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RESTORATION OF DEFECTIVE CELLULAR FUNCTIONS BY SUPPLY OF DNA POLYMERASE I TO PERMEABLE CELLS OF ESCHERICHIA COLI

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SUMMARY: Plasmolysed cells of polA mutants of Escherichia coli acquired ultraviolet resistance when the cells were exposed to high concentrations of E. coli DNA polymerase I. An impared ability of the mutant to support the growth of λred phage was restored to a certain level on the enzyme treatment. These results suggest that E. coli DNA polymerase I may be inserted into viable plasmolysed cells.

Artificial introduction of active protein molecules into viable cells may provide a useful means for analysing cellular functions and also for assaying certain proteins whose activity cannot be measured by ordinary biochemical methods. We were thus led to explore the possibility of making permeable cells which still retain cell viability and introducing biologically active proteins into such cells.

By examining various procedures which are known to increase permeability of bacterial cells, we found that plasmolysis is most suitable for this purpose. We have shown that plasmolysed cells of Escherichia coli strains having mutations in uvr genes acquired ultraviolet resistance after treatment of the cells with T4 endonuclease V (1). It was suggested that the T4 enzyme, taken up into permeable cells, can function in vivo to replace defective uvr functions.

Since T4 endonuclease V is small and, unlike most proteins, is positively charged at neutral pH (2), it was uncertain whether this method was applicable to other proteins. In the present study we have investigated effect of externally supplied E. coli DNA polymerase I on functions of permeable cells of polA mutants.

MATERIALS AND METHODS

Bacteria and phages: E. coli N611 (polA, ilv, trp, gal, strA) and N17-9 (uvrA54, trp, gal, strA) are derivatives of W3623 (trp, gal, strA) and were obtained from Drs. K. Shimada and H. Ogawa, respectively (3, 4). Strain

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P3478 (polA1, thyA36) is a derivative of W3110T (thyA36) and was furnished by Dr. J. Cairns (5). Phage λbio11 was provided by Dr. K. Shimada.

Media: The composition of L-broth, M9S and BSSE (balanced salt solution supplemented with EGTA) was described previously (1). TG medium was as described by Wickner and Hurwitz (6).

Enzymes: E. coli DNA polymerase I (Fraction VII, 9170 units/mg protein) was purified according to Jovin et al. (7) and kindly provided by Dr. K. Hori. The product of Behringer Manheim Gmbh was also used. The polymerase activity was determined as described by Richardson et al.(8). T4 endonuclease V was purified as described (1, 2) and used after dialysis at 0°C for 6 hr against 1,000 volumes of BSSE.

Preparation of plasmolysed cells: Cells were grown in L-broth at 37°C and harvested during the late logarithmic phase of growth. After washing with cold 40 mM Tris·HCl buffer (pH 8.0), cells were suspended in ice-cold plasmolysing buffer at a cell density of 5 ~ 10 x 10^9/ml and kept at 0°C for 30 min. The plasmolysing buffer, which contains 2.4 M sucrose, 20 mM EGTA and 40 mM Tris·HCl (pH 8.0), was prepared immediately before use. The ultracentrifugation-grade of sucrose was used.

Reactivation of UV-irradiated cells: A portion of a suspension of plasmolysed cells was added to 10 volumes of BSSE containing E. coli DNA polymerase I or T4 endonuclease V. After standing at 0°C for 30 min, the cell suspension was diluted 10 fold with M9S. Portions (0.2 ml each) of the diluted cell suspension were transferred to holes (7 mm in diameter) of a microtiter plate and irradiated with UV. In some experiments, cells were preirradiated, permeabilized and treated with the enzyme. To avoid photoreactivation, all operations were carried out under a yellow dim light. Other procedures were as described in the previous paper (1).

RESULTS

Reactivation of UV-irradiated polA mutants: PolA mutants of E. coli exhibit increased sensitivity to UV when compared with wild type strains (5, 9). It was expected, therefore, that effective introduction of DNA polymerase I into polA mutants should increase survivals of the cells after UV irradiation.

Bacteria which had been plasmolysed in high concentrations of sucrose solution were exposed to E. coli DNA polymerase I and then the UV sensitivity was determined. We calculated the reactivation index by dividing the survival fraction (ratio of the number of colonies of UV-irradiated sample to that of non-irradiated sample) of enzyme-treated cells by the surviving fraction of non-treated cells.

As can be seen in Table 1, the highest reactivation index was obtained with cells plasmolysed in 2.4 M sucrose. Since strain P3478 and N611, used in the present studies, were relatively resistant to osmotic shock, we used this concentration of sucrose to prepare permeable cells.

