JOURNAL OF BACTERIOLOGY, June 1995, p. 3420–3426 Vol. 177, No. 12
0021-9193/95/$04.00+0
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Energy Buffering of DNA Structure Fails when *Escherichia coli* Runs Out of Substrate

PETER RUHDAL JENSEN,† LEINE LOMAN, BOB PETRA, COEN VAN DER WEIJDEN,‡ AND HANS V. WESTERHOFF*

Division of Molecular Biology, The Netherlands Cancer Institute, NL-1066 CX Amsterdam, The Netherlands

Received 23 January 1995/Accepted 17 April 1995

To study how changes in the [ATP]/[ADP] ratio affect the level of DNA supercoiling in *Escherichia coli*, the cellular content of H^+-ATPase was modulated around the wild-type level. A relatively large drop in the [ATP]/[ADP] ratio from the normal ratio resulted in a small increase in the linking number of our reporter plasmid (corresponding to a small decrease in negative supercoiling). However, when cells depleted their carbon and energy source, the ensuing drop in energy state was accompanied by a strong increase in linking number. This increase was not due to reduced transcription of the DNA in the absence of growth substrate, since rifampin had virtually no effect on the plasmid linking number. To examine whether DNA supercoiling depends more strongly on the cellular energy state at low [ATP]/[ADP] ratios than at high ratios, we used cells that were already at a low energy state after substrate depletion; after the addition of an uncoupler to these cells, the [ATP]/[ADP] ratio decreased further, which resulted in a strong increase in plasmid linking number. Our results suggest that the strong thermodynamic control of DNA supercoiling takes over at low [ATP]/[ADP] ratios, whereas at high ratios homeostatic control mechanisms attenuate thermodynamic control.

Chromosomal DNA isolated from prokaryotic cells is normally found in a negatively supercoiled state (29), which may play an important role in DNA tracking processes such as transcription and DNA replication. For instance, changes in DNA supercoiling affect the expression of a large number of *Escherichia coli* genes in either direction (6, 15a, 20, 21a). The supercoiled conformation of the DNA is maintained by the opposite activities of at least two topoisomerases: topoisomerase I relaxes negatively supercoiled DNA, whereas DNA gyrase introduces negative supercoils into the DNA, using ATP to drive the thermodynamically unfavorable process (5a).

Changes in growth conditions, such as increased temperature, anaerobiosis, or osmotic shock, have been shown to affect the level of negative supercoiling of bacterial DNA (4, 7, 8). The mechanism of how these changes affect the level of DNA supercoiling in the cell is not fully understood. Regulatory mechanisms, e.g., those involving the levels of transcription of the genes encoding topoisomerase I and DNA gyrase, have been found (17, 23). In vitro studies with isolated DNA gyrase from *E. coli* showed that the activity of this enzyme was sensitive to the ratio between the concentrations of ATP and ADP around the enzyme, whereas the absolute concentrations of the nucleotides did not have any effect on the enzyme activity (26). Because the Gibbs free energy of ATP hydrolysis also depends on the ratio of ATP to ADP concentration and not on their total concentration, this would constitute thermodynamic control of DNA structure. There are some indications that in vivo, changes in the growth conditions that normally lead to a change in DNA supercoiling are accompanied by changes in the [ATP]/[ADP] ratio in *E. coli* (9, 10, 25), but a direct link between cellular energy metabolism and DNA supercoiling has not yet been established.

In earlier experiments, uncouplers of oxidative phosphorylation were added (25), the cells were shifted to anaerobiosis (9), or the medium's ionic strength was changed (10). Perhaps the most direct way of examining if cellular energy metabolism controls DNA supercoiling is to change the cellular capacity for ATP synthesis and measure the change in DNA supercoiling along with the [ATP]/[ADP] ratio. In this study, we modulate the concentration of the H^+-ATPase and measure the effect on DNA supercoiling in growing cells and in cells depleted of carbon source. We show that the dependence of DNA structure on cellular energy state varies strongly with the energy state.

MATERIALS AND METHODS

Bacterial strains. The *E. coli* K-12 strains used in this study, PJ4000, PJ4002, and PJ4004, were derived from strains LM3112, LM3113 (14), and LM3118 (11), respectively, by transformation with plasmid pBR322. All these strains have the genotype F^+ *asnB thi-1 relA1 spoT1 lacUV5 lacY*(Am). The promoters of the chromosomal *atp* genes in strain PJ4000 have been replaced with a single inducible *lacUV5* promoter (*atpI*::p*lacUV5*), and these promoters in strain PJ4002 have been replaced with a *tacI* promoter (*atpI*::p*tacI*).

Materials. Hybond membranes were obtained from Amersham, 2,4-dinitrophenol was from Merck, IPTG (isopropyl-β-D-thiogalactoside) was from Boehringer, rifampin was from Gist Brocades, and phosphoenolpyruvate and chloroquine were from Sigma.

Growth of bacterial cultures. Batch cultures were grown at 37°C in the morpholinepropanesulfonic acid (MOPS)-buffered minimal medium, prepared as specified by Neidhardt and coworkers (18), supplemented with thiamine (1 μg/ml), ampicillin (100 μg/ml), and either glucose or succinate at the concentrations specified in the figure legends.

