise from an impaired mechanism of nucleoid segregation itself. We know that cell division is perturbed by the absence of HU, that a high percentage of anucleate cells (10–15%) are produced (11), and that the normal segregation of the bacterial nucleoid is affected.

In conclusion, our results clearly demonstrate that a small histone-like protein, HU, plays a role in protecting the bacterial chromosomal DNA against lethal effects of radiation damage. It will be of interest to determine whether histones and HMG proteins play a similar role in eukaryotic living cells.

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