Reversible Induction of ATP Synthesis by DNA Damage and Repair in Escherichia coli

IN VIVO NMR STUDIES

(Received for publication, February 20, 1998, and in revised form, September 3, 1998)

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Early metabolic events in Escherichia coli exposed to nalidixic acid, a topoisomerase II inhibitor and an
inducer of the SOS system, were investigated by in vivo NMR spectroscopy, a technique that permits monitoring
of bacteria under controlled physiological conditions. The energetics of AB1157 (wild type) and of its isogenic,
SOS-defective mutants, recBC, lexA, and ΔrecA, were studied by 31P and 19F NMR before, during, and after
exposure to nalidixic acid. The content of the NTP in E. coli embedded in agarose beads and perfused at 36 °C
was found to be 4.3 ± 1.1 × 10−18 mol/cell, yielding a concentration of ∼2.7 ± 0.7 mm. Nalidixic acid induced in
the wild type and mutants a rapid 2-fold increase in the content of the NTP, predominantly ATP. This induction
did not involve synthesis of uracil derivatives or breakdown of RNA and caused cell proliferation to stop. Re-
moval of nalidixic acid after 40 min of treatment rescued the cells and resulted in a decrease of ATP to control
levels and resumption of proliferation. However, in ΔrecA cells, which were more sensitive to the activity of
the drug, ATP elevation could not be reversed, and ATP content continued to increase faster than in control
cells. The results ruled out association between the elevation of ATP and the induction of the SOS system and
suggested involvement of a process reminiscent of apoptosis in the stimulation of ATP synthesis. Thus, the
presence of the RecA protein was found to be essential for reversing the ATP increase and cell rescue, possibly
by its function in repair of DNA damage.

Several antitumor and antibacterial drugs were shown to induce a rapid elevation in the total pool of the NTPs in pro-
karyotes and eukaryotes (1–7). In Escherichia coli, bleomycin and UV radiation, which are also bacterial SOS activators,
were shown to induce a rapid, transient, and 2-fold increase in ATP concentration (1, 2). The pronounced elevation of ATP was
shown to be independent of oxidative phosphorylation and was therefore attributed to an unknown intracellular phosphoryla-
tion pathway (1). In addition, with UV a delayed and sub-
tially smaller increase in ATP was found to occur in the recBC mutant (1), whereas a complete inhibition of this increase

* This work was supported by German Israel Foundation Grant 0368.
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and $^{19}$F was applied to monitor fluoronucleotides derived from 5-fluorouracil (FUra)\(^3\) incorporation. The involvement of the SOS response in the observed metabolic alterations was investigated by using recA, lexA, and recBC mutants in which the SOS system cannot be induced. Nalidixic acid was shown to induce a rapid increase in the content of the NTPs, predominantly ATP. This induction was found to be independent of the SOS response and appeared to be associated with an apoptotic-like process.

**EXPERIMENTAL PROCEDURES**

**E. coli Mutants**—The bacterial strains used were derivatives of *E. coli* K-12 (Table I). A deletion of recA was introduced into AB1157 cells by P1 transduction using WBM535 (20) as the donor strain (21). The presence of the recA genotype was verified by the extreme sensitivity of the mutant to UV irradiation at 254 nm.

**Cell Cultures**—The standard growth medium for culturing the bacteria contained 1.46 mM NaHPO\(_4\), 0.73 mM KH\(_2\)PO\(_4\), 82.6 mM Tris base, 100 mM NaCl, 19 mM NH\(_4\)Cl, 20 mM KCl, 10 mg/ml thiamine (B1), 1 mM MgSO\(_4\), 0.1 mM CaCl\(_2\), 0.012 mM FeCl\(_3\), 11 mM glucose, and amino acid solution (100 ml/liter; Sigma). The pH of this medium was adjusted to 7.5. The concentration of P, in this medium, was low, to bring the intensity of the P\(_i\) signal close to the intensities of the intracellular phosphate signals of the \(^{31}\)P NMR spectra. We verified that this concentration was sufficient to maintain the same growth rate as in the high phosphate medium (generation time, ~45 min for wild type, lexA3, and rec A mutants and 60 min for the recBC mutant). In experiments with FUra (Sigma), cells were cultivated for several generations in the standard growth medium supplemented with 10–100 \(\mu\)M FUra.