Extraction of ATP, ADP, and total DNA. Part (0.9 ml) of the cell culture was mixed with 0.9 ml of (80°C) phenol (equilibrated with 10 mM Tris–1 mM EDTA, pH 8) and immediately vortexed vigorously for 10 s. After 1 h at room temperature, the sample was vortexed again for 10 s and then either stored at −20°C or processed immediately as follows: the two phases were separated by centrifugation at 12,000 × *g* for 15 min, and the phenol in the water phase was then removed by extraction with 1 volume of chloroform. Fifty microliters of the water phase was used for subsequent measurement of ATP and ADP (see below); the DNA in the rest of the water phase was recovered by a standard isopropanol

* Corresponding author. Present address: Department of Microbial Physiology, Free University Amsterdam, de Boelelaan 1087, NL-1081 HV Amsterdam, The Netherlands. Phone: (31) 20 444 7230. Fax: (31) 20 444 7229.

† Present address: Department of Microbiology, Technical University of Denmark, DK-2800 Lyngby, Denmark.

‡ Present address: Department of Microbial Physiology, Vrije Universiteit, de Boelelaan 1087, 1081 HV Amsterdam, The Netherlands.

precipitation (16). This DNA preparation contains plasmid DNA, RNA, and chromosomal DNA. The RNA was removed by RNase treatment, prior to loading the DNA on the gel. The chromosomal DNA did not interfere with the subsequent gel assay.

Besides being less laborious than other methods, this method has several advantages in the present context: (i) the cellular metabolism is quenched quickly and efficiently; (ii) the DNA and metabolites are recovered quantitatively; (iii) the conditions are mild with respect to DNA and metabolite stability, and ATP and ADP concentrations and plasmid supercoiling can be determined in the same sample.

Measurement of ATP and ADP concentrations. A luciferin-luciferase ATP monitoring kit was obtained from and used as recommended by LKB, except that 3 mM phosphoenolpyruvate was added. The concentration of ATP was measured first. Subsequently, the ADP in the same sample was converted to ATP by adding pyruvate kinase and recorded as the concomitant increase in luminescence. Especially with high ratios of ATP to ADP, it was necessary to correct for 4 to 5% quenching of the ATP signal caused by the presence of glycerol in the pyruvate kinase preparation. However, the ratios above 10 are still subject to large errors, because small amounts of ADP are then determined on top of a large amount of ATP.

Quantification of changes in plasmid supercoiling. Electrophoresis of total DNA preparations was carried out on 1.4% agarose gels, using Tris-phosphate-EDTA buffer (16) and in the presence of 10 μg of chloroquine per ml, unless otherwise stated. After electrophoresis, the DNA was transferred to Hybond membranes and hybridized to pBR322 labeled with ^{35}S-dATP. The degree of DNA supercoiling was then quantified relative to that of a standard preparation of pBR322 by a method that will be described in detail elsewhere. Basically, the method calculates the average linking number of the supercoiled plasmid population relative to that of open circular DNA by weighting the intensities of topoisomer bands with their electrophoretic mobilities, calibrating the latter in terms of linking number, and then scaling with the total content of topoisomers in the sample. The more usual method interprets the heights of the individual topoisomer peaks (e.g., 3, 21) or of fitted integrals of topoisomer peaks in terms of a Gaussian distribution. The method we use here gives virtually the same results (not shown) but is in principle independent of the form of the topoisomer distribution. Using this method, we could estimate the relative average linking number of a plasmid sample with an accuracy of less than 0.2 linking number, or a specific linking difference ($\Delta\sigma$) of $(0.2 \times 10.4)/4{,}363$ bp = 0.0005. Samples with a linking number difference greater than 4 to 6 compared with the standard pBR322 preparation were given the linking number of the most intense band in the preparation and are thus subject to larger errors (± 0.5 to 1 linking numbers).

RESULTS

Dependence of DNA supercoiling on the energy state of growing cells. In order to change the (Gibbs free) energy state of the cells around the normal level, we used *E. coli* strains in which the chromosomal *atp* genes (encoding the H^+-ATPase) are transcribed from inducible (*lac*-type) promoters. In strain PJ4000, the promoter is weak but tight (p*lacUV5*), and using this strain allows us to change the cellular concentration of H^+-ATPase from virtually no expression up to the wild-type level by changing the concentration of the inducer IPTG in the growth medium (14). In strain PJ4002, the promoter is strong but leaky (p*tacI*), and with this strain, we can manipulate the cellular concentration of H^+-ATPase from 15% and up to 400% of the wild-type level. Changes in the [ATP]/[ADP] ratio were used to monitor changes in the cellular energy state, and we used changes in the linking number of a plasmid isolated from the cells as a probe for changes in DNA supercoiling.

If the cells are grown with succinate as the sole energy source, then the energy state of the cells should depend on the activity of the H^+-ATPase (15), because the cells must rely on oxidative phosphorylation for their ATP synthesis. Figure 1 shows an experiment with strain PJ4000: the cells were grown overnight in minimal medium supplemented with succinate and 0.5 mM IPTG to obtain cells with the wild-type H^+-ATPase concentration (see above). The culture grown overnight was centrifuged, and the pellet was resuspended in fresh medium without IPTG at time zero. The cells then started growing, initially with a growth rate close to that of the wild-type cells but then slower and slower as the H^+-ATPases that were present in the culture grown overnight were divided among the daughter cells in the culture. After 8 h, the growth

