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THE MODULATING INFLUENCE OF THE FLUIDITY OF CELL MEMBRANE ON EXCISION REPAIR OF DNA IN UV-IRRADIATED ESCHERICHIA COLI

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SUMMARY: When the extent of liquid holding recovery (LHR) was measured as a function of the temperature at the time of liquid holding and the Arrhenius plot was made, two distinctive phases for the LHR were demonstrated in UV-irradiated RecA$^-$ derivative of E. coli ole28E$_1$, which are unable to synthesize and degrade unsaturated fatty acids. The inflection temperatures were 17-18°C, 23-24°C and 28-30°C for linoleate-, oleate- and elaidate-grown cells, respectively. These temperatures well corresponded to the phase transition temperatures of the cell membrane supplemented with the fatty acid. It is therefore concluded that at least a component involved in in vivo excision repair in E. coli is associated with cell membrane.

We have reported that the drugs such as chlorpromazine and procaine, which bind to cell membranes causing changes in the structural and functional organization (1-3), inhibit excision repair of DNA in UV-irradiated E. coli (4,5). The results suggest that cell membrane may be involved in the DNA repair process in E. coli. In order to obtain further evidence for the involvement of cell membrane in excision repair process, it is necessary to know whether or not the fluidity of cell membrane modulates the extent of excision repair. Since the fatty acid composition relates the fluidity of membranes (6-8), we examined the influence of fatty acid composition on liquid holding recovery (LHR) in UV-irradiated E. coli ole28E$_1$, an unsaturated fatty acid auxotroph (9), whose membrane fatty acid composition can be partly controlled. In this experiment we used a RecA$^-$ derivative of E. coli ole28E$_1$, because excision repair alone is sufficient for LHR in RecA$^-$ strains (10,11).

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Abbreviations: LHR, liquid holding recovery; AC, acriflavine;
Tc, phase transition temperature

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MATERIALS AND METHODS

Bacteria

Escherichia coli ole28E_1, an unsaturated fatty acid auxotroph (9), were kindly supplied by Dr. K. Izui, Kyoto University. A RecA$^-$ derivative, oleUV-10, was isolated by the treatment of the parental strain with N-methyl-N'-nitro-N-nitrosoguanidine in our laboratory.

Media

Bacterial cells were grown at 37°C with aeration in medium E (12) supplemented with 0.5 % glycerol, 0.25 mg/ml casamino acids, 0.05 % Triton X100 and 0.05 % desired unsaturated fatty acids. Nutrient broth agar supplemented with 0.05 % Triton X100 and 0.05 % oleate was used for measurement of survival.

UV Irradiation and Liquid Holding

The bacterial cells in exponentially growing phase were washed twice, resuspended in medium E buffer (medium E without glucose) at the concentration of about 2 X 10^8 cells/ml and then irradiated with UV light (254 nm) emitted from a germicidal lamp (15 W). The fluence rate was 0.05 J/m^2/sec, estimated with a UV intensity meter (Topcon UVR254, Tokyo).

UV-irradiated cells were subsequently held in medium E buffer in the dark at desired temperatures for up to 3 hr.

Survival Assay

After liquid holding, the cell suspensions were appropriately diluted and plated on nutrient broth agar containing 0.05 % Triton X100, 0.05 % oleate and 2 μg/ml of acriflavine (AC). AC was added to reduce the repair on the plates, since it inhibits excision repair by binding to DNA (12,13). After incubation at 37°C for about 48 hr, the number of viable colonies was counted to estimate survival.

Fatty Acid Analyses

Lipids were extracted from whole cells by the method of Ames (14). Fatty acid methyl esters were prepared by transestirification with boron trifluoride in methanol. Fatty acid composition was determined by analysis of the fatty acid methyl esters by gas chromatography on an instrument (Shimadzu GC-7APF, Kyoto) with a hydrogen flame ionization detector.

Chemicals

Fatty acids (potassium salts) and the standard mixtures of fatty acid methyl esters were obtained from Sigma Chemicals (St. Louis, USA) and Serdary Research Laboratories Inc. (Ontario, Canada), respectively. AC was the product of Wako Pure Chemicals (Osaka). The other reagents used in the present experiments were of the highest purity commercially available.

