# Prophage Induction by Alkylating Ethyl Methylaminosulfonate

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Treatment of the lysogenic strain *M. lysodeikticus* 53-40 (N5) with ethyl methylaminosulfonate results in a slight increase in infective center concentration of N5 phages which is only detectable once disturbing cells had been eliminated by lysozyme. After conversion of ethyl methylaminosulfonate with radioactive labelled guanosine at 37 °C, N7-ethylguanine could be ascertained. Therefore the biological activity of the drug may be due to an ethylation of DNA.

Lysogenic phages can insert their chromosome in that of the host. This latent form is called prophage. It remains dormant for many cell generations until some metabolic alteration or an external agent supervenes. Agents causing prophage induction are mostly mutagens and carcinogens. According to Heinemann [1] out of 89 prophage inducing agents 45% possess a mutagenic effect and 28% a carcinogenic one. Screening for the capability to induce prophages therefore helps to make classifying the harmfulness of a drug fast and easy. If the agents inactivate both free phages and bacteria to a high extent and the inducing effect is lower than the tenfold, then no prophage induction can be detected, especially if a high spontaneous induction counteracts to the measurement.

In the case of ethyl methylaminosulfonate $CH_3NHSO_2OC_2H_5$ we show a method which allows to detect slight prophage inductions. Alkyl alkylaminosulfonates are mutagens as could be proved with strains of *E. coli* [2]. By using ethyl methylaminosulfonate 0.3% auxotrophic mutants out of 0.5–1.0% survivors could be found after treatment with 0.04 mol mutagen/l under optimal conditions (paper in preparation). Field and Naylor ascertained that dimethylsulfate induces the development of phage N5 in the lysogenic strain *M. lysodeikticus* 53–40 (N5) [3]. Therefore we used this strain in our experiments. As the cells tend to form clumps, a short sonication was necessary before treating them with a mutagen [3]. The cells were then incubated for 30 minutes with the mutagen of different concentrations at 30 °C in CP-buffer pH 7 [2] or in ML-medium [3]. After washing the bacteria once they were incubated for another four hours and plated on ML-plates with the indicator strain *M. lysodeikticus* 53–5. The size of prophage induction results from the ratio

$$J = \frac{\left(\frac{\text{titer after 4 h}}{\text{titer after 30 min}}\right)_{\text{test}}}{\left(\frac{\text{titer after 4 h}}{\text{titer after 30 min}}\right)_{\text{control}}}$$

(presupposing proportional cell numbers in the test and the control). Without any further treatment one keeps a background of more than 90% irrelevant plaques on plates which is due to the high spontaneous induction. The small number of plaques produced by drug induction is neither measurable nor morphologically distinguishable from those originating from spontaneous induction. The appearance of plaques without a central colony can be explained by spontaneous induction of one lysogenic cell of the arising central colony after many cell generations and phage multiplications in surrounding indicator bacteria which leads to multiple infection and to the loss of immunity or to "lysis from without" [4] of the remaining lysogenic cells. Drug induction, however, causes lysis of the cell within the first generation. Therefore, three different events can be held responsible for a plaque without a central colony: infection by free phages, spontaneous induction, if the preincubation is not used (see below) or drug induction.

In order to eliminate any remaining lysogenic cells the following methods are to be considered: *Elimination of lysogenic cells by centrifugation* is out of the question, because of the tendency of forming clumps and as only a small prophage induction is to be expected. Therefore, Field and Naylor [3] developed a *preincubation method* with *M. lysodeikticus* 53–40 (N5) in which samples of lysogenic treated resp. untreated bacterial suspensions are poured on soft agar plates, incubated for 24 hours at 30 °C, afterwards sprayed with indicator bacteria and again incubated. After the second incubation one observes phage plaques both with and without central colony. The plaques with central colony are caused by lysogenic cells, while the plaques without central colony originated from free phages. The sensibility of this method seemed to be too low based on the great spontaneous induction. Also a smearing on the plates has to be prevented.

*Plating with streptomycin resistant indicator bacteria* on agar containing streptomycin inhibits

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growth and spontaneous induction of the lysogenic cells [5].

Almost similarly effective is the *plating of polyauxotrophic lysogenic cells with prototrophic indicator cells* on unsupplemented minimal agar.

Better results are obtained with *M. lysodeikticus* when treating induced lysogenic bacteria with lysozyme (20 µg/ml, 10 min at 42 °C). It has been known for some time that *M. lysodeikticus* is a test substrate for lysozyme [6]. As the lysogenic bacteria are lysed, the disturbing growth does not occur. By means of this lysozyme method with ethyl methylaminosulfonate in a concentration of about 1% (0.09 mol/l) a 2.7-fold increase of prophage induction could be found compared with the untreated control (Fig. 1). With this method we realized a 70-fold increase in infective center concentration using ultraviolet light and with dimethylsulfate a 13-fold one.