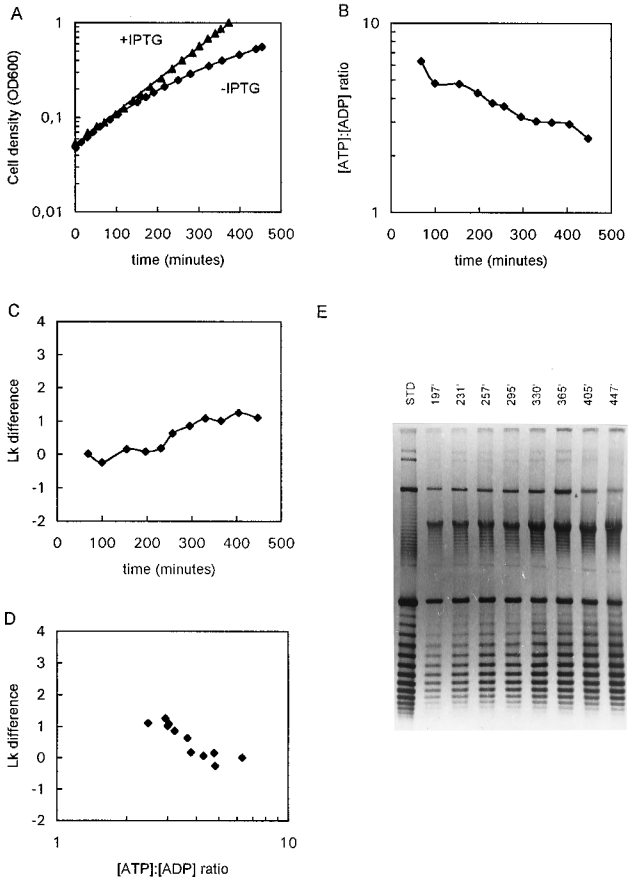

FIG. 1. A decrease in the [ATP]/[ADP] ratio and increase in the plasmid linking number (less negative supercoiling) follows repression of the *atp* genes. Strain PJ4000 (*atpI*::p*lacUV5*) was grown in minimal medium supplemented with 20 mM succinate and 0.5 mM IPTG so as to give expression of the *atp* genes close to the wild-type level. At time zero, the cells were centrifuged and the pellets were resuspended in medium with or without IPTG. (A) Optical density (measured at 600 nm [OD600]) versus time. (B) [ATP]/[ADP] ratio versus time. (C) Change in negative supercoiling (linking number [Lk] difference) of the reporter plasmid versus time (a positive value means a change toward a higher linking number or less negative supercoiling, compared with the reference point at time zero). (D) Correlation between the [ATP]/[ADP] ratio and linking number (Lk) difference. (E) Agarose gel electrophoresis of plasmid preparations from various time points of the growth curve. The time (in minutes) is given over each lane. Lane STD contains a standard preparation of pBR322 plasmid.

rate of the culture had decreased to approximately 25% of the initial rate and the [ATP]/[ADP] ratio had dropped from 8 to 2.5. Figure 1C and E show what happened to the degree of supercoiling of our reporter plasmid: as the growth rate and energy state decreased, the topoisomer distribution shifted slightly upward in the gel. Under the conditions used here, this means that the average linking number of the plasmid population isolated from these cells increased, which corresponds to a decrease in the level of negative supercoiling. Because the change in linking number is relatively small, we had to develop an accurate method for quantifying the average linking number of the plasmid population (see Materials and Methods). Using this method, we find that the plasmid supercoiling in this experiment decreased by approximately 1 to 2 linking numbers as the [ATP]/[ADP] ratio dropped from 8 to 2.5 (Fig. 1D). Thus, the change in linking number was in the direction that was expected for thermodynamic control of DNA gyrase activity, but the linking number difference was smaller than what

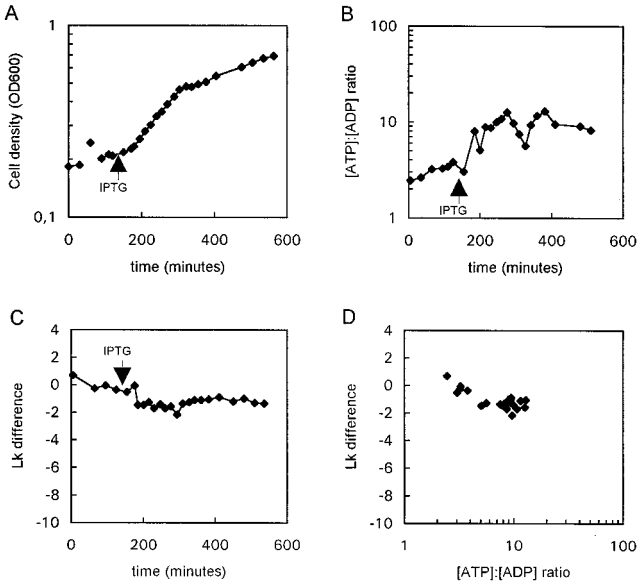

FIG. 2. Induced biosynthesis of H^+-ATPase increases the [ATP]/[ADP] ratio and decreases the plasmid linking number (Lk) of growing *E. coli* cells. Strain PJ4002 (*atpI*::p*tacI*) was grown in minimal medium supplemented with 20 mM succinate. At t = 140 min, 0.5 mM IPTG was added to the culture to give maximal expression of the *atp* genes. These results were published previously in a preliminary form (13) (see Results and the legend to Fig. 1 for details of experimental methods and explanation of graphs and abbreviations.)

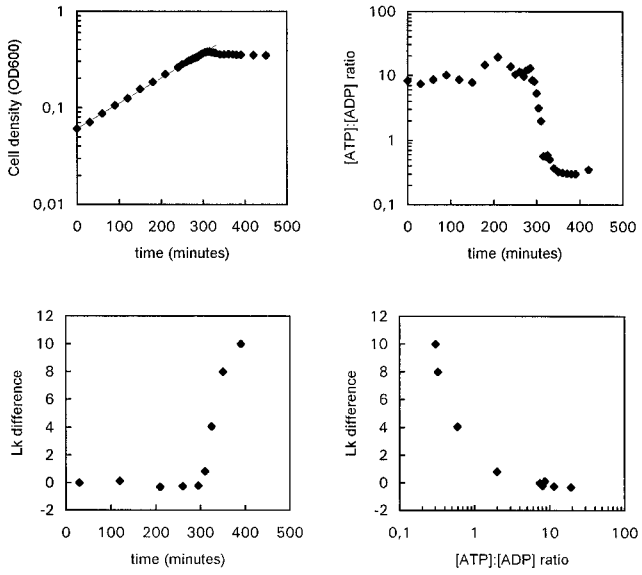

FIG. 3. [ATP]/[ADP] ratio and plasmid linking number (Lk) difference during succinate depletion. PJ4004 (wild-type strain) was grown in minimal medium supplemented with a limiting concentration of succinate (5 mM) (see the legend to Fig. 1 for details).

we expected from in vitro measurements of the sensitivity of DNA gyrase toward changes in the [ATP]/[ADP] ratio (26).