RESULTS AND DISCUSSION

Since AC inhibits almost completely the repair on agar plates (12,13), the addition of it to the plates allows us to observe the repair processes that occur just during liquid holding. Fig. 1 shows that a gradual increase in the number of cells able to form colonies on the AC plates in UV-irradiated *E. coli* oleUV-10 (RecA$^-$) with time of liquid holding at 30°C. AC reduced completely the LHR in this mutant when added at 2 μg/ml to the LHR buffer. These results are consistent with the findings that excision repair alone is sufficient for LHR in RecA$^-$ strains (10,11). Thus, by examining the extent of LHR under various conditions, it can be

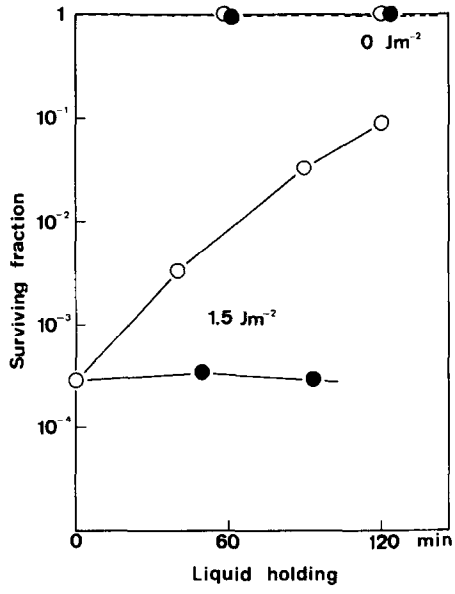

Fig. 1. LHR in UV-irradiated E. coli oleUV-10 ($RecA^-$). The cells grown with oleate were irradiated with 1.5 J/m^2 and then held in buffer at 30°C with (●) or without (○) AC.

determined whether the membrane fluidity has played a role in the in vivo excision repair in E. coli.

Unsaturated fatty acid auxotrophs of E. coli can synthesize saturated fatty acids, but lose the biosynthetic capacity for unsaturated fatty acids. The gas chromatography data of fatty acid composition of oleUV-10 cells grown in medium E supplemented with either oleate, linoleate or elaidate are presented in Table I. The only unsaturated fatty acid detected in cellular lipids was the one supplied.

Table I. Fatty acid composition of cell membrane of E. coli oleUV-10 grown with either oleate, linoleate or elaidate

Fatty acid classes	Percentage of fatty acids		
	Grown with oleate	Grown with linoleate	Grown with elaidate
14 : 0	7.5	1.3	9.7
16 : 0	45.5	52.1	35.0
16 : 1 (cis)	1.3	5.2	-
18 : 1 (cis)	42.5	-	-
18 : 1 (trans)	-	-	39.1
18 : 2 (cis, cis)	-	39.1	-
Others	3.2	2.3	3.8