Fig. 1 shows UV survivals of plasmolysed cells of polA mutant which had been treated with two different levels of E. coli DNA polymerase I. About 16 fold reactivation for irradiation of 45 J/m^2 was observed with plasmolysed cells treated with 36 units/ml of the enzyme. No reactivation was induced

Table 1. Effect of sucrose concentration on the reactivation of UV-irradiated cells by DNA polymerase I

Concentration of sucrose (M)	Viability (%)	Reactivation index (fold)
0	71	1.1
1.0	39	—
1.5	30	—
2.0	18	8.3
2.4	15	17.4

Cells of N611(polA) were suspended in 0.3 ml of 20 mM EGTA-40 mM Tris·HCl (pH 8.0) containing various concentrations of sucrose at a cell density of 5 x 10^9/ml and kept at 0°C for 30 min. To 10 µl of the suspension 0.1 ml of cold BSSE with or without E. coli DNA polymerase I (36 units/ml) was added. The mixture was stood in ice for 30 min, diluted and the UV sensitivity was measured (UV dose, 40 J/m^2).

when the plasmolysed cells were treated with an enzyme preparation which had been heated at 50°C for 20 min.

A similar result was obtained when bacteria were first irradiated and then treated with the enzyme (Fig. 2). With increasing concentrations of DNA polymerase I, survivals of UV-irradiated cells increased while number of unirradiated cells decreased slightly. It seems, therefore, that UV-damaged cells are actually reactivated by the enzyme treatment.

Specific action of enzymes: Two UV-sensitive strains, polA and uvrA mutants, which are defective in different steps of excision repair were compared for their responce to E. coli DNA polymerase I and T4 endonuclease V. The results are summarized in Table 2.

Application of T4 endonuclease V to plasmolysed cells of a polA mutant did not cause any increase in the UV resistance, whereas the mutant was reactivated with DNA polymerase I. Conversely, plasmolysed cells of a uvrA mutant was reactivated by endonuclease V but not by DNA polymerase I. Thus, the phenotype of each mutant is suppressed, at least in part, by supply of a specific enzyme.

Reproduction of λred phage: It has been shown that the burst size of λred phage is low in polA mutant (10). We have examined whether the impaired ability to support the growth of λred can be restored by supply of DNA polymerase I.

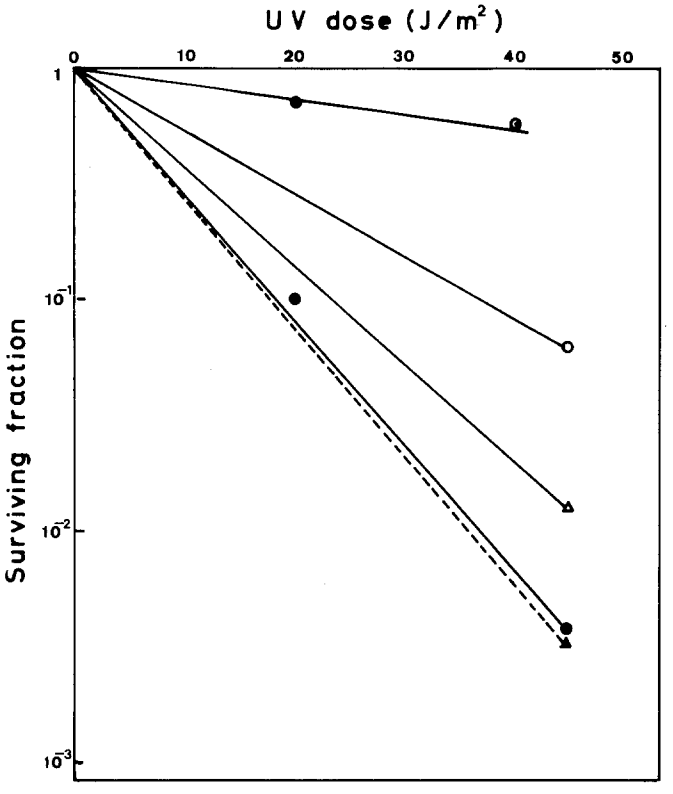

Fig. 1. Acquisition of UV resistance by polA mutant on introduction of DNA polymerase I. Cells of E. coli strain P3478 (polA1) were plasmolysed, treated with various amounts of DNA polymerase I and then irradiated with UV. (●) P3478 cells without enzyme treatment; (Δ) P3478 cells treated with 7.2 units/ml of polymerase; (○) P3478 cells treated with 36 units/ml of polymerase; (▲) P3478 cells treated with the heated enzyme (at 50°C for 20 min); (⊙) W3110T (polA$^+$) cells without enzyme treatment.

Since plasmolysed cells could not be infected with phage, bacteria were infected with λbio11 and then plasmolysed and treated with the enzyme. As shown in Table 3, the burst size of λred in enzyme-treated polA$^-$ cells increased twice the level of non-treated polA$^-$ cells. The increment amounted to about 20% of the burst size in polA$^+$ cells.

