

Salmonella/Microsome Mutagenicity of 1-Nitropyrene-2-ol, a Nitropyrene Phenol Formed in the Photolysis of 1-Nitropyrene

Göran Löfroth^{***}, Lena Nilsson^{**}, Eva Agurell^{**}, and Akio Yasuhara^{***}

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1-Nitropyrene-2-ol is not detectably mutagenic in the *Salmonella* test in the absence of mammalian activation. It is activated by the rat liver microsome-containing preparation S9 and gives a mutagenic response similar to that of benzo(a)pyrene.

Introduction

Airborne particulate matter contains organic compounds that are mutagenic in short-term bioassays and carcinogenic in animal tests [1]. Some of these compounds are the conventional polycyclic aromatic hydrocarbons (PAH) such as benzo(a)pyrene. A large part, however, is derivatives which may be oxygenated and/or nitrated polycyclic hydrocarbons as judged by their mutagenic features in the *Salmonella*/microsome test and by their polarity.

Pyrene is easily nitrated and mutagenic nitro-pyrenes have attracted considerable interest but neither these compounds nor similar nitroarenes seem to account for the mutagenic potential of the more hydrophilic fraction of airborne particulate matter.

We have studied the mutagenic behavior of a doubly derivatized PAH, 1-nitropyrene-2-ol, which has been isolated after photolysis of 1-nitropyrene [2].

Materials and Methods

The compound has been isolated from an irradiated acetonitrile solution of 1-nitropyrene [2]. A

small amount, about 2 mg, was transported in solid form and subsequently dissolved in dimethyl sulfoxide for the mutagenicity tests. The assays were performed by the plate incorporation method [3] with the *Salmonella* strains TA98 and TA100 in the absence and in the presence of mammalian activation and with the enzyme deficient strains TA 98 NR and TA 98/1,8-DNP₆ [4] in the presence of mammalian activation. The mammalian activation was made by the addition of 0.5 ml S9-mix containing necessary cofactors and either 20 or 50 µl S9 which had been obtained from the livers of Aroclor 1254-induced male Sprague-Dawley rats. Assays were also performed with an incomplete S9-mix to which no NADP had been added. The final mutagenicity results are based on at least three independent tests. All assays included tests with single doses of positive control compounds which were a commercial preparation of 1-nitropyrene (Koch-Light Ltd, UK) and quercetin (Sigma Chemical Co, USA) in the absence of S9 and benzo(a)pyrene (Sigma Chemical Co, USA) in the presence of S9. The bacterial strains were checked for appropriate characteristics by spot tests with positive control compounds, by sensitivity to crystal violet, by resistance to ampicillin and by their spontaneous reversion frequency (20–50 revertants per plate for TA 98, TA 98 NR and TA 98/1,8-DNP₆ and 150–200 revertants per plate for TA 100).

Results

1-Nitropyrene-2-ol is not detectably mutagenic in the absence of mammalian metabolic activation (Table I) with doses up to 25 µg per plate which shows that it is neither a direct-acting mutagen nor is solely activated by bacterial enzymes to mutagenic metabolites. Mutagenicity was also not detected with anaerobic incubation (data not shown) which means that the bacterial oxygen-sensitive nitroreductase(s) does not activate the compound to mutagenic metabolites [5, 6].

In the presence of S9, however, the compound is mutagenic (Fig. 1) having a response of 100–200 revertants per µg which is of the same order of magnitude as that of benzo(a)pyrene (Table I). The activity is highest in the TA 100 strain and it is not strongly dependent on the amount of S9 added within the commonly used interval of S9 amounts (4–10 v/v %). Assays using an S9-mix without

* Nordic School of Public Health, S-413 46 Gothenburg, Sweden

** Department of Radiobiology, Wallenberg Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden

*** Division of Chemistry and Physics, National Institute for Environmental Studies, Yatabe-machi, Tsukuba-gun, Ibaraki 305, Japan

Reprint requests to G. Löfroth (Stockholm).
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Table I. The mutagenic response of 1-nitropyrene-2-ol, its precursor 1-nitropyrene and positive control compounds in different *Salmonella* strains.

Compound	Dose range µg per plate	S9 µl per plate	Revertants per µg			
			TA 98	TA 98 NR	TA 98/1,8-DNP ₆	TA 100
1-nitropyrene-2-ol	0.25–25	—	< 2	—	—	< 2
1-nitropyrene-2-ol	0.5–5	20	100	—	—	180
1-nitropyrene-2-ol	0.5–5	50	85	65	50	160
1-nitropyrene ^a	0.05–2	—	1900	140	1500	600
1-nitropyrene ^a	0.05–2	20	—	600	—	—
quercetin	50	—	11	10	10	—
benzo(a)pyrene	2.5	20	140	—	—	270
benzo(a)pyrene	2.5	50	110	—	—	270

^a Data from ref. 6 on a high purity sample.