**Survival Assay**—*E. coli* in mid-logarithmic growth (\(A = 0.5\) at 595 nm) were exposed for 40 min to low concentrations of nalidixic acid (Sigma): 0.4 \(\mu\)g/ml and 1.4 \(\mu\)g/ml, equivalent to 1.6 \(\times\) 10\(^{-9}\) and 5 \(\times\) 10\(^{-9}\) \(\mu\)g/cell, respectively, or a high concentration of 40 \(\mu\)g/ml, equivalent to 1.6 \(\times\) 10\(^{-7}\) \(\mu\)g/cell. Aliquots (100 \(\mu\)l) of the treated and untreated cultures were plated on Luria agar plates. The surviving colonies were counted after an overnight incubation at 37 °C.

**Transmission Electron Microscopy**—*E. coli* was grown in Luciani medium to the mid-logarithmic phase of growth. Bacteria were treated with nalidixic acid (40 \(\mu\)g/ml) for 40 min. Control and treated bacteria were washed twice with Luciani medium and then centrifuged. The pellets were fixed in 2.5% glutaraldehyde in cacodylate buffer (pH 7.4) and post-fixed in 1% osmium tetraoxide for 2 h. The samples were then dehydrated in graded ethanol solutions and embedded in Epon (Polared 812 resin, Polaron, Watford, United Kingdom). Ultra thin sections were constructed with uranyl acetate and lead citrate and were examined with a Jeol JEM 1200 EXII microscope at 80 kV.

**NMR Experiments**—Encapsulation of *E. coli* for NMR experiments: This method was adapted from the encapsulation method of the alga *Dunaliella* (22). A day before an NMR experiment, ~9 \(\times\) 10\(^{10}\) cells were harvested in the mid-logarithmic phase of growth, resuspended in 3 ml of fresh growth medium and incubated briefly (~5 min) at 37 °C. The suspension was then mixed thoroughly with 3 ml of 6% low gelling paraffin oil and washed several times with fresh growth medium and kept at 4 °C overnight. The next day, 2.5 ml of the harvested agarose beads were transferred to a 10-mm NMR tube. The sample was placed in the spectrometer, and the cells embedded in the agarose beads were perfused at 36 ± 1 °C under sterile conditions, as described below.

**Medium for NMR**—The standard growth medium described above was slightly modified for NMR use, containing a higher concentration of glucose (90 mM) and Tris base (165 mM) and a lower concentration of NaCl (57 mM). We verified that the growth rate in this modified medium was the same as in the standard medium with a generation time of ~45 min. The increased glucose supply and increased buffer capacity (because of Tris) ensured adequate nutrition and a stable pH during the NMR experiments. In NMR experiments with FUra, the perfusion medium also contained 15 \(\mu\)M of the drug.

Nalidixic acid was added at a concentration of 140 \(\mu\)g/ml, which was equivalent to a dose of ~9.3 \(\times\) 10\(^{-9}\) \(\mu\)g/cell. Below this concentration no change in the spectra could be observed, whereas a higher dose (~50%) yielded similar results.

**Perfusion System**—A perfusion system, previously developed in our laboratory (22), was adapted for the NMR studies (Fig. 1). The cells were perfused unidirectionally at a flow rate of 3 ml/min to ensure an adequate supply of glucose and oxygen. A bubble trap was added in the delivery line to prevent bubbles from entering the tube and perturbing the homogeneity of the magnetic field. The temperature in the NMR tube was adjusted to 36 ± 1 °C during the NMR experiments, using the variable temperature control unit (Bruker). The medium container outside of the spectrometer was saturated with humidified oxygen (100%) and kept at 39 ± 1 °C. Medium was changed by replacing the whole container with a new one containing the desired medium (e.g., with a fresh medium or one containing the drug).

**NMR Measurements**—NMR measurements were performed with a Bruker AM-500 spectrometer equipped with a quadrupole-nuclei (\(^1\)H, \(^{19}\)F, \(^{13}\)C, and \(^{15}\)N) probe or a Bruker AMX-400 spectrometer equipped with a triple-tuned (\(^1\)H, \(^{19}\)F, and \(^{13}\)C) probe and software-controlled probe. \(^{31}\)P spectra recorded at 202.5 MHz were obtained by collecting 760 transients within 13 min, applying 60° pulses with a 1-s repetition time, and proton decoupling with a composite pulse decoupling sequence of ~1 watt. \(^{31}\)P spectra recorded at 164 MHz were obtained by collecting 1900 transients within 8.8 min, applying 55° pulses with a 0.42-s repetition time, and composite pulse decoupling during acquisition as above. \(^{19}\)F spectra recorded at 376 MHz were obtained by collecting 1400 transients within 8.5 min, applying 60° pulses with a 0.24-s repetition time, and composite pulse decoupling during acquisition. The \(^{19}\)F and \(^{31}\)P spectra were recorded sequentially. In both spectrometers, the temperature of the sample was maintained at 36 ± 1 °C with a Bruker variable temperature control unit.