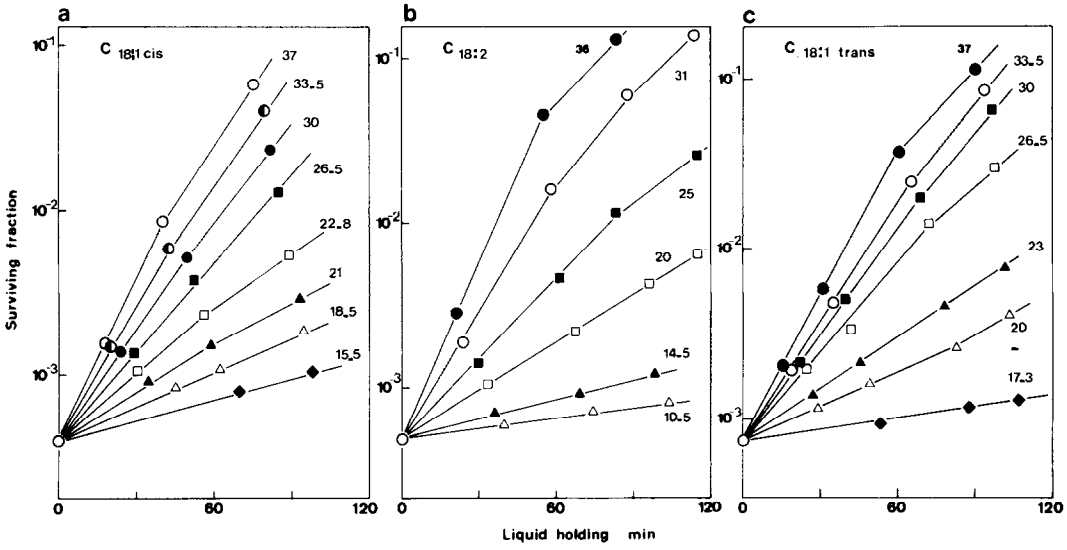

Fig. 2. LHR in UV-irradiated E. coli oleUV-10 under different conditions of liquid holding. The cells were grown with oleate (a), linoleate (b) or elaidate (c). UV fluence was 1.5 J/m^2. UV-irradiated cells were subsequently held in buffer at temperatures indicated.

In order to obtain evidence for the modulating influence of membrane fluidity on excision repair of DNA, the Arrhenius plot was made for the extent of LHR-temperature profile in UV-irradiated E. coli oleUV-10 supplemented with various fatty acids. The cells were grown with either oleate, linoleate or elaidate, irradiated with UV and then held in buffer at various temperatures. Fig. 2 shows the increase in the number of cells able to form colonies on the AC plates under different conditions of liquid holding. The Arrhenius plots for the extents of LHR at 1 hr were found to be discontinuous and biphasic (Figs. 3 and 4). The two line segments could be extraporated to an intersection point that was dependent on the unsaturated fatty acid to support growth. The inflection temperatures observed varied widely, that is, 23-24°C and 28-30°C for oleate- and elaidate-grown cells, respectively. In this experiment there appeared no discontinuity in the Arrhenius plot for linoleate-grown cells (Fig. 5), but our separate experiments have revealed that the discontinuity could be observed at 17-18°C for linoleate-grown cells (15).

The physical state of membrane lipids markedly depends upon temperature. The thermotropic order $\rightleftharpoons$ disorder phase transition has been well

