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EVOLUTION OF CELLULAR ATP CONCENTRATION AFTER UV-MEDIATED INDUCTION OF SOS SYSTEM IN Escherichia coli

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UV-irradiation of E. coli induces a two fold increase in ATP pool in the first 20 min. Afterwards, in RecA$^+$ strains ATP level drops quickly below values of non irradiated cells. Mutants of E. coli defective in RecA protein or with either RecA protease activity deficient or protease resistant LexA repressor do not present this decrease, showing that it is due to cleavage of LexA repressor by RecA protease. The ATP increase produced in the first 20 min is dependent on RecBC exonuclease activity and it must be due to substrate level phosphorylation since an uncoupler such as dinitrophenol does not affect it.

Following DNA damage by radiation, chemical carcinogens or treatments that inhibit DNA replication, E. coli displays a complex set of metabolic reactions, the SOS response, in which a number of cellular functions are coordinately expressed after the damage (1). This response is dependent on recA, lexA and ssb genes and includes: mutagenesis, filamentation, inhibition of cell respiration, prophage induction and amplification of RecA protein (2). The process of SOS system induction requires firstly the activation of protease ability of the RecA protein by a yet unknown inducing signal. Afterwards, RecA protease cleaves the LexA repressor (3) which in non-induced cells is blocking the transcription of the SOS genes (4). However, expression of the SOS system is not an all-or-none process, since some SOS functions may be induced by some treatments but not by others (5,6). It is known that the cleavage in vitro of LexA and Lambda prophage repressors by the RecA protease requires ATP or ATP-γ-S as a cofactor (7). Nevertheless, evolution of ATP pool in the cell during UV-mediated SOS system induction has not been extensively studied. Here we report the

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results obtained on a wild type and other strains carrying different recA alleles i.e. recA13 (RecA defective protein), recA430 (formerly lexB30; specific defect in RecA protease activity) and recA441 (formerly tif-1; RecA protease activity spontaneously activated at 40° C) and lexA1 mutant (harboring a protease resistant LexA repressor).

MATERIALS AND METHODS

Bacterial strains and growth conditions. Strains of E. coli K12 used in this work are the following: AB1157 (Rec+), AB2463 (recA13), IC41 (recA430), AB2494 (lexA1), JM12 (recA441), IC53 (recB21, recC22) and AB 1886 (uvrA6). All of them are isogenic except by the repair markers and have been described elsewhere (8) except AB2494, JM12, IC53 and AB1886 which were obtained from M. Blanco. Cultures were grown at 30°C or 37°C with aeration in LB medium (5).

UV Irradiation. The bacterial cells in exponentially growing phase were washed by centrifugation, resuspended in 0.01 M $MgSO_4$ at the concentration of about 2 x 10^8 cells/ml and then irradiated in a glass Petri dish in thin layers with swirling with a General Electric GY1578 germicidal lamp, at a rate of 0.5 J/m^2/s (determined with a Latarjet dosimeter). Afterwards, bacterial suspensions were diluted at 10^8 cells/ml by the addition of an equal volume of double strength LB medium and were incubated at the desired temperature. All experiments with UV-irradiated cells were performed under yellow light or in the dark to prevent photoreactivation.

Determination of the intracellular ATP concentration. After UV-irradiation or temperature shift treatment, cell aliquots of 1 ml were added to 4 ml of 0.025 M Tris-HCl buffer (pH 7. 75) and boiled in a boiling water bath for 5 min. Then, the suspension was centrifuged for 10 min at 4°C and 8,000 x g. ATP present in the supernatant was immediately measured by the firefly luciferin luciferase assay (9).