**Analysis of NMR Data**—The \(^{31}\)P and \(^{19}\)F chemical shifts were determined in reference to the \(vNTP\) signal at ~10.04 ppm and to the FUra signal at ~171 ppm, respectively. Changes in the areas or the intensities of each signal were directly proportional to changes in the content of the corresponding metabolite. The areas of the signals were determined either by line shape simulation using the Bruker GLINFIT program or by applying the integral mode of the spectrometer. A comparative analysis of the relative changes in areas or intensities of the NTP signals yielded similar results that were within experimental error. NTP content per cell was estimated from the signal area of \(vNTP\) in the \(^{31}\)P spectra of perfused cells (for example, see Fig. 2) in reference to the signal area of P\(_i\) attributable predominantly to the 2.18 mM inorganic phosphate in the medium, taking into account saturation effects as a result of the NMR acquisition parameters and the differences in the \(T_1\) relaxation rates of \(vNTP\) (\(T_1 = 0.7\) s) and P\(_i\) (\(T_1 = 5.4\) s).

\(^3\) The abbreviations used are: FUra, 5-fluorouracil; NDP, nucleoside diphosphate.
RESULTS

In this study, the high cell density in the NMR sample and the high metabolic activity of the cells required a rapid and continuous supply of nutrients and oxygen, as well as a fast removal of inhibitory products and prevention of medium acidification. These demands were met by perfusing cells embedded in agarose beads unidirectionally, at a high rate (3 ml/min), with medium saturated with oxygen, containing a high concentration of glucose (90 mM) and Tris base (165 mM) to achieve high buffer capacity.

The optimal concentrations of glucose and the buffer as well as the rate of perfusion were determined by modifying these parameters and measuring the NTP levels of the perfused cells by $^{31}$P NMR, aiming to reach maximal and reproducible levels within consecutive recordings (each for 12 min). The initial number of cells in the beads was measured before the encapsulation. However, it was not possible to determine exactly the number of cells at the end of the experiments, because isolation of the cells from the beads led to substantial cell death. At the start of each NMR experiment, the intensities of the phosphate signals were very low, close to the noise level. However, within the first 1–2 h of adaptation to the NMR perfusion conditions (at 36 °C), the intracellular phosphate signals including the three NTP resonances became detectable. From the area of the NTP signal relative to that of the external Pi signal (2.18 mM), taking into account saturation effects, the amount of NTP per cell was found to be $4.3 \pm 1.1 \times 10^{-18}$ mol/cell, yielding a cellular NTP concentration of $2.7 \pm 0.7$ mM. In control experiments, under constant perfusion conditions for 7 h, a large increase in the NTP signals was observed (Figs. 2–4). This increase can be attributed only to an increase in the number of cells, because cellular NTP concentration remains the same under constant conditions (e.g. constant supply of nutrients and oxygen, constant pH, and constant temperature). The increase in NTP was logarithmic and reached saturation levels as expected for cell growth curves. From this logarithmic increase a generation time of 100 min was determined for E. coli entrapped in beads. This generation time was twice as long as that measured in suspension (~45 min), presumably because of the high density of the bacteria in the agarose beads.

The $^{31}$P NMR spectra of cells perfused at 36 °C exhibited in addition to the NTP signals phosphomonoester signals, Pi signal predominantly attributable to the medium Pi (2.18 mM), and NAD and uridine diphosphosugar signals (Fig. 2). The signals of αNDP and βNDP overlap with the signals of αNTP and γNTP, respectively. The area of the γNTP plus βNDP signal was the same, within experimental error, as that of βNTP. We can therefore conclude that the content of NDP under the NMR perfusion conditions is below detection level, namely, [NDP] is <20% of [NTP]. The uridine diphosphosugar concentration in the cells was similar to the concentration of NTP.