If the gradual decrease in negative DNA supercoiling that we observed as the H^+-ATPase content of the cells decreased (Fig. 1) was caused by the change in energy state, then we should find an increase in negative supercoiling when the [ATP]/[ADP] ratio is varied in the other direction, i.e., from low to high ratios. To test this hypothesis, we used strain PJ4002, which carries the *tacI* promoter in front of the chromosomal *atp* genes. PJ4002 cells were grown in minimal medium supplemented with succinate, initially in the absence of IPTG. These conditions cause the cells to have an H^+-ATPase level that is 15% of that of wild-type cells (14), and the growth rate is much lower than that of the wild-type cells. These cells had an [ATP]/[ADP] ratio of approximately 4. At t = 140 min, IPTG was added to the culture to give maximal expression of H^+-ATPase, and a few minutes later, the [ATP]/[ADP] ratio increased abruptly from 4 to 10 or 12. The increase in [ATP]/[ADP] ratio was indeed paralleled by an increase in negative DNA supercoiling by 1 linking number (Fig. 2). Three hours after the addition of IPTG, the growth rate slowed down again, probably as a result of excessive amounts of H^+-ATPase in the cells (cf. the effect of ATPase on growth rate [15]). However, the [ATP]/[ADP] ratio and the level of negative supercoiling remained higher than before induction with IPTG.

Changes in DNA structure upon substrate depletion. We also analyzed how the plasmid supercoiling changed during substrate depletion and monitored how the energy state of the cells changed. The wild-type cells were grown exponentially with a small amount of substrate, and samples were withdrawn frequently for the determination of the optical density of the cell culture. Around the time when the cells were expected to deplete the substrate, samples were withdrawn at 1-min intervals for analysis of plasmid supercoiling and of ATP and ADP concentrations.

Figure 3 shows the result of a substrate depletion experiment in which wild-type cells were grown in the presence of a limited amount of succinate. After the cells had used up the succinate, growth stopped and the [ATP]/[ADP] ratio dropped from 9 to 0.2. Interestingly, these results were accompanied by a strong decrease in negative supercoiling; the plasmid linking number increased by 10 linking numbers.

Effect of inhibition of transcription on DNA supercoiling. Why does the DNA structure change so strongly when the cells run out of their growth substrate? One possibility is that the change in DNA supercoiling that we observed when cells had depleted their carbon and free energy source was caused by a drop in transcriptional activity and therefore in transcription-driven DNA supercoiling (30). To test whether inhibition of transcription had an effect on DNA supercoiling, we added rifampin to cells growing on succinate and measured the effect on plasmid supercoiling. As can be seen from the experiment shown in Fig. 4, rifampin had no or very little effect on the DNA supercoiling of the reporter plasmid, when it was added at a concentration that was sufficient to inhibit the growth of the cells. Interestingly, we observed an increase in the [ATP]/[ADP] ratio immediately after rifampin was added to the culture. This increase in [ATP]/[ADP] ratio and its effect on DNA supercoiling could in principle have masked a negative effect of rifampin on DNA supercoiling. In order to be able to compare cells with and without rifampin at the same [ATP]/[ADP] ratios, we therefore added rifampin to cells at different time points after IPTG had been removed from a culture of strain PJ4000 grown on succinate. As explained above, this procedure results in a culture which has a decreasing [ATP]/[ADP] ratio over time (Fig. 2) and allows for comparison of the level of DNA supercoiling with and without rifampin at the same [ATP]/[ADP] ratio. Also when compared in this way, the effect of rifampin on plasmid supercoiling was smaller than 1 linking number (not shown). We therefore conclude that the strong changes in DNA supercoiling during substrate depletion are not just caused by abolition of transcription-driven supercoiling.

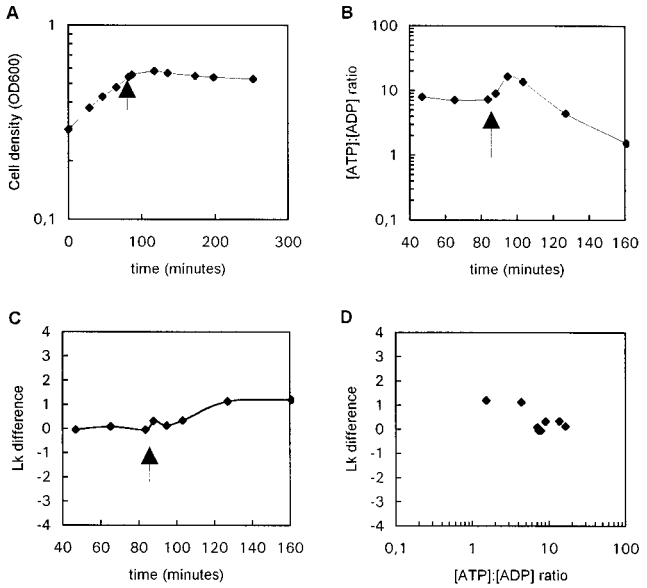

FIG. 4. Effect of inhibition of transcription on the [ATP]/[ADP] ratio and plasmid linking number (Lk) difference. Strain PJ4000 (*atpI*::p*lacUV5*) was grown, initially in minimal medium supplemented with succinate (20 mM) and 0.5 mM IPTG. At time zero, the IPTG was removed, and at the time point indicated by the arrow, rifampin (100 μg/ml) was added to the culture (see Results and the legend to Fig. 1 for details).