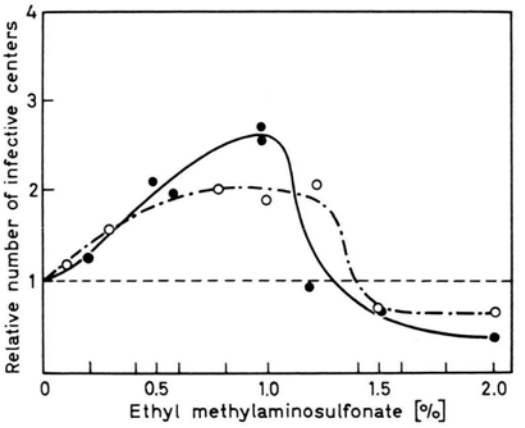


Fig. 1. 0.1 vol. of an overnight culture of *M. lysodeikticus* 53-40 (N5) in ML-medium [3] were diluted and grown to log-phase. These cells were sonicated and treated with different concentrations of ethyl methylaminosulfonate in CP-buffer [2] (○– – –○) or ML-medium (●– – –●) for 30 min by shaking at 30 °C (total volume 5.3 ml). The cells were centrifuged and washed once with ML-medium. After 4 hours of incubation at 37 °C in a shaking water bath, the cells were lysed with lysozyme (final concentration 20 µg/ml) and after suitable dilution plated on ML-agar plates with cells of the indicator strain *M. lysodeikticus* 53-5. In the same way control experiments without any treatment with mutagen were carried out (– – –).

The biological activity of ethyl methylaminosulfonate may be caused by an alkylation of nucleic bases of the DNA.

Radioactively labelled guanosine was converted with ethyl methylaminosulfonate at 37 °C. The conversion products were hydrolyzed and then chromatographed on cellulose thin-layer plates. Chromatograms were developed in *n*-butanol-glacial acetic acid-water (4 : 1 : 5), ethanol-water-conc. ammonia (80 : 18 : 2) or *n*-butanol (water saturated). The products were identified by means of autoradiography and thin-layer scanning by using authentic substances. N7-ethylguanine could be ascertained as an alkylating product, whereas N7-methylguanine was not observed. *Vice versa*, the corresponding conversion with methyl ethylaminosulfonate resulted in N7-methylguanine in a relative high yield, whereas N7-ethylguanine was not formed (Fig. 2).

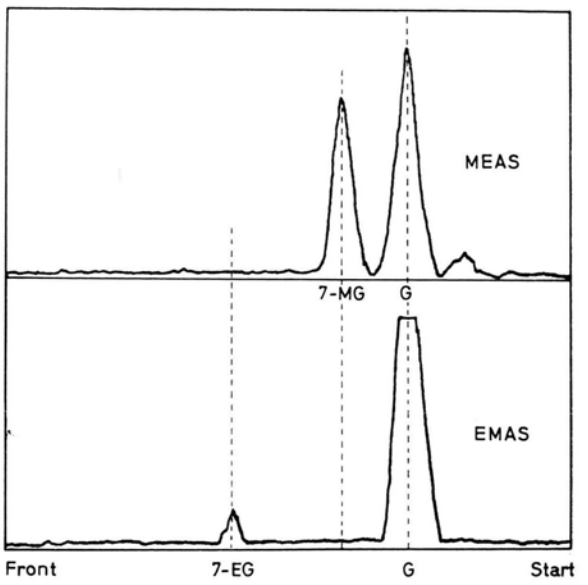


Fig. 2. MEAS, methyl ethylaminosulfonate; EMAS, ethyl methylaminosulfonate; 7-MG, N7-methylguanine; 7-EG, N7-ethylguanine; G, guanine. 0.005 µmol [8-$^{14}$C]guanosine (specific activity 49.2 mCi/mmol) were converted with 0.03 µmol ethyl methylaminosulfonate resp. methyl ethylaminosulfonate in citrate-phosphate buffer of pH 7 [2] at 37 °C for 48 hours (total volume 0.25 ml). The reaction products were hydrolyzed in 1 N HCl for 20 min at 80 °C, then neutralized with ammonia and visualized after chromatography on cellulose thin-layer plates in *n*-butanol-glacial acetic acid-water (4:1:5) by radio-thin-layer scanning.

From these results we conclude that the following reaction did occur:

$$\text{Guanosine} + R_1 - NH - SO_2 - OR_2 \rightarrow$$
$$N7 - R_2 - \text{Guanosine} + R_1 - NH - SO_2 - OH.$$

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