Changes in the phosphate metabolites induced by exposure to nalidixic acid were studied by $^{31}$P NMR in wild type, and the recBC, lexA, and ΔrecA mutants. In each experiment, two separate samples of the same cell preparation were monitored one after the other. The first sample was treated transiently with nalidixic acid (140 μg/ml), whereas the second one was continuously perfused with nalidixic acid-free medium and served as a control. Administration of nalidixic acid (for 40 min or 3 h) induced a rapid increase of ~2-fold in the NTPs of all the strains tested (Figs. 3 and 4). Specifically, the extent in NTP elevation was $2.00 \pm 0.20$ (n = 6) for E. coli wild type, $2.10 \pm 0.08$ (n = 2) for recBC, $2.2 (n = 1)$ for lexA, and $1.7 \pm 0.03$ (n = 6) for ΔrecA.

FIG. 1. Scheme of a system designed to perfuse E. coli in a conventional NMR spectrometer.

FIG. 2. Representative $^{31}$P NMR spectrum and increase in NTP during growth of E. coli in the NMR spectrometer. AB1157 cells embedded in agarose beads were perfused in the NMR spectrometer at 36 ± 1 °C as described under “Experimental Procedures.” The spectrum was recorded 100 min after initiation of the NMR experiment. 760 transients were accumulated during 12 min. Exponential multiplication with a line broadening of 15 Hz was applied before the Fourier transformation. A.U., arbitrary unit; UDPS, uridine diphosphosugar; PME, phosphomonoesters.
**ATP Synthesis Induction in E. coli**

The main induction occurred during the first 20 min of exposure to nalidixic acid. At 40 min after the removal of nalidixic acid, the level of the NTPs remained high and did not return to control levels, and a further increase occurred thereafter, two to three times faster than in control cells (Fig. 3A). After removal of the nalidixic acid, the level of the NTPs decreased to that of controls and was then followed by a gradual increase. In parallel, the incorporation of FUra was retarded induction of NTPs, no change in the fluorinated uracil derivatives was observed.

**Fig. 5. The effect of 5-fluorouracil on the growth of AB1157 cells.** Cells were grown in suspension, and varying doses of FUra (5-FU) were added at 180 min as indicated by the arrow. ■, control; ● and ○, in the presence of 10 and 100 μM FUra, respectively.

**Fig. 3. Modulation in the γNTP signal intensity of E. coli in the course of short term (40 min) exposure to nalidixic acid.** A, AB1157 (wild type); B, JC5519 (recB21 recC22); C, DM49 (lexA3); D, ABE10 (ΔrecA). Cells embedded in beads were perfused in the NMR spectrometer at 36 ± 1°C. Filled arrows indicate the addition and open arrows the removal of nalidixic acid (140 μg/ml). The insets in A and D represent γNTP content in control cells, not exposed to the drug.

**Fig. 4. Modulation in the γNTP signal intensity of AB1157 cells in the course of long term (3-h) exposure to nalidixic acid.** Cells embedded in beads were perfused in the NMR spectrometer at 36 ± 1°C. Addition and removal of nalidixic acid (140 μg/ml) are indicated by filled and open arrows, respectively. Open and filled circles refer to control cells and to cells exposed to nalidixic acid, respectively. A.U., arbitrary unit.

3) for ΔrecA, and substantially higher than the increase attributable only to the growth of the bacteria (Figs. 3 and 4, insets). The main induction occurred during the first 20 min of exposure to the drug. In the cells treated for 3 h with nalidixic acid, the initial 2-fold increase was followed by an additional but slower increase for 2 more h (Fig. 4).

Removal of nalidixic acid from the perfusion medium after 40 min caused in E. coli wild type, recBC, and lexA a reduction in the induced NTP content to a level similar to that of untreated, control cells. This recovery was then followed by a gradual increase in the NTPs caused by normal cell growth. Exceptionally, in ΔrecA cells, despite removal of the nalidixic acid, the level of the NTPs remained high and did not return to control level, and a further increase occurred thereafter, two to three times faster than in ΔrecA control cells (Fig. 3D). Thus E. coli ΔrecA cells were not able to recover from the nalidixic acid insult, and the induction of ATP synthesis could not be reversed. Long term treatment with nalidixic acid of E. coli wild type also inhibited rescue of the cells (Fig. 4). The cells did not recover; on the contrary, despite removal of nalidixic acid after 3 h of exposure, a drastic ∼4 fold decrease in NTP had occurred, indicating cell death (Fig. 4). Thus, it appears that during the longer exposure to the drug, the cells entered a process from which they could not be rescued anymore.