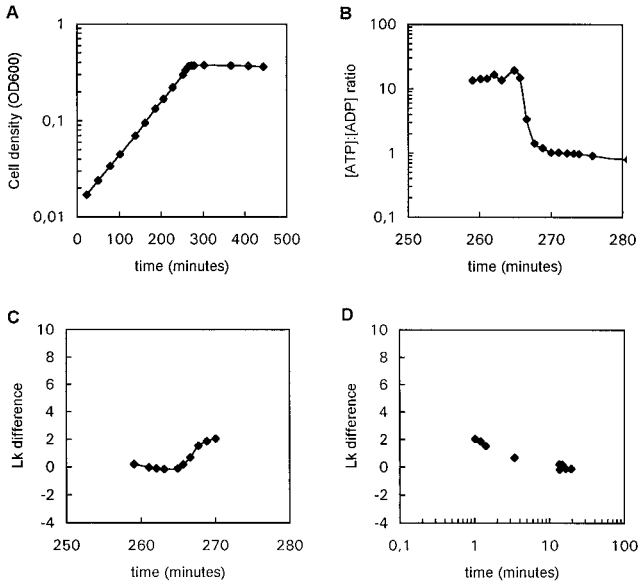

FIG. 5. [ATP]/[ADP] ratio and plasmid linking number (Lk) difference during glucose depletion of wild-type cells. Strain PJ4004 was grown in minimal medium supplemented with a limiting concentration of glucose (2 mM) (see the legend to Fig. 1 for details).

Substrate depletion with glucose. The strong effect on DNA supercoiling observed when cells run out of succinate could also be a phenomenon somehow specifically related to cells growing on this substrate. For instance, when *E. coli* is grown on glucose, there is a small amount of acetate excreted from the cells as a by-product, which will in principle allow the cells to continue ATP synthesis by oxidative phosphorylation, after glucose is depleted (this is not the case for growth on succinate, where wild-type *E. coli* normally does not excrete any by-product other than CO_2 [1]). One might therefore expect that when cells run out of glucose, the effect on the energy state will be less drastic than when they run out of succinate. In Fig. 5 we show the effect of glucose depletion on supercoiling and [ATP]/[ADP] ratio in wild-type *E. coli* cells. Indeed, upon glucose depletion, the [ATP]/[ADP] ratio dropped to 1, rather than the 0.2 observed in the case of succinate, within a few minutes and then remained fairly constant for several hours. Thus, it seems that glucose depletion had a less dramatic effect on the energy state of the cells than succinate depletion did. If the change in DNA supercoiling were somehow linked to the energy state, we should also see a smaller effect on DNA supercoiling than when cells run out of succinate. This result was indeed found: the drop in energy state was accompanied by a simultaneous drop in the supercoiling of the reporter plasmid but this time only by approximately 2 linking numbers.

If the relatively small change in energy state observed for cells depleted of glucose is due to the cells' oxidative phosphorylation capacity and residual acetate, then the drop in energy state should be stronger in cells which do not contain H^+-ATPase and are thus unable to synthesize ATP by oxidative phosphorylation. Strain PJ4000 carries a *lac* promoter in front of the *atp* genes, and in the absence of inducer, virtually no H^+-ATPase is present in the cells (14). These cells grew with a growth rate on glucose equal to 75% of the wild-type growth rate (Fig. 6), and the energy state of the cells was somewhat lower than that of the wild-type cells ([ATP]/[ADP] = 8). Indeed, when these cells were allowed to run out of glucose, the [ATP]/[ADP] ratio dropped to a much lower value ([ATP]/[ADP] = 0.3) than that observed with the wild-type strain. As expected, the effect on DNA supercoiling was also much stronger than with the wild-type cells: here the drop in [ATP]/[ADP] ratio to 0.3 was accompanied by a reduction of DNA supercoiling by 6 linking numbers.

We also carried out the glucose depletion experiment using

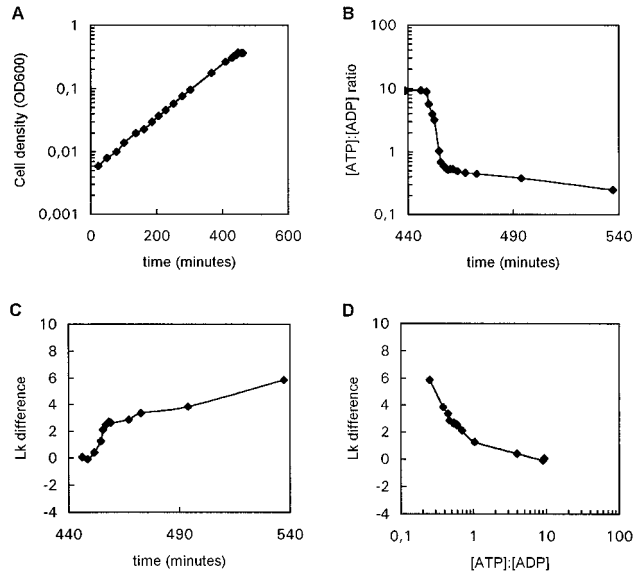

FIG. 6. [ATP]/[ADP] ratio and plasmid linking number (Lk) difference during glucose depletion of cells without H^+-ATPase. Strain PJ4000 was grown in minimal medium without IPTG, supplemented with 4 mM glucose (4 mM glucose was chosen in order to have the cells run out of glucose at approximately the same cell concentration as that of the wild-type cells [see Fig. 5] because of the lower yield of cell mass obtained with cells that have no H^+-ATPase [cf. 12]) (see the legend to Fig. 1 for details).

