Inhibition of Nitrogen Mustard After-Effect in $F^-$-Cells by the Factor Produced by $E. coli$ K-12 Fertile Strains

D. M. GOLDFAIB, L. S. CHEBIN and Yu. K. FOMITCHEV
Institute of General Genetics, USSR Academy of Sciences, Moscow,
Byelorussian State University, Minsk

Received January 4, 1969

Summary. 1. $Hfr$, $F^+$ and $RTF$ strains of $E. coli$ produce an extracellular inhibitor of nitrogen mustard after-effect (NMAE) in NM-treated $F^-$-cells.
2. The NMAE inhibiting factor is a non-dialyzable protein.
3. The NMAE inhibiting factor is inactive in $F^{-}rec^-$ and $F^{-}uvr^-$-cells treated with NM.

Among the peculiarities of the nitrogen mustard (NM) effect on phages and bacteria is the so called after-effect. The nitrogen mustard after-effect (NMAE) manifests itself in a decrease of viability of NM-treated phages and bacteria during a post-treatment incubation in a liquid medium.

LOVELESS and STOCK (1959b) concluded that the NM-inactivation of $T$-phages and $E. coli$ is a stepwise process. The first step is the primary alkylation, i.e. an interaction of one of the active NM groups with DNA. The next step is the secondary alkylation proceeding at the expense of the second alkylating group. These two reactions do not occur simultaneously and the after-effect is thought to be due to the latter step. This conclusion was inferred from the experiments on the protective effect of sodium thiosulphate which appeared to be caused by the blockage of the second functional NM group (LOVELESS, STOCK, 1959a). The authors (LOVELESS, STOCK, 1959a) stated that the sodium thiosulphate failed to protect the cells damaged by a monofunctional alkylating agent.

We have previously described (GOLDFARB, FOMITCHEV, LEBANOK, 1968) the inhibition of NMAE in $F^-$-cells after conjugation of the damaged cells with intact $Hfr$ and $F^+$-strains of $E. coli$. It was also established that the NMAE inhibition occurred only when the treated $F^-$-cells were $rec^+$. It remained obscure whether this phenomenon depended on the transfer of an episome or a fragment of the donor chromosome into the damaged recipient cell, or whether the contact of the surfaces of the intact and damaged cells accounted for the NMAE inhibition. It was possible that the "curing" donor cells produce an unknown factor capable of inhibiting NMAE in $F^-$-cells.

All the above suggestions needed experimental verification which is the subject of the present communication. The following questions were studied:

1. whether the previously described NMAE inhibition during conjugation is caused by transfer of an episome or a fragment of a donor chromosome into the damaged recipient cells;
2. whether the ability of donor cells to inhibit NMAE in $F^-$-cells is dependent on the kind of an episome carried by the donor cells;
3. whether the donor strains are capable of producing an extracellular factor inhibiting NMAE.

Materials and Methods

1. NM-Treated Test-Bacteria

*E. coli K-12 JC 411 F-* (leu lac gal his str-r mal zyl mtl arg met rec+ uren+) kindly supplied by Dr. J. Clark; neomycin-resistant *JC 411 F- nm-r* (20 units/ml) induced by nitrosoguanidine from strain *JC 411 F-*, recombination-deficient mutant of *E. coli K-12 JC 1553 F-rec* (Clark, Margulies, 1965); *E. coli CR-34, KMBL-91 F-* (thr leu lac pyr thy str-r thi dar 2) kindly provided by Dr. A. Rösh.

2. Donor-Bacteria Used for the "Curing" NM-Treated Test-Cells

*E. coli K-12 H/J C* (met str-s) kindly supplied by Dr. W. Hayes; *E. coli K-12 S F* wild type kindly sent by Dr. L. Alfoldy; *E. coli K-12 CSH-2 (222) F-Rfi+* (met str-r cm-r te-r su-r) kindly supplied by Dr. T. Watanabe; *E. coli B; Shigella dysenteriae Flexneri 170; E. coli K-12 JC 411 F-.

3. Media

a) Rich medium — a standard meat-peptone broth (MPB); b) 1.5% meat-peptone agar (MPA) with streptomycin (200 units/ml) or neomycin (20 units/ml); c) Buffer salt solution contained 0.89% KCl — 150.0 ml, 0.66 M Na$_2$HPO$_4$ — 60.0 ml, 0.66 M KH$_2$PO$_4$ — 40.0 ml, distilled water to 1,000.0 ml; d) M-9 medium supplemented with Casamino acids: NH$_4$Cl — 1.0, KH$_2$PO$_4$ — 3.0, Na$_2$HPO$_4$ — 6.0, MgSO$_4$ — 0.13, glucose — 4.0, Casamino acid (Schuchardt München) — 1.0, distilled water to 1,000.0 ml. pH — 7.2. MgSO$_4$ and glucose were separately sterilized and added to a chilled M-9 medium; e) Medium for selecting the recipient $F^-$-cells with a R-factor contains 1.5% MPA with streptomycin (200 units/ml), tetracycline (100 µg/ml) and chloramphenicol (100 µg/ml).

4. NM-Treatment of Test-Bacteria

The cells grown overnight in MPB were inoculated into the fresh MPB and grown under shaking for 2 hours up to 3—5 × 10$^8$ cells/ml. The logarithmically growing culture was exposed to NM for 5 min at 37°C at a final concentration of 300 µg/ml. In order to eliminate NM the cells treated were either centrifuged or diluted 10$^9$—10$^{10}$-fold in a fresh pre-warmed broth.

5. Preparation of Bacterial Filtrates

Donor bacteria were grown up to 5 × 10$^8$ cells/ml in broth or in M-9 medium supplemented with Casamino acids and were filtered through 1.42—1.48 µ Millipore filter (type 117G5). Filtrates obtained retained their activity for about one month while stored at —20°C.

6. Preparation of Bacterial Extracts (Adler et al., 1966)

$H/J C$ or $JC 411 F$-strains were grown with a slow shaking to about 6.0—8.0 × 10$^8$ cells/ml at 37°C and then centrifuged at 4,000 rev/min for 20 min. The pellet was resuspended in an equal volume of 0.067 M phosphate buffer and then centrifuged once more. The pellet formed was resuspended in a 50-fold reduced buffer volume. The concentrated cells were frozen at —70°C and passed twice through a hydraulic press at 150 atm. The extract was centrifuged for 30 min at 20,000 rev/min at 4°C. The supernatant was used in the experiments.