Labeling of the NTP pool can provide a tool to monitor the source of the induction as a result of nalidixic acid administration. It was previously shown that when E. coli were grown in the presence of FUra, −70 to 95% of the FUra was incorporated into RNA (23, 24). Herein the incorporation of FUra was used as a tool to monitor changes in FUra derivatives during elevation of NTPs by nalidixic acid. The FUra derivatives included the soluble nucleotides F-UMP, F-UDP, and F-UTP, all of which can be monitored intracellularly by 19F NMR. In the presence of a low concentration of FUra, the growth of E. coli wild type was only slightly inhibited (Fig. 5). For the NMR experiments, wild type (n = 2) and ΔrecA (n = 2) were cultivated in the presence of a low concentration (15 μM) of FUra for 2–3 generations. The soluble nucleotides of the cells were then monitored by recording sequentially both 31P spectra, which measured the total NTP pool, and 19F spectra, which measured the content of fluorinated derivatives (Fig. 6).

31P spectra of wild-type cells demonstrated the transient induction in NTP level by nalidixic acid (Figs. 6A and 7A) as was found for FUra-free cells. The 19F spectra of these FUra-labeled cells exhibited two signals at 165.3 and 165.45 ppm, which were assigned, based on their chemical shift, to 5-FUra derivatives (Fig. 6B). These two separate, but close, signals increased during cell growth before the addition of nalidixic acid, in parallel to the increase in NTPs. However, in the presence of nalidixic acid no change in either of the two fluorinated derivatives was observed, even though there was a substantial increase in NTP (Figs. 6B and 7B). After removal of the nalidixic acid, the level of the NTPs decreased to that of controls and was then followed by a gradual increase. In parallel, the incorporation of FUra was restored, and a gradual increase in the fluorinated uracil derivatives was observed (Fig. 7B). Similar experiments with the ΔrecA mutant also showed that during the nalidixic acid-mediated induction of NTPs, no change in the fluorinated uracil derivatives was observed.

Nalidixic acid is known to act on E. coli as a bacteriocidal agent (25). However, to rule out the possibility that during the elevation in the ATP content nalidixic acid induced a faster cell growth, we have studied the effect of nalidixic acid on cell growth and survival. The ability of wild type and the ΔrecA mutant to survive in the presence of varying doses of nalidixic acid was monitored in cell suspensions, after treatment of the cells with the drug for 40 min, as in the NMR experiments. At the low doses of nalidixic acid (0.4 μg/ml = 1.6 × 10−9 μg/cell, and 1.4 μg/ml = 5 × 10−9 μg/cell) the growth of wild-type
bacteria was not affected, and the number of cells increased as in untreated cells (Fig. 8), whereas the growth of ΔrecA cells was arrested, and only a marginal increase in cell number had occurred. At the high dose (40 μg/ml = 1.6 × 10⁻⁷ μg/cell) the growth of wild-type bacteria was arrested, with no change in the initial cell number (Fig. 8), whereas a decrease in cell number indicating cell death had occurred in ΔrecA cells (Fig. 8). The dose applied in the NMR experiments was 140 μg/ml = 9 × 10⁻⁹ μg/cell. In terms of amount of drug per cell, it was closer to the lower doses in suspension (1.4–5 × 10⁻⁹ μg/cell) than to the high one (1.6 × 10⁻⁷ μg/cell). Indeed, the NMR studies of cells treated with nalidixic acid for 40 min did not indicate cell death (e.g. decline of ATP) during the experiments, suggesting that the survival under the NMR conditions was similar to that of cells in suspension treated with the low dose of nalidixic acid. However, during the longer duration of treatment (3 h) with nalidixic acid, the bacteriocidic capacity of this drug was exhibited by a marked decrease in NTP (Fig. 4).

In addition to the survival of cells in suspension, we also investigated morphological changes induced by nalidixic acid using transmission electron microscopy. Control E. coli demonstrated the typical elongated structure surrounded by an external envelope. The cytoplasm exhibited a bright nonconfined area in the center of the bacterium that contained a nucleoid with a dispersed appearance. The peripheral cytoplasm displayed an increased density with a granular appearance attributable to the large number of ribosomes (Fig. 9, A and C). After treatment with nalidixic acid, the cells appeared more dense with the nucleoid less dispersed and more localized (Fig. 9, B and D–F). Also, the difference between the density of the nucleoid and the density of the area with the ribosomes was less pronounced. In the majority of the nalidixic acid-treated cells we also observed inclusion bodies in the cytoplasm (Fig. 9, E and F). These inclusion bodies exhibited a crystalline-like structure and were generally linked to the cell membrane.

