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Mutagenic activity in *Salmonella typhimurium* mutants of the dust organic extract and its five fractions derived from an aluminum electrolytic plant

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Abstract—The dust derived from an aluminum electrolytic plant was collected on a filter, then extracted with benzene. The benzene-soluble material was separated into five fractions, namely organic acid, organic alkali, aliphatic hydrocarbon, polycyclic aromatic hydrocarbon and polar compounds. The mutagenic activities of these fractions were examined with two strains of *Salmonella typhimurium* mutants. It was demonstrated that the dust organic extract showed a very strong mutagenic activity. Among the five fractions tested, three fractions, namely organic acid, polycyclic aromatic hydrocarbon and polar compounds, played an important role in the mutagenic activity of the dust organic extract. The other two fractions, organic alkali and aliphatic hydrocarbon had no mutagenic activities. Therefore, dust abatement is an important measure in aluminum plants for controlling carcinogens, mutagens and protecting the environment and human health.

Keywords: dust organic extract; *Salmonella typhimurium*; mutagenic activity.

1 Introduction

The process of aluminum electrolysis is the main source of environmental pollution of aluminum industry. With the increasing market demand for aluminum and the unceasing expansion of aluminum production, the pollution caused by toxic matter from aluminum electrolysis to the environment is broadening on a daily scale and its potential effect on human health is becoming more and more serious. Epidemiology studies have suggested that, compared to common people, the aluminum smelter workers and surrounding inhabitants more easily suffer from lung cancer, bladder cancer and skin cancer, and the mortality caused by tumor is higher (Becher, 1984; Chu, 1996; Graham, 1979; Theriault, 1984). The rates of chromosome aberration, micronucleus (MN) and sister-chromatic exchange (SCE) in aluminum smelter workers are much higher (Wu, 1985). In addition, the urine from aluminum smelter workers possesses the mutagenicity (Heussner, 1985). To our knowledge, however, most of the presently available reports studied the mutagenic activity of air particles derived from city smog (Houdt, 1989), inside and outside home (Houdt, 1984), gas plant (Zhu, 1988), steel plant (Lu, 1988) and electric power plant (Kubitschek, 1979). But, little information exists on the mutagenic effects of airborne particulates derived from aluminum electrolytic plant. Helmes (Helmes, 1982) and Schlipkoter (Schlipkoter, 1983) believed that airborne particulate matters from heavy industrialized areas contain more than 500 chemical substances including mutagens/carcinogens, such as polycyclic aromatic

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hydrocarbons, chlorinated hydrocarbons, aromatic amine and others. It is necessary to study the mutagenic activities of the different fractions of the dust. For these reasons, we studied the mutagenic activities of the dust organic extract and its five fractions derived from an aluminum electrolytic plant with Ames test. We divided the dust organic extract into five fractions, in order to find out the genetic toxicants with stronger mutagenic activity.

2 Materials and methods

2.1 Sample collection and fractionation

Samples of the dust were collected on a fiberglass filter within the scope of the operator's breathing air. Four samplers (CG-2A Model) were equidistantly placed in the electrolytic plant. The sampling time lasted for 2 days. Before and after each sampling period, the filter was conditioned at constant relative humidity (50% ± 2%) and temperature (22 ± 2℃) for 24h and weighted to obtain the weight of suspended dust. The samples volume was 1583 m^3. The dust-laden filter was placed in a Soxhlet extractor and the organic material was extracted for 8h with 250 ml of spectrograde benzene. The benzene solution was filtered, concentrated to 10 ml and then lyophilized to have the dust organic extract (F). The dust organic extract (F, 500 mg) was fractionated by a method modified from Wynder and Hoffmann (Wynder, 1965) into three fractions, organic acidic (F1), organic alkali (F2) and neutral. The neutral part was divided into aliphatic hydrocarbon (F3), polycyclic aromatic hydrocarbon (F4) and polar compounds (F5). The percentages of each fractions in the dust organic extract (F) were F1 18.5%, F2 7.1%, F3 20.4%, F4 26.3% and F5 9.8%, respectively. The dust organic extract and its five fractions were dissolved in DMSO and stored in the dark at 4℃.