DISCUSSION

We have demonstrated that adequately permeabilized cells of polA mutants of E. coli acquired considerable degree of UV resistance after treatment of the cells with DNA polymerase I. Since the enzyme must have an access to the sites of action, namely UV-damaged chromosomal DNA, to exert such effect, the observed phenomenon can be taken as a proof that an active enzyme is inserted into viable cells. Although the precise mechanism of permeation is

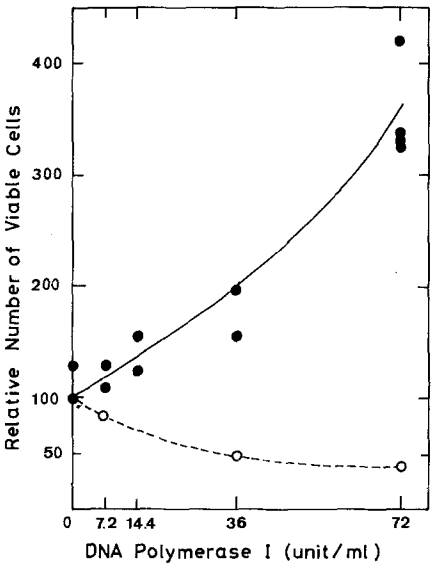

Fig. 2. Reactivation of UV-irradiated, plasmolysed cells by DNA polymerase I. N611 (polA) cells were suspended in M9S at 1 x 10^8 cells/ml and irradiated with approximately 30 J/m^2. Cells were washed with 1/10 volume of cold 40 mM Tris·HCl (pH 8.0) and plasmolysed with 1/250 volume of plasmolysis solution at 0°C. Non-irradiated cells were plasmolysed in a simialr manner. To 2 µl of the cell suspension were added 20 µl of BSSE containing various amounts of DNA polymerase I. After standing in ice for 30 min, the suspension was diluted and plated on L-broth plates. (•) UV-irradiated cells; (o) non-irradiated cells.

unknown, it is possible to suppose that in the absence of Ca^{2+} the membrane structure of plasmolysed cell becomes transiently porous so that large protein molecules in a medium can enter into cell when the cell is swollen by abrupt decrease in osmotic pressure.

From slopes of UV survival curves for enzyme-treated $polA^-$ cells and for non-treated $polA^-$ and $polA^+$ cells, it was roughly estimated that the UV resistance level of polA mutant increased to 14% of wild type level on treatment with 36 units/ml of DNA polymerase I. It has been shown that approximately 400 molecules of DNA polymerase I are present in normal E. coli cells (7, 8). Thus, if we assume that the resistance level is proportional to the amount of the polymerase present in cell, we may suppose that about 50 molecules of polymerase were introduced into the permeable cell.
A similar estimation was performed with recovery of reproduction of λred phage; in this case, it was calculated that about 70 molecules of polymerase were inserted into cell on treatment with 72 units/ml of the enzyme. This is, of course, a maximum estimation, and the actural values might be lower than those estimated.

Table 2. Responce of polA and uvr mutants to E. coli DNA polymerase I and T4 endonuclease V

Cells	Treatment	Surviving fraction after UV irradiation	Reactivation (fold)
N611 (polA)	None (control)	1.3×10^{-4}	1.0
	Polymerase I (36 units/ml)	1.1×10^{-3}	8.5
	Endonuclease V (25 units/ml)	1.3×10^{-4}	1.0
N17-9 (uvrA)	None (control)	1.3×10^{-4}	1.0
	Polymerase I (72 units/ml)	1.3×10^{-4}	1.0
	Endonuclease V (25 units/ml)	3.6×10^{-3}	27.7

Cells were plasmolysed and treated with BSSE containing either E. coli DNA polymerase I or T4 endonuclease V. The UV sensitivity of N611 and N17-9 was determined at 45 J/m^2 and 20 J/m^2, respectively.

Table 3. Effect of DNA polymerase I treatment on reproduction of λred phage

Strain	Treatment	Burst size
P3478 (polA)	None	8.1 ± 0.2
P3478 (polA)	DNA polymerase I	17.8 ± 3.0
W3110T (polA$^+$)	None	60.8 ± 1.0

Cell cultures in late-logarithmic phase were concentrated 10 fold in 10 mM $MgSO_4$-4 μg/ml thymine and infected with λbio11 at a multiplicity of 0.2 by standing at room temperature for 30 min. Infected cells were washed once with 10 volumes of cold 10 mM $MgSO_4$-4 μg/ml thymine and then plasmolysed. To 2 μl of infected, plasmolysed cells were added 20 μl of BSSE with or without DNA polymerase I (72 units/ml) and stood at 0°C for 30 min. Samples were diluted to 10 ml of prewarmed (37°C) TYM medium (0-time) and incubated further at 37°C for 90 min. Cells were lysed on incubation at 37°C for 15 min after addition of 0.1 ml of chloroform. Number of infective centers was determined by plating with E. coli C600 as an indicator.

The characteristic of the present system is that a considerable fraction (about 15%) of permeable cells retain cell viability. Although Wickner and Hurwitz (6) have demonstrated that plasmolysed cells were permeable to some proteins, such as pancreatic DNase I and trypsin, their plasmolysed cell preparations were practically non-viable (less than 0.1% of original viability). In the present study, by controlling conditions for plasmolysis and selecting adequate bacterial strains, we were able to prepare viable plasmolysed cells. Since the integrity of cellular organization is conserved in such cells, this system may allow one to investigate complex biological reactions which operate in close association with organized cell structure. By using modifications of this method, it might be possible to assay regulatory proteins for cell division or proteins which are involved in processes of induction and fixation of mutation. An attempt was recently made to assay an activity of the umuC gene product (11).

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