**DISCUSSION**

Changes in the energetics of E. coli cells after exposure to the SOS activator nalidixic acid were monitored in vivo using ³¹P and ¹⁹F NMR spectroscopy. The results demonstrated that nalidixic acid induced a rapid 2-fold increase in the content of the intracellular NTPs, predominantly ATP. A similar elevation of ATP level was previously demonstrated in E. coli treated with UV radiation (1, 3), as well as after treatment with bleomycin (2). However, we were not able to verify the effect of bleomycin in vivo, because concentrations up to 100 μg/ml did not cause a significant increase in NTP (data not shown).

The experimental procedure used previously to determine ATP concentration in E. coli was based on boiling the cells and
extracting the ATP. A calculation of the concentration of ATP in the cells, based on the results presented previously (1–3), indicated a very low content of ATP of \( \approx 4 \times 10^{-20} \) mol/cell, equivalent to a concentration of \( \approx 0.025 \) mM. Thus, it appears that this extraction method measured a small fraction of the total ATP pool, estimated to be \( \approx 3 \) mM (26). In contrast, we have determined by the NMR in vivo method an NTP content of \( 4.3 \times 10^{-18} \) mol/cell, which is equivalent to \( 2.7 \) mM. The concentration of ATP is \( \approx 70\% \) of the total NTP concentration, 1.9 mM, close to previous determinations (26).

We therefore attribute the discrepancy between our results and the results reported previously (1–3) to the difference in the methodology of the measurement.

It is well known that the induction of the SOS regulatory network is controlled by a complex circuitry that involves the RecA and LexA proteins (27). Thus, the nalidixic acid-induced increase in NTP in \( \Delta \text{recA} \) and \( \text{lexA} \) mutants clearly indicated that the elevation of NTP is independent of the SOS activity of the drug. The nalidixic acid-mediated induction of NTP in \( \text{rec}BC\)-defective strains further supports this conclusion, because it is known that nalidixic acid is unable to induce an SOS response in the absence of the RecBCD enzyme (12, 13). When the \( \text{rec}BC\)-defective mutant was exposed to UV irradiation or bleomycin, which also damaged DNA and induced the SOS response, the intracellular ATP did not increase markedly and rapidly (1, 2). This disagreement with our results could arise from variations in the sites of the DNA damage exerted by nalidixic acid and by UV irradiation.

In wild type and \( \text{lexA} \) and \( \text{rec}BC \) mutants treated transiently for 40 min with nalidixic acid, the level of the NTP pool declined to that of controls after removal of nalidixic acid. It was previously reported that in the \( \text{lexA}1 \) mutant the ATP level, with exposure to UV radiation or bleomycin, did not decline and remained elevated (2). As in the induction phase of NTP, our findings differ in the recovery phase as well and show a decline in \( \text{lexA} \) mutant after removal of nalidixic acid. Thus, our in vivo studies do not support the hypothesis previously proposed by Barbé and colleagues (1, 2) that the decrease in ATP during the recovery phase is attributable to cleavage of the LexA repressor by the RecA protease. This discrepancy, as the others described above, might be related to differences in the site of the DNA damage.

We have also followed in vivo changes induced by nalidixic acid in Fura-derived nucleotides using 19F NMR. It was previously demonstrated that in the presence of Fura the derived F-UTP is readily incorporated into all types of RNA, replacing mainly uracil (28). The incorporation of Fura into RNA over several generations provides a method for observing fluoronucleotides derived from RNA breakdown. At a relatively high dose of Fura (100 \( \mu\)M), incorporation into RNA was shown to modify cellular metabolism (28, 29). In the present study, a high dose of Fura retarded cell growth. However, at a low dose (\( \approx 10–20 \) \( \mu\)M) the rate of cell growth was only slightly reduced, and the energetics, exhibited by the phosphate profile of the cells, was not altered. Using this low dose of Fura enabled us to find out whether uracil nucleotides are involved in the NTPs induction by nalidixic acid and whether the NTPs originate from breakdown of RNA. The results clearly demonstrated that although nalidixic acid induced an increase in the NTPs, the F-UTP remained constant, and the Fura uptake and phosphorylation were totally inhibited. This further indicated that an enhanced synthesis of UTP from its soluble precursors, obtained either from breakdown of RNA or from uptake of Fura, is not involved in the nalidixic acid-induced increase of the NTPs. Previous results showing that nalidixic acid did not induce breakdown of RNA but rather caused DNA degradation (25) are in agreement with our results.