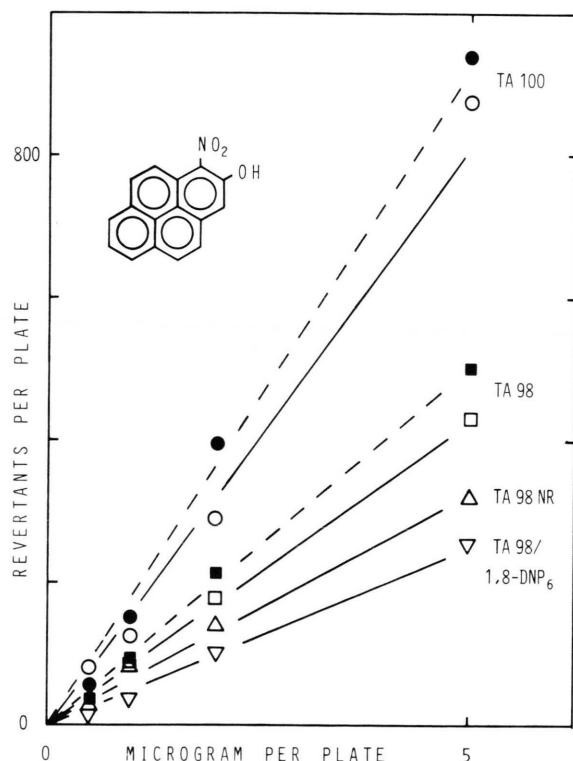


Fig. 1. Dose response curves for 1-nitropyrene-2-ol in different *Salmonella* strains in the presence of S9. Revertants per plate after subtraction of the spontaneous reversion frequency is plotted against the amount per plate. Dashed lines and filled symbols, ●■, 20 µl S9 per plate. Solid lines and open symbols, ○□△▽, 50 µl S9 per plate.

NADP do not result in any mutagenic activity showing that this cofactor is essential in the activation. The difference between the responses in TA 98, TA98 NR and TA98/1,8-DNP₆ is too small to be used as a basis for judging whether or not bacterial enzymes also are involved in the activation.

Discussion

The mutagenic behavior of 1-nitropyrene-2-ol in the *Salmonella* test is distinctly different from that shown by a number of other nitropyrenes. Whereas 1-nitropyrene-2-ol requires mammalian activation and is neither activated by the classical bacterial nitroreductase nor by the oxygen-sensitive bacterial nitroreductase, all other investigated nitropyrenes are activated to mutagenic metabolites by the bacteria. Such compounds are 1- and 2-nitropyrene [6], di-, tri- and tetranitropyrenes [7] and 1-nitropyrene-3-ol, -6-ol and -8-ol [8, 9].

The metabolism of nitropyrenes can either proceed by nitroreduction or by ring oxidation. For 1-nitropyrene, nitroreduction is the predominant pathway in *Salmonella* [10] and intestinal bacteria [11, 12] and it can also occur in mammalian systems under reduced oxygen concentration [9, 13, 14]. Ring oxidation is a major pathway with microsomal S9 under aerobic conditions [9]. 1-Nitropyrene has in addition to its S9-independent mutagenicity also an S9-dependent mutagenicity in the *Salmonella* test [6 (see Table I), 15, 16] but it is not known if the latter is due to ring oxidation or nitroreduction.

Nitroarene mutagenicity in the *Salmonella* test in the absence of microsomal S9 generally implies nitroreduction. The absence of a mutagenic effect by 1-nitropyrene-2-ol may simply be due to that the nitro group becomes inaccessible to enzymatic attack by the presence of the neighboring hydroxy group which also forms a chelate structure with the nitro group by intramolecular hydrogen bonding.

The finding that the mutagenic response in the presence of S9 is higher in TA 100 than in TA 98 indicates that the ultimate mutagen is an arene dihydro epoxide as such epoxides mostly have a

higher response in TA 100 than in TA 98. In contrast to pyrene which is not detectably mutagenic in the *Salmonella* assay although its metabolism includes epoxide intermediates [17], a dihydro epoxide from 1-nitropyrene-2-ol, *e.g.* at the 4,5-bond, might be sufficiently stable to give mutagenic effects.

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