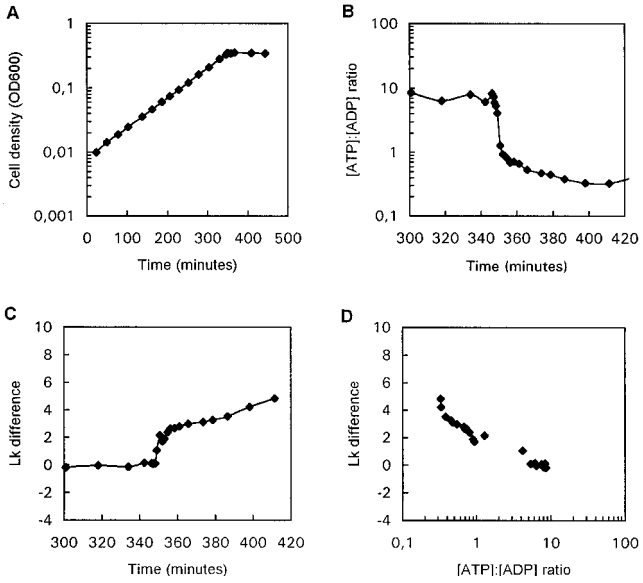

FIG. 7. [ATP]/[ADP] ratio and plasmid linking number (Lk) difference during glucose depletion of cells with 15% of the wild-type H^+-ATPase content. Strain PJ4002 was grown in minimal medium supplemented with a limiting concentration of glucose (3 mM) (see the legends to Fig. 1 and 6 for details).

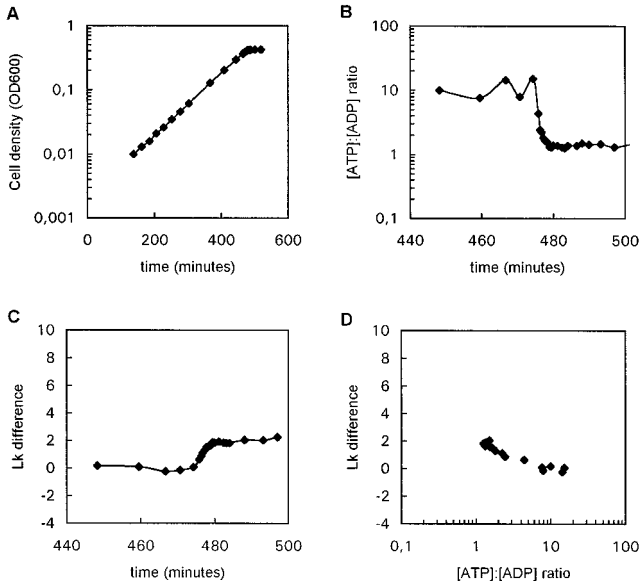

FIG. 8. [ATP]/[ADP] ratio and plasmid linking number (Lk) difference during glucose depletion of cells which overexpress the H^+-ATPase approximately fourfold. Strain PJ4002 was grown in minimal medium supplemented with a limiting concentration of glucose (2 mM) and 0.5 mM IPTG to give maximal expression of the *atp* genes (400% of the wild-type H^+-ATPase content) (see the legend to Fig. 1 for details).

cells with an intermediate concentration of H^+-ATPase. Strain PJ4002 (see above) contains approximately 15% of the H^+-ATPase level present in the wild-type *E. coli* cell. These cells had a growth rate equal to 85% of the wild-type growth rate, and for this strain, the energy state was also lower than in the wild-type cell ([ATP]/[ADP] = 8). After glucose was exhausted, the [ATP]/[ADP] ratio in these cells dropped to a value (0.4 to 0.5) which was intermediate to the results obtained with the wild-type strain and the strain without H^+-ATPase. The effect on DNA supercoiling was also intermediate (Fig. 7).

Strain PJ4002 also allowed us to grow cells with increased concentrations of H^+-ATPase compared to that of the wild-type cells. In Fig. 8 we show the result of an experiment in which cells were grown in the presence of a concentration of IPTG that gives maximal expression of the *atp* genes, equivalent to approximately four times the wild-type H^+-ATPase concentration (14). When these cells had depleted the glucose, the [ATP]/[ADP] ratio dropped to 1.3, which is a slightly higher value than in the wild-type cells; what happened to supercoiling was again in the direction expected if the level of DNA supercoiling were influenced by the energy state.

Dependence of DNA supercoiling on the [ATP]/[ADP] ratio at low-energy states. From the above experiments, it appeared that the changes in DNA supercoiling resulting from changes in the [ATP]/[ADP] ratio were much stronger at low [ATP]/[ADP] ratios than at high ratios. In this scenario, the DNA supercoiling in the starved cells should be strongly dependent on the [ATP]/[ADP] ratio. We therefore added an uncoupler of oxidative phosphorylation (2,4-dinitrophenol) to starved cells to see if we could lower the energy state even further. This was indeed the case: shortly after the uncoupler was added, the [ATP]/[ADP] ratio decreased from 1 to 0.2 and less, and this decrease was accompanied by a strong change in DNA supercoiling (7 linking numbers) (Fig. 9).