The initial response of \( \Delta \text{recA} \) cells to nalidixic acid was similar to that of the wild-type cells and the other mutants. However, the \( \Delta \text{recA} \) cells, unlike the other cells, could not recover after the removal of nalidixic acid, and the increase in ATP could not be reversed. Similar findings were previously reported for a mutant defective in RecA protein and for another mutant with deficient RecA protease activity (1, 3). It is known that RecA function is required for homologous recombination and for repair of DNA damage caused by DNA-damaging drugs and by UV radiation (30, 31). Furthermore, the recA gene product promotes recognition of homology and strand exchange between two homologous DNAs during both recombination and repair (32, 33). Thus, the inability of the \( \Delta \text{recA} \) mutant to recover from the DNA damage appeared to be related to the repair function of RecA; namely, in the absence of RecA the failure to repair DNA damage is responsible for the continuous irreversible increase in ATP.

It is interesting to note that the mammalian homologue of RecA is Rad51, which regulates the recombination and double-
stranded DNA repair in mammalian systems (34, 35). RecA and Rad51 proteins were shown to be similar in both structure and function; the primary sequences of the two proteins showed significant homology, and both displayed ATP-dependent DNA binding (36). The failure to properly repair DNA damage in the absence of Rad51 appears to be in the pathway of the p53 checkpoint, leading to cell cycle arrest or apoptosis of mammalian cells (34, 37).

It has been recently recognized that programmed cell death is a well-established process in the microbial world too (38). For example, binding of microcin B17 and CcdB to DNA gyrase (E. coli topoisomerase II) can cause double-stranded DNA breaks (39) and inhibition of the gyrase (40). These changes triggered the SOS response followed by cell death, reminiscent of apoptosis (40). It is important to note that DNA gyrase, the cellular target of microcin B17 and CcdB protein, is also the target of nalidixic acid, and therefore DNA degradation and cell death induced by nalidixic acid might be likened to apoptosis too. Moreover, CcdB-induced cell killing does not require the host enzymes RecA and RecBC, which are needed for the SOS response (41), a situation similar to that of the nalidixic acid induction of NTP. The morphological changes induced by nalidixic acid showing condensation of the cytoplasm (Fig. 9) could reflect a process reminiscent of apoptosis as the condensation of the cytoplasm in apoptotic mammalian cells (42). Furthermore, the inclusion bodies with a crystalline structure inside the cytoplasm (Fig. 9, E and F), similar to those observed in various bacterial cells in response to stress conditions (43, 44), may also suggest the presence of a specific pathway of cell death.

In summary, we have shown that nalidixic acid, a topoisomerase II inhibitor, induces in E. coli wild type and in mutants deficient in the SOS response a rapid and marked increase in ATP. This induction is reversible when the treatment with nalidixic acid is transient (40 min), except in ΔRecA cells. The mechanism associated with the specific elevation in ATP level appears to be unrelated to the SOS response but might be related to an apoptotic-like process.

Acknowledgments—We thank Prof. W. Mueller-Klieser and Dr. B. Schick for carefully reviewing the manuscript.