RESULTS AND DISCUSSION

Fig 1. shows evolution of the intracellular ATP in RecA+ and RecA- strains of E. coli after UV-irradiation. Under these conditions, an increase occurs

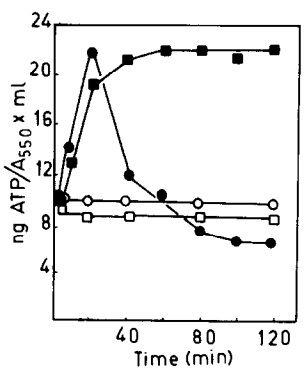

Fig. 1. Evolution of cellular ATP in RecA+ (●) and recA13 (■) strains of E. coli after UV-irradiation at 20 J/m^2. The ATP levels of non irradiated cultures of both RecA+ (○) and recA13 (□) strains are also shown as a control.

in the ATP level of both RecA+ and RecA- strains during the first 20 min. Afterwards, ATP level drops below the values of unirradiated cells of RecA+ strain but not in RecA- mutant which maintains the level achieved in the initial 20 min. The fall in the ATP level of the UV-irradiated RecA+ strain is presumably due to cleavage of LexA protein by the activated RecA protease. This is because, although after UV-irradiation lexA (protease-resistant LexA repressor) and recA430 (RecA protein deficient in protease activity) mutants both show the increases in the ATP level neither, unlike the RecA+ strain, show the post-20 minute fall (Fig. 2). In this respect, the slight recA-independent decrease of respiration rate previously reported for recA13 (10), recA430 (8) and lexA1 (10) mutants after UV-irradiation may be explained by the higher concentration of cell ATP under these conditions and by the normal coupling that exists between respiration rate and ATP level (11). Furthermore, with the recA441 mutant, when the RecA protein is activated by raising the temperature to 40°C there is between 10 and 20 minutes a progressive decrease in ATP level (Fig. 3). Nevertheless, this same recA441 strain does not show any increase in the ATP level at 40°C.

These results suggest that a relationship exists between the DNA damage and the increase in ATP level during the first 20 minutes. For this reason, we

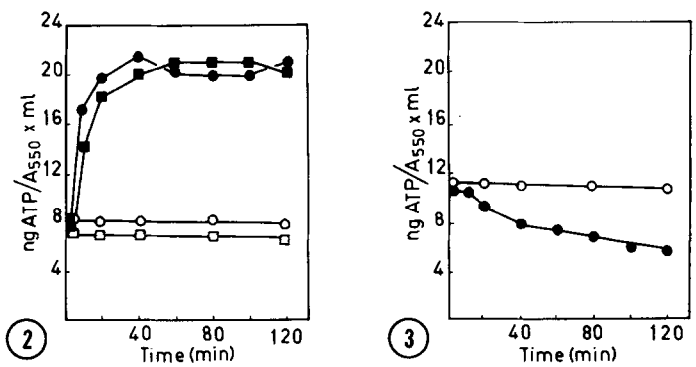

Fig. 2. Evolution of cellular ATP in recA430 (●) and lexA1 (■) mutants of E. coli after UV-irradiation at 20 J/m². The ATP levels of non irradiated cultures of both recA430 (○) and lexA1 (□) mutants are also shown as a control.

Fig. 3. Evolution of cellular ATP in the recA441 mutant of E. coli growing at 30°C (○) and after the temperature shift to 40°C (●) .

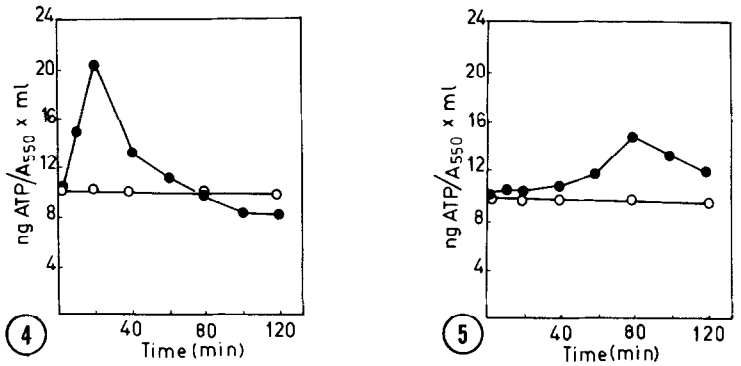

Fig. 4. Evolution of cellular ATP in both irradiated (●) and non irradiated (○) cultures of strain AB1886 (uvrA6) of E. coli. The dose of UV radiation used was 20 J/m^2.