In Figure 10 we have plotted the datum points for plasmid linking number versus [ATP]/[ADP] ratio for the four glucose depletion experiments (Fig. 5 to 8). Although these data were obtained with different strains, there seems to be a good correlation between the results from the different experiments: at high [ATP]/[ADP] ratios, the level of DNA supercoiling varies weakly with the energy state, whereas at low [ATP]/[ADP] ratios the DNA supercoiling and the energy state of the cell are more strongly correlated.

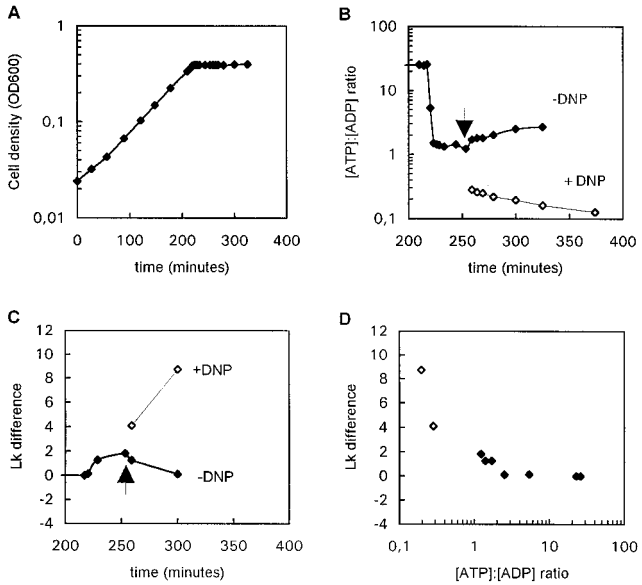

FIG. 9. Effect of uncoupler on the [ATP]/[ADP] ratio and plasmid linking number (Lk) difference of starved cells. Strain PJ4004 was grown in minimal medium supplemented with 2 mM glucose. At t = 222 min, the cells had depleted the glucose, and at t = 257 min, an uncoupler was added (2 mM 2,4-dinitrophenol [DNP]) to lower the [ATP]/[ADP] ratio (see the legend to Fig. 1 for details).

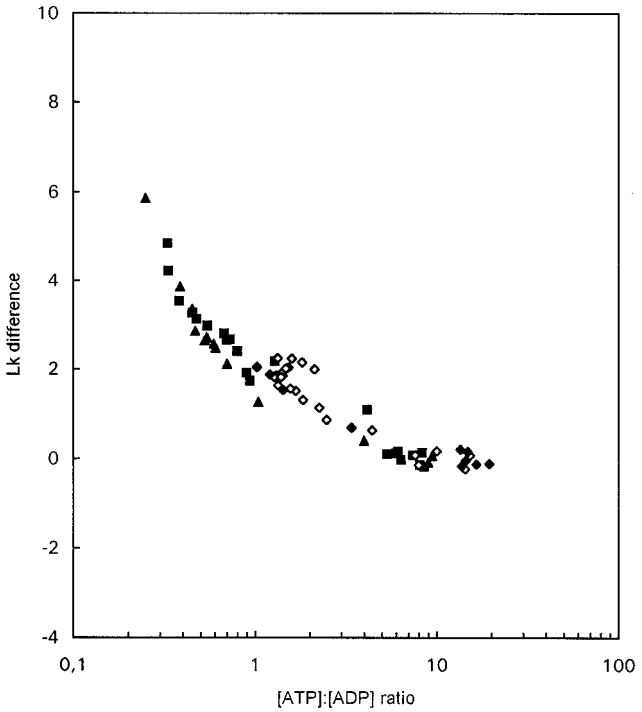

FIG. 10. Correlation between the [ATP]/[ADP] ratio and plasmid linking number (Lk) difference during substrate depletion. Pooled data from the four glucose depletion experiments (Fig. 5 to 8).

DISCUSSION

We have analyzed the relationship between DNA supercoiling and the cellular energy state by changing the capacity of the cells' energy metabolism. We find that when the energy state of growing cells is changed around the wild-type level, then the level of DNA supercoiling changes in the direction that one would expect from thermodynamic control of DNA supercoiling. During substrate depletion experiments, when the energy state of the cells dropped dramatically, the correlation between the [ATP]/[ADP] ratio and DNA supercoiling was in the same direction and much stronger. DNA gyrase uses ATP to introduce supercoils into the DNA, and the enzyme has been shown to be sensitive to changes in the [ATP]/[ADP] ratio around the enzyme in vitro (26). We therefore suggest that the changes in DNA supercoiling that we observe when the cells' energy metabolism is modulated are due to changes in the activity of DNA gyrase, mediated by changes in the [ATP]/[ADP] ratio (cf. 9, 10, 26).

In the in vitro studies by Westerhoff et al. (26), a decrease in the [ATP]/[ADP] ratio from 6 to 2.5 resulted in a change in DNA supercoiling of the plasmid pBR322 of +4 linking numbers, or a specific linking difference, $\Delta\sigma$ of $(4 \times 10.4)/4{,}363$ bp $= 0.01$. This result was obtained in the presence of spermidine; in the absence of spermidine, the decrease in DNA supercoiling was only 1.5 linking numbers. It is not yet clear which of these two situations is closest to the in vivo reaction of DNA gyrase, but it is possible that spermidine enhances the coupling of the gyrase reaction, and the in vitro experiments with spermidine may therefore be closest to the in vivo situation. In our experiments, we saw a difference of 1 to 2 linking numbers as the [ATP]/[ADP] ratio was changed from 2.5 to 6, significantly less than in the in vitro experiments with spermidine.