2.2 Ames testing

Two *Salmonella typhimurium* strains TA98 and TA100 were used for the present study. Based on the results of four steps method, they accorded with the experimental standard. A 9000 × g liver supernatant (S9) was prepared from Aroclor 1254-induced male Wistar rats. In tests of metabolic activation, 50 μl of S9 was used in a plate. Ames test was performed essentially according to the procedure described by Maron and Ames (Maron, 1983). 3 doses of the test were 200 μg/plate, 400 μg/plate and 800 μg/plate, respectively. Meanwhile, blank and positive controls were used, each treatment was in triplicate. All of the tests were repeated 3 times.

2.3 Statistical analysis

The data were showed using mean ± deviation. The results of mutagenic activity were decided according to the standard by Ames *et al*. (Ames, 1983).

3 Results

Mutagenic activities of the dust organic extract and its five fractions on two test strains are presented in Table 1. Table 1 shows that mutagenic activities of the dust organic extract and its five fractions were different from one another, although within the range of the test, the dust organic extract and its five fractions were all increased in revertants for TA98 and TA100. After addition of S9, revertants increased slightly. Mutagenic activity of dust organic extract was very strong on TA98 and TA100, and after added S9, it was higher than that without S9. Polycyclic aromatic hydrocarbon showed a mutagenic activity on TA98 and TA100 with and without S9. All polar compounds, were detected on TA98 with and without S9, showed mutagenic activities, but

they could result in mutagenic activity on TA100 at the concentrations of 400—800 μg/plate only. Organic acid showed mutagenic activity only at the range of 400—800 μg/plate on TA98 and TA100 with and without S9. Both organic alkali and aliphatic hydrocarbon had no mutagenic activities either on TA98 and TA100 with and without S9. In a word, mutagenic activities are mainly in the dust organic extract and its three fractions, namely organic acid, polycyclic aromatic hydrocarbon and polar compounds.

Table 1 Ames test results of the dust organic extract and its five fractions derived from an aluminum electrolytic plant ($\bar{X} \pm S$)

Samples	Dose, μg/plate	TA98-S9	TA98 + S9	TA100-S9	TA100 + S9
N. Control					
DMSO	0.00	23.4±1.7	25.8±3.6	112.8±17.5	129.5±18.3
F	200	66.0±5.1*	74.7±7.1*	259.4±6.3*	306.7±4.5*
	400	87.3±7.4**	98.7±9.5**	342.4±9.0**	393.1±9.5**
	800	96.7±9.3**	103.2±8.4**	415.7±8.4**	444.0±7.6**
Rev./μg		0.330	0.373	1.297	1.533
F1	200	45.0±3.0	49.5±7.5	177.0±5.5	180.7±6.5
	400	61.2±2.5*	68.0±7.7*	226.0±7.8*	268.8±8.3*
	800	77.7±3.7**	93.0±6.6**	302.0±7.8*	325.8±5.4*
Rev./μg		0.225	0.247	0.885	0.903
F2	200	28.3±3.4	32.7±5.7	125.7±7.1	142.7±8.1
	400	31.2±6.2	39.3±4.1	154.7±7.1	171.7±6.5
	800	38.8±3.5	45.5±6.1	182.3±7.2	198.6±5.5
Rev./μg		0.141	0.163	0.628	0.713
F3	200	32.3±4.6	34.7±4.9	144.0±8.5	160.7±7.0
	400	37.8±6.8	42.0±2.0	176.4±7.6	195.2±4.7
	800	44.0±5.7	47.5±6.4	199.3±8.7	217.3±7.8
Rev./μg		0.161	0.173	0.720	0.803
F4	200	56.7±3.9*	69.5±3.8*	228.3±11.6*	259.8±14.2*
	400	79.5±3.6**	81.8±3.4**	314.7±12.1*	328.9±19.5*
	800	89.0±3.8**	98.3±6.1**	387.7±8.7**	437.1±7.7**
Rev./μg		0.283	0.347	1.141	1.299
F5	200	49.0±2.7*	61.2±3.3*	190.3±8.6	197.7±7.0
	400	67.8+3.6*	73.9+2.8*	238.0±7.8*	278.3±4.2*
	800	87.5±3.6**	96.7±5.2**	314.3±3.2*	345.1±4.2*
Rev./μg		0.245	0.306	0.951	0.988
P. Control					
NaN_3	1.00			998.3±45.14**	
AF-2	10.00		944.1±57.3**		965.1±69.5**
2.7-AF	100.00	1226.9±7.1**			

*: $P<0.05$ **: $P<0.01$

4 Discussion

The utilization of short-term bioassays which detect mutagenic and possibly carcinogenic activities of environmental chemicals has increased dramatically in recent years. The wide acceptance of the mutagenesis bioassay using bacterial strain *Salmonella typhimurium* developed by Ames *et al*. (Ames, 1975) has been a catalyst in the expanded growth of the discipline of genetic toxicology. Because of the sensitivity and accuracy of this assay, it has been extensively used for the detection of mutagens in complex environmental sample.

