Induction of SOS Functions in *Escherichia coli* and Biosynthesis of Nitrosamine in Rabbits by Nitrogen Dioxide

by Hiroaki Kosaka,* Mitsuro Uozumi,* and Taichi Nakajima**

Nitrogen dioxide induced SOS functions in *Salmonella typhimurium* and *Escherichia coli* K-12 and was mutagenic in *Escherichia coli* WP2. When a rabbit was administered aminopyrine intravenously and administered nitrogen dioxide by inhalation, N-nitrosodimethylamine was detected in its blood. Analysis was conducted with [15N-nitrosodimethylamine as an internal standard by a combination of capillary gas chromatography and mass spectrometry. Accompanying administration of cystamine increased the blood concentration of N-nitrosodimethylamine in the rabbit, suggesting inhibition of its metabolism. Concurrent sulfur trioxide inhalation increased N-nitrosodimethylamine formation in the rabbit.

**Introduction**

Oxides of nitrogen, oxides of sulfur, and ozone occur in the air above the major cities of the world. Nitrogen dioxide (NO₂) is among the most toxic of the nitrogen oxides, but its genetic effects have not been investigated as much as those of ozone. NO₂ has been demonstrated to induce proliferative changes of the terminal bronchiolar and alveolar epithelium. SOS functions, such as enhanced capacity for DNA repair and mutagenesis, inhibition of cell division, and prophage induction (1), were induced by NO₂ but not by NO (2) in *Salmonella typhimurium* (2) and *Escherichia coli* (3).

NO₂ or its dimer (N₂O₄) also reacts with amines to yield diazo and N-nitrosamine products even under aqueous neutral and alkaline conditions (4–9). According to Challis (5), nitrosation by gaseous N₂O₃ and N₂O₄ in aqueous solution is a recent finding, probably because both had been expected to undergo rapid hydrolysis at pH > 5 to innocuous nitrite. Hydrolysis does occur, but less rapidly than the nitrosation of many amines. N₂O₃ and N₂O₄ react about 2000 times more rapidly with most amines than with H₂O.

This article briefly reviews our previous investigations on the induction of the SOS functions and mutation by NO₂ (2,9) and nitrosamine formation by NO₂ (7–9).

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**Materials and Methods**

**Induction of SOS Functions and Mutation**

The plasmid pSK1002 (10), which has a *umuC–lacZ* fusion gene, was introduced into *Escherichia coli* KY700 [Δ(pro-lac) thi ara met srlC300::Tnl10] (11). The gene produces hybrid RecA-LacZ protein when the SOS functions are induced. Beta-galactosidase activity was assayed by Miller’s method (12). *Escherichia coli* WP2 (trpE65) was used for mutagenesis. Exponentially growing bacteria were used after washing.

**Nitrosamine Formation**

Air containing NO₂ was prepared with a Standard Gas Dilution System (Model 302, Seiitetsu Kagaku Works, Ltd.). A rabbit was tied and treated without anesthesia. A venous blood sample (about 4 mL) was mixed with 1 N KOH (1 mL) and an internal standard [15N-nitrosodimethylamine (15N-NDMA)]. Each sample was placed in a dialysis tube and dialyzed against 80 mL of dichloromethane two times for 30 min each with shaking (90 strokes/min). The dialyzed samples were concentrated to about 5 mL using a K-D evaporator, then left in a water bath (45°C) under a stream of nitrogen for further concentration to 0.1 to 0.3 mL. The 1 μL aliquot was injected onto a fused silica capillary column (liquid phase PEG 20M, length 25 m, ID 0.25 mm, Nihon Chromato Works, Ltd.), which was mounted in a Hewlett Packard 5710A gas chromatograph with a capillary inlet system 18740B. The carrier gas was helium at a pres-
sure of 10 psi. The injector and oven temperatures were 200°C and 70°C, respectively. The effluent was directed via an all-glass jet separator (200°C) into the electron ionization ion source of a Jeol 300 DX mass spectrometer set to monitor the ions at m/z 74 and m/z 75 after peak position adjustment using 14N-NDMA and 15N-NDMA, respectively. The peak height was used for the calculation.

**Results**

The expression of umu operon was examined with the *Escherichia coli* strain K-12 carrying the umuC–lacZ fusion plasmid by measuring the levels of β-galactosidase. As shown in Figure 1, 90 μL/L NO2 induced the expression of umuC operon after bubbling of the gas at 100 mL/min for 30 min. The mutagenicity of NO2 was also investigated. Figure 2 demonstrates that NO2 increased the induced mutation frequency in a dose-dependent fashion in *Escherichia coli* strain WP2 by reversion to Trp+.

Nitrosodimethylamine (NDMA) was detected in the blood of a rabbit administered aminopyrine IV and NO2 (50 μL/L) by inhalation. Interruption of the NO2 supply led to an immediate decrease in the NDMA level, in agreement with the report that the blood concentration of NDMA declines with a half-life of 11 min (18). Accompanying IV administration of cystamine (200 mg/kg/hr), which is speculated to inhibit NDMA metabolism (14), increased the concentration of NDMA in the blood, as shown in Figure 3. Concurrent sulfur trioxide (SO3) inhalation accelerated the formation of NDMA in the blood (Fig. 4), where SO3 was added by passing the gas over a solution (0.3 mL) of 60% fuming sulfuric acid.

**Discussion**

NO2 induced the SOS functions in *Salmonella typhimurium* (2) and *Escherichia coli* K-12 (Fig. 1) and also mutagenesis in *Escherichia coli* WP2 (Fig. 2), although the levels of mutagenesis were low. The weak mutagenesis may have been due to the low level of induction of the SOS functions. Alternatively, NO2 may be one of the so-called weak mutagens which induce SOS repair activity by arresting DNA replication but do not otherwise induce premutagenic lesions (1). Isomura et al. (15) found that NO2 induced mutagenicity in *Salmonella typhimurium* TA100 and TA1535, and that mutations and chromosome aberrations were induced in lung cells following in vivo exposure of rats to NO2. Tsuda et al. (16) also reported that NO2 could induce chromosome aberrations in cultured Chinese hamster V79-H3 cells.

In the rabbit, IV administration of aminopyrine and
pyrrole might inhibit the metabolism of NDMA, as aminopyrine is metabolized by cytochrome P-450, yielding formaldehyde. One observation concerning NDMA metabolism that has puzzled many investigators is that NDMA demethylase activity is not induced by classical inducers and is not significantly inhibited by classical monoxygenase inhibitors, such as SKF-525A and metyrapone, but is inhibited by compounds such as 3-amino-1,2,4-triazole and 2-phenylethylamine. Cytochrome P-450 LM₃₉ isolated from liver microsomes of ethanol-treated rabbits (23) should help resolve these conflicting observations.

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