Changes in DNA supercoiling have previously been shown to occur when *E. coli* is shifted to anaerobiosis or to a high salt concentration (4, 8). In either case, the shift in growth conditions was accompanied by changes in the energy state of the cells, and it was suggested that the [ATP]/[ADP] ratio affected DNA gyrase in vivo as it does in vitro (9, 10). Changing the [ATP]/[ADP] ratio of growing cells around the wild-type ratio had only a small effect on the level of DNA supercoiling compared with the results of in vitro studies of gyrase sensitivity to the [ATP]/[ADP] ratio in the presence of spermidine (26). Therefore, our results would suggest that in vivo, the effect of changes in the [ATP]/[ADP] ratio on DNA supercoiling is smaller than in vitro.

DNA supercoiling in *E. coli* is, to some extent, homeostatically controlled: the expression of the *gyrA* and *tyrB* genes (encoding DNA gyrase) are repressed by high levels of negative supercoiling (17), and the expression of *topA* (the gene encoding topoisomerase I) is stimulated by negative supercoiling (23). In the experiments in which we diluted the cellular content of H^+-ATPase (Fig. 2), the changes occurred sufficiently slowly to allow for changes in the expression of the genes encoding DNA gyrase and topoisomerase I and therefore could have masked some of the effects of changes in the [ATP]/[ADP] ratio on supercoiling. However, in our substrate depletion experiments, the changes in [ATP]/[ADP] ratio and DNA supercoiling occurred virtually simultaneously, and in this case, it is unlikely that homeostatic regulation at the level of gene expression, would have modulated the effect of the [ATP]/[ADP] ratio on supercoiling.

In general, we find that when the energy state of the cell is already low (i.e., when the [ATP]/[ADP] ratio is less than 1), we see a stronger dependence of supercoiling on the energy state. For instance, the change in linking number that followed glucose depletion of the wild-type cells (Fig. 5) was much smaller than the change in linking number that followed the addition of uncoupler to the starved cells (Fig. 9), although in both cases the [ATP]/[ADP] ratio changed by a factor of approximately 10. These results suggest that the cell has regulatory mechanisms to maintain a constant level of DNA supercoiling when the [ATP]/[ADP] ratio changes around the normal ratio, but when the energy state is very low, these mechanisms fail and the state of the DNA then becomes more sensitive to changes in the [ATP]/[ADP] ratio. It is likely that such regulatory mechanisms would involve strong (and opposite) elasticities (28) of the two major topoisomerases toward negative supercoiling, as has previously been shown in vitro (22, 24). Alternatively, incomplete coupling of the DNA gyrase reaction at high extents of negative supercoiling, as observed in the absence of spermidine, could be responsible (26).

Balke and Gralla (2) also studied the effect of changes in the carbon source on DNA supercoiling but concluded that the effects were not mediated by changes in the [ATP]/[ADP] ratio, since uncouplers had no effect on DNA supercoiling. However, these investigators did not measure the cellular concentrations of ATP and ADP and since the experiments were performed in the presence of glucose, it is likely that the uncoupler did not have a strong effect on the energy state of the cell; because the cells can synthesize ATP by substrate-level phosphorylation, protonophores may not have yielded the low [ATP]/[ADP] ratios that seem to be necessary in order to observe (free energy-linked) changes in DNA supercoiling.

Earlier, it has been shown that DNA supercoiling is affected by changes in [ATP]/[ADP] ratio brought about by uncouplers (25), anaerobiosis (9), and changes in the ionic strength of the medium (10). Here we have specifically interfered with ATP synthesis. It seems that DNA supercoiling is, to some extent, controlled by the balance between ATP synthesis and ATP

consumption, as reflected in the [ATP]/[ADP] ratio. In terms of metabolic control analysis (27), these results then suggest that some of the control of DNA supercoiling resides in processes, such as oxidative phosphorylation, and some control residues in the anabolic processes that consume ATP. An interesting question then is why bacteria would have evolved so as to have their DNA structure linked to the energy state. Negative supercoiling facilitates the opening of the double helix, necessary for growth-related processes such as transcription and DNA replication, and a possible role of the link to the energy state therefore is to achieve a general inhibition of these processes when energy becomes scarce.

It has been proposed that much of the DNA supercoiling in vivo depends on active transcription, DNA gyrase mainly acting in front of RNA polymerase (30). In our experiments, much of the supercoiling appeared independently of transcription. Indeed, the minor drop in supercoiling following rifampin addition could be explained in terms of the (ultimate) parallel drop in the [ATP]/[ADP] ratio. In line with the results of Cook et al. (2a), DNA gyrase and topoisomerase I activities may suffice to relax all topological constraints generated by RNA polymerase. DNA supercoiling may well be determined by the balance between transcription-independent DNA gyrase and topoisomerase I activities.

The expression of many of *E. coli*'s genes is affected by changes in DNA supercoiling (6, 15a, 20, 21a). We may therefore speculate that the changes in DNA structure which occur when the cells run out of their growth substrates stimulate the expression of some genes and repress the expression of other genes so as to reorganize the pattern of gene expression to adjust to the new conditions of starvation. For instance, the promoters of ribosomal genes are stimulated by DNA gyrase activity (19). Therefore, the changes in supercoiling that we observe when the cells run out of their growth substrate should repress the synthesis of more ribosomes, which makes sense if growth is to stop anyway. Likewise, the initiation of DNA replication requires DNA gyrase activity, and a decrease in gyrase activity upon substrate depletion may therefore play a role in abolishing initiation of chromosome replication under these conditions.

ACKNOWLEDGMENTS

This study was supported by The Netherlands Organisation for Scientific Research (NWO), the European Communities (Biotechnology programme), the Danish Natural Research Council (SNF), and Danish Center for Microbiology (CM).

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