The results showed that the range of mutagenicity of the dust organic extract derived from aluminum electrolytic plants are very wide, it contains not only base pair substitution mutations but also frameshift mutations. Therefore, the broad mutations of the dust organic extract indicate that the diversity and complexity of the carcinogenic and mutagenic chemical compounds. The mutations of its five fractions are different each other. Polycyclic aromatic hydrocarbon has the highest mutagenic activity, polar compounds have the lower, organic acid has the lowest, and organic alkali and aliphatic hydrocarbon have no mutagenic activity at all. The results are just the same as the results of unscheduled DNA synthesis (UDS), micronucleus (MN) and sister-chromatic exchange (SCE) tests in human lymphocytes in vitro (Wang, 1996a; 1996b).

So far, aluminum electrolysis mainly uses the electrolytic method of salt solutions. The dust containing aluminum oxide and fluorine will be emitted while the materials are added into and melted in electrolytic cells. In the process of electrolysis, powdered-coke, pitch and petroleum tar of carbonanode are continually burning, and pitch smoke and powdered-coke produced are filling the air. Therefore, the dust contacted by aluminum smelter workers are mixture, and the organic extract from the dust is complex mixture which composed of a large variety of substances. Epidemiology investigations and animal test proved that coal tar pitch can cause cancer (Cheng, 1985). The nocuous matter, such as pitch smoke and powdered-coke, discharged from aluminum plants contained polycyclic aromatic hydrocarbon and nitrogen-oxygen-sulfur heterocyclic compounds (Glushko, 1990). Polycyclic aromatic hydrocarbons are the kinds of environment pollutant, which are of the greatest quantity and of extensive distribution, closely related to human and seriously threaten to human health. Polycyclic aromatic hydrocarbons have been ensured to have mutagenicity and carcinogenicity (Hadnagy, 1986). The studies of epidemiology have suggested that polycyclic aromatic hydrocarbon is the main cause of many professional cancers such as lung and skin cancers. Aluminum electrolytic plants belong to the line of professional contaction of polycyclic aromatic hydrocarbon (Jongeneelen, 1984). The results of this study also confirmed that polycyclic aromatic hydrocarbon have the strongest mutagenicity in the five fractions. In addition, our results indicates that the mutagenicities are obtained both for organic acid and polar compounds. It is known that some intermediate metabolic products of the polar compounds may be carcinogenic; organic acid-like matters have the effect of promoting cancers (Cheng, 1980). Previously, people only emphasized the hazard of polycyclic aromatic hydrocarbon to the environment and human health, they overlooked the hazard of polar compounds and organic acid. According to this paper, we should pay close attention to the potential hazards of polar compounds and organic acid to the environment and human health, as we take the polycyclic aromatic hydrocarbon as the main hazard of the dust organic extract derived from aluminum electrolytic plants.

In Ames test, Ames estimated that there are the close correlations between mutagents and

carcinogens (Ca, 83%). Thus, dust organic extract and organic acid, polycyclic aromatic hydrocarbon, polar compounds can be regarded as substance with carcinogenic danger, they have potential endanger for human health. In the past decade, however, some researchers only have studied considerably the influence of fluorides from aluminum electrolytic process on the environment and human health (Kongerud, 1990; Sheng, 1987; Zhang, 1987). We think besides the direct hazard of fluorides, it is very important to pay close attention to the hazard of genetic toxicity of the dust derived from aluminum electrolytic plants, because it causes not only short-term but also seriously forward hazards, that is, long-term effect can cause carcinogenicity, aberration and mutagenicity, it affects this generations as well as the following ones. Some protective measures should be taken to abate the dust in aluminum plants and control carcinogens and mutagens, in order to protect the environment and human health.

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