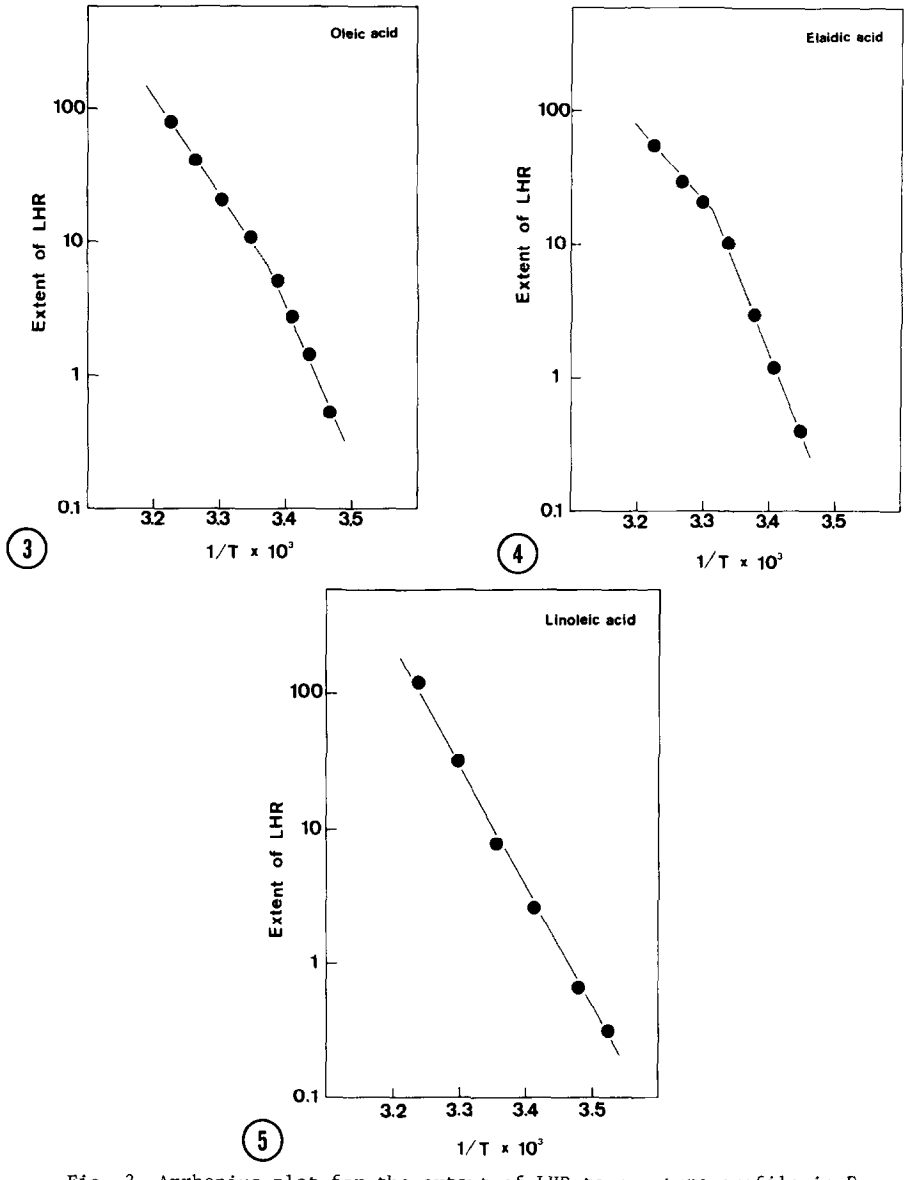

Fig. 3. Arrhenius plot for the extent of LHR-temperature profile in E. coli oleUV-10 grown with oleate.

Fig. 4. Arrhenius plot for the extent of LHR-temperature profile in E. coli oleUV-10 grown with elaidate.

Fig. 5. Arrhenius plot for the extent of LHR-temperature profile in E. coli oleUV-10 grown with linoleate.

documented in various membranes (16,17). Below the phase transition temperature (Tc), lipids exist in a highly ordered, gel state, in which the fatty acyl chains are relatively immobilidized, whereas, above the Tc, a disordered, fluid state exists with a conformational freedom for

the fatty acyl chains in membranes (5,16,17). The phase transition of membrane lipids has been shown to modulate membrane functions in general, e.g., membrane permeability and activities of membrane-bound enzymes (6-8, 18). The Tc depends upon the fatty acid composition of membrane lipids and is in increasing order when determined in unsaturated fatty acid auxotrophs of E. coli grown with linoleate (cis, cis-dienoic, 18:2), oleate (cis-monoenoic, 18:1) and elaidate (trans-monoenoic, 18:1) (6-8,17,18). Discontinuities in the Arrhenius plot for membrane-associated functions have been interpreted in terms of phase transition of membrane lipids (6-8). The inflection temperatures in the Arrhenius plot for the extent of LHR-temperature profiles (Figs. 3 and 4, 15) were in satisfactory agreement with those for membrane permeability and furthermore with the Tc of each unsaturated fatty acid-supplied cell membrane determined with the aid of physico-chemical probes (6-8,18). These results clearly indicate that at least a component of the in vivo excision repair of DNA in E. coli is associated with the cell membrane.

A major mechanism for the repair of DNA damaged by UV is an error-free, multienzymatic excision repair (19-21). In E. coli, this scheme is initiated by an endonucleolytic cleavage of a phosphodiester bond at or near the site of pyrimidine dimers produced by UV in DNA. Subsequently, the dimers can be excised by an exonuclease and the resulting gaps filled in and sealed by the action of DNA polymerase and DNA ligase (19-21). The molecular mechanisms of the modulation of excision repair by the fluidity of cell membrane could not be elucidated in the present experiments. However, it is likely that the initial step, incision and/or excision step may be modulated by the membrane fluidity, since membrane-specific drugs like procaine and lidocaine have been shown to inhibit the removal of thymine dimers from DNA in E. coli (5). At least an enzyme involved in these steps might be bound to cell membrane. Alternatively, excision repair process is a coordinated series of enzymatic reactions mentioned above. Although the manner in which all of the steps are co-

ordinated is not understood, cell membrane may be involved and the fluidity may play an important role in this coordination. On the other hand, some metabolic pathways such as energy production are known to be associated with cell membrane in E. coli (1,6). A more trivial explanation would be that energy production is modulated by the membrane fluidity, which might be the cause of the change in excision repair capacity. There may be another possible explanations for the involvement of cell membrane in the DNA repair. Work along with these lines is currently being undertaken in our laboratory.

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