REFERENCES
1. Barbé, J., Villaverde, A., and Guerrero, R. (1983) Biochem. Biophys. Res. Commun. 117, 556–561
2. Guerrero, R., Llagostera, M., Villaverde, A., and Barbé J. (1984) J. Gen. Microbiol. 130, 2247–2251
3. Barbé, J., Villaverde, A., Cairo, J., and Guerrero, R. (1986) J. Bacteriol. 167, 1055–1057
4. Neeman, M., Eldar, H., Rushkin, E., and Degani, H. (1990) Biochim. Biophys. Acta 1052, 255–263
5. de Jong, S., Mulder, N. H., de Vries, E. G. E., and Robillard, G. T. (1991) Br. J. Cancer 63, 205–212
6. Bergmanns, K., Ruiz-Caballo J., Simpkins, H., Andrews, P. A., and Cohen, J. S. (1992) Biochem. Biophys. Res. Commun. 183, 114–120
7. Kowk, J. B. J., and Tattersall, M. H. N. (1992) Br. J. Cancer 65, 503–508
8. Dahan-Grobeld, E., Margalit, R., and Degani, H. (1995) in Proceedings of The Society of Magnetic Resonance, Third Scientific Meeting and Exhibition and European Society for Magnetic Resonance in Medicine and Biology Twelfth Annual Meeting and Exhibition, p. 1698, Nice Aeropoli, Nice
9. Chen, G. L., and Liu, L. F. (1988) Ann. Rep. Med. Chem. 23, 257–261
10. Hoff, A. C., and Kreeger, R. N. (1990) J. Biol. Chem. 265, 20196–20505
11. Kupfer, G., Bodley, A. L., and Liu, L. F. (1987) NCI (Nat. Cancer Inst.) Monogr. 4, 37–40
12. Kars, A. E., and Belk, E. D. (1982) Mol. Gen. Genet. 185, 275–282
13. Chaudhury, A. M., and Smith, G. R. (1985) Mol. Gen. Genet. 201, 525–528
14. Ugurkılı, K., Shulman, R. G., and Brown, T. (1979) in Proceedings of The Society of Magnetic Resonance (Shulman R. G., ed) pp. 527–539, Academic Press, New York
15. Ugurkılı, K., Rottenberg, H., Glynn, P., and Shulman, R. G. (1982) Biochemistry 21, 1068–1075
16. Alam, K. Y., and Clark, D. P. (1989) J. Bacteriol. 171, 6213–6217
17. Briareo, C. A., Karel, S. F., and Robertson, C. R. (1990) Biotechnol. Bioeng. 36, 887–901
18. Kushner, S. R., Nagaishi, H., Templin, A., and Clark, A. J. (1971) Proc. Natl. Acad. Sci. U. S. A. 68, 824–827
19. Mount, D. W., Low, K. B., and Edmiston, S. J. (1972) J. Bacteriol. 112, 886–893
20. Cohen-Fix, O., and Livneh, Z. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 3300–3304
21. Silhavy, T. J., Berman, M. L., and Enquist, L. W. (1984) in Experiments with Gene Fusions, pp. 107–111, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
22. Bentall, M., Pick, U., Avron, M., and Degani, H. (1990) Eur. J. Biochem. 188, 111–116
23. Horowitz, J., Ofengand, J., Daniel, W. E., Jr., and Cohn, M. (1977) J. Biol. Chem. 252, 4418–4420
24. Marshall, A. G., and Smith, J. L. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 5624–5627
25. Mount, D. W., Low, K. B., and Edmiston, S. J. (1972) J. Bacteriol. 112, 886–893
26. Cohen-Fix, O., and Livneh, Z. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 3300–3304
27. Silhavy, T. J., Berman, M. L., and Enquist, L. W. (1984) in Experiments with Gene Fusions, pp. 107–111, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
28. Bentall, M., Pick, U., Avron, M., and Degani, H. (1990) Eur. J. Biochem. 188, 111–116
29. Horowitz, J., Ofengand, J., Daniel, W. E., Jr., and Cohn, M. (1977) J. Biol. Chem. 252, 4418–4420
30. Marshall, A. G., and Smith, J. L. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 5624–5627
31. Witkin, E. M. (1969) Annu. Rev. Microbiol. 23, 487–514
32. Richa, A. I., and Cox, M. M. (1990) Crit. Rev. Biochem. Mol. Biol. 25, 415–456
33. Kowalczykowski, S. C. (1991) Annu. Rev. Biophys. Biophys. Chem. 20, 539–575
34. Lim, D. S., and Hasty, P. (1996) Mol. Cell. Biol. 16, 7133–7143
35. Scully, R., Chen, J., Flug, A., Xiao, Y., Weaver, D., Feunteun, J., Ashley, T., and Livingston, D. M. (1997) Cell 88, 265–275
36. Shinohara, A., Ogawa, H., and Ogawa, T. (1992) Cell 69, 457–470
37. Dravides, G., and Guise, D. (1979) J. Bacteriol. 135, 185–190
38. Marzilinsky, M. B. (1995) Science 267, 836–837
39. Liu, J. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 4618–4620
40. Jansen, R. B., and Gerdes, K. (1995) Mol. Microbiol. 17, 205–210
41. Hall, J. M., and Keates, E. (1996) Mol. Microbiol. 23, 224–231
42. Brüggemann, H., Schmitt, H., and Keates, E. (1996) Mol. Microbiol. 23, 224–231
43. Brüggemann, H., Schmitt, H., and Keates, E. (1996) Mol. Microbiol. 23, 224–231
44. Brüggemann, H., Schmitt, H., and Keates, E. (1996) Mol. Microbiol. 23, 224–231
45. Brüggemann, H., Schmitt, H., and Keates, E. (1996) Mol. Microbiol. 23, 224–231
46. Brüggemann, H., Schmitt, H., and Keates, E. (1996) Mol. Microbiol. 23, 224–231
47. Brüggemann, H., Schmitt, H., and Keates, E. (1996) Mol. Microbiol. 23, 224–231