Fig. 5. Evolution of cellular ATP in both irradiated (●) and non irradiated (○) cultures of strain IC53 (recB21, recC22) of E. coli. The dose of UV radiation used was 20 J/m^2.

studied the evolution of cell ATP concentration in the uvrA (repair excision defective) and recBC (defective in exonuclease V) mutants. The data obtained are shown in Figs. 4 and 5 where it can be seen how the uvrA strain presents a behavior similar to that of RecA$^+$, although the increase in ATP concentration is slightly lower than in the wild type strain. On the other hand, the recBC mutant shows an ATP constant level at least after 60 min following UV-irradiation when there is a very slight increase, which may be due to the action of other exonucleases, and that drops slowly 20 min later.

Therefore, it can be concluded that the increase in ATP level of RecA$^+$ strain after UV-irradiation depends on UV-induced DNA degradation via the RecBC exonuclease. To determine the mechanism by which DNA degradation produces the ATP increase, we have studied the ATP pool of RecA$^+$ and RecA$^-$ strains after UV-irradiation in the presence of the uncoupler dinitrophenol. At a concentration of 250 µM this reagent causes a dramatic decrease in ATP levels of both non irradiated RecA$^+$ and RecA$^-$ cells (Fig. 6). Nevertheless, dinitrophenol is unable to prevent the recBC-dependent ATP increase in the RecA$^+$ and RecA$^-$ strains, showing that it is not generated by the phosphorylation oxidative pathway. This fact suggests that its origin must be a substrate level phosphorylation, probably by metabolizing activity of the DNA degradation products. In this way, the cell can use the products not

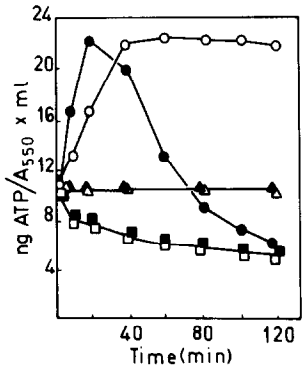

Fig. 6. Effect of the uncoupler dinitrophenol on the cellular ATP levels of RecA+ (●) and recA13 (○) strains of E. coli following UV-irradiation at 20 J/m^2. Dinitrophenol was added immediately after irradiation. The ATP levels of both non irradiated RecA+ (closed symbols) and recA13 (open symbols) strains in the absence (△,▲) or in the presence (□,■) of dinitrophenol are shown as a control. In all cases in which it was used, the concentration of dinitrophenol was 250 µM.

only to trigger SOS system in some cases (12) but also to produce energy to stimulate RecA protease activity.

The recA and lexA-dependent decrease in concentration of ATP in vivo reported in this paper, gives support to the results found by other authors (3) who have shown that the active RecA protease requires ATP to cleave in vitro LexA repressor. Nevertheless, our results are not fully in agreement with the data reported by Craig and Roberts (13) who found that ATP-γ-S is not hydrolyzed efficiently during Lambda repressor cleavage in vitro, despite the fact that this analogue can support a much higher cleavage rate than does ATP in this reaction. The basis of this difference may be found in the fact that the in vivo pathway of RecA protein activation must differ from that in vitro, in which case the results with purified RecA protein may not fully reflect the in vivo situation where RecA protein could function in association with other DNA binding proteins such as has been proposed by some authors (14). Moreover, the fact that the decrease in ATP begins about 20 min after UV-irradiation suggests that the SOS system-inducing signal requires at least this time to be produced or to generate the RecA protease active conformation. Related to this, it has been shown that induction of several SOS genes such as uvrB (15), sfiA (16) and umuC (17) occurs about 30

min after UV irradiation. Also, Lambda prophage induction begins 30 min after UV treatment (18). Furthermore, in the recBC mutant after UV-irradiation, both slight increases and decreases in ATP level are found 60 min later than in the wild type strain which is also in agreement with the delay of 1 h reported in the induction of the SOS system in the exonuclease V mutants (19).

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