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Altered Protein Synthesis in Rat Kidney Cells Exposed to Semiconductor Materials

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It is thought that the extensive industrial use of arsenic, gallium and indium, which have applications as the materials for III–V semiconductors, will increase human exposure to these compounds in the near future. We have undertaken the development of new biological indicators for assessing exposure to these elements. Element-specific alterations in protein synthesis patterns were expected to occur following exposure to arsenic compounds. We examined alterations in protein synthesis in primary cultures of rat kidney proximal tubule epithelial cells by sodium arsenite, gallium chloride and indium chloride, utilizing two-dimensional gel electrophoresis. After incubation with the chemicals for 20 h, newly synthesized proteins were labeled with [^{35}S]methionine. A protein with a molecular weight (M_r) of 30 000 was markedly induced on exposure to 10 μM arsenite or 300 μM gallium chloride, and synthesis of proteins with M_r values of 85 000, 71 000, 65 000, 51 000, 38 000 and 28 000 were also increased by exposure to arsenite and gallium chloride. No significant changes were observed upon exposure to indium. Some of these increased proteins could be heat-shock proteins.

Keywords: Arsenite, arsenic, gallium, indium, kidney tubule epithelial cells, primary culture, heat-shock protein, stress protein, semiconductor, rat

INTRODUCTION

Arsenic, gallium and indium are major components of III–V semiconductors and solar cells. For example, gallium arsenide (GaAs) will be an essential material for high-speed microcircuits in millimeter telecommunication systems and supercomputers in the near future[1], because this material has a distinct advantage in speed over silicon semiconductors. As components of semiconductors, the lead will pass from silicon to gallium arsenide and indium arsenide. The industrial applcations of arsenic, gallium and indium have increased dramatically for this reason. Table 1 indicates the production and trade of arsenic and gallium in Japan from 1978 to 1990.[2] The amount of arsenic produced in and imported into Japan increased about 10-fold during 1980s. Demand for gallium has also increased dramatically since 1984. The consumption of these compounds will further increase in the next decade, with increased human exposure to arsenic and gallium. This may result in new occupational and environmental chemical hazards in the near future, because these elements have been documented as toxic chemicals.

Numerous compounds containing arsenic have been recognized as toxic substances, but studies of the toxic effects of gallium-containing compounds such as gallium arsenide have only recently been undertaken, after these elements were used as materials for microelectronic devices. Since airborne particles of gallium arsenide have been shown to have pulmonary toxicity in experimental animals, exposure to airborne particles of this compound might be a potential health hazard in the microelectronics industry. Webb *et al.* reported an inflammatory response and pneumonocyte hyperplasia in rat lungs in which particles of gallium arsenide had been intratracheally instilled.[3–5] Aside from the pulmonary effects, urinary excretion of uroporphyrin was elevated in rats dosed with gallium arsenide. The pharmacokinetics of gallium arsenide in hamsters was investigated by Yamauchi *et al.*, after oral or intraperitoneal administration.[6] Inorganic arsenic, dimethylarsinic acid and methylarsinic acid were major metabolites of arsenic, and were excreted in the urine. Goering *et al.*

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Table 1 Production and trade of gallium, indium and arsenic in Japan (in tons)

	Gallium	Indium		Arsenic		
Year	Demand	Production	Imported	Production	Imported	Exported
1978	—[a]	6.5	—	6	—	—
1979	—	4	—	4	—	—
1980	—	10	—	13	—	—
1981	—	15	—	—	—	—
1982	—	15	—	15	—	—
1983	—	15	—	28	—	—
1984	30	15	1	40	—	—
1985	36	16	8	40	—	—
1986	32	—	21.5	40	—	—
1987	38	27	31	40	—	—
1988	47	48	30	40	9	5
1989	54	49	32	42	30	14
1990	63	48	36	40	95	13

[a] —, Statistics incomplete.

studied the biochemical effects of intratracheal administration of gallium arsenide and found that δ-aminolevulinic acid dehydratase (ALAD) in the blood, kidney and liver was inhibited with a concomitant increase in urinary excretion of aminolevulinic acid.[7] Sikorski *et al.* observed that gallium arsenide caused immunosuppression in mice after a single intratracheal dose.[8,9] The arsenic component of gallium arsenide was the major contributor to immunosuppression.[10]

Although the toxicity of arsenic- and gallium-containing compounds has been documented, the mechanisms of toxicity manifestation remain to be investigated. Our goal is to reveal the biological and biochemical changes caused by such compounds, to clarify the toxicity mechanism of these elements. We have also aimed to develop new biological indicators for the assessment of exposure to these elements in order to minimize or eliminate chemical hazards in the workplace as well as in the environment.[11]

Proteins induced specifically by environmental stress (including chemical exposure) are defined as stress proteins. Heat-shock proteins are a good example of stress proteins. They are induced not only by high-temperature stress but also by arsenic-containing compounds, metals and various other chemicals. The heat-shock protein family comprises several proteins with molecular weight (M_r) 90 000, 70,000 and 20 000–30 000, and the induction patterns of these proteins differ depending on the nature of the stress.[12] We examined element-specific alterations in protein synthesis following *in vitro* exposure of primary cultures of rat kidney proximal tubule cells to sodium arsenite, gallium chloride and indium chloride. The kidney proximal tubules were chosen because they are considered to be a primary target of metal and metalloid toxicity.

Protein synthesis in the exposed cells was analyzed by two-dimensional gel electrophoresis. This meethod is a powerful tool for identification of new or altered protein synthesis as a result of chemical exposure, because it can separate several hundred proteins. We found that the synthesis of several proteins was either stimulated or inhibited on exposure to sodium arsenite and gallium chloride. In particular, a protein with M_r 30 000 was markedly induced by these chemicals.

MATERIALS AND METHODS

The details of the methods used here were reported previously.[11]

Materials

Solutions of 3 mM gallium chloride and 3 mM indium chloride were prepared in 0.9% sodium chloride. A solution of 0.1 M sodium arsenite was prepared in distilled water. Waymouth's culture medium containing 10% (v/v) fetal calf serum, 100 units cm^{-3} penicillin and 100 μg cm^{-3} streptomycin was used.

Primary culture of proximal tubule epithelial cells exposed to chemicals

Fragments of the proximal tubule were isolated from the kidneys of rats (Fisher F-344; body weight, 200 g) and were inoculated into a six-well culture plate with 1.5 cm^3 of Waymouth's medium containing 10% fetal calf serum as previously reported.[11] The cells became confluent after five days of culture in a carbon dioxide (5%) incubator.

Cultures of confluent cells were incubated for 20 h in 15 cm^3 of Earle's balanced salt solution containing 10 mM Hepes/NaOH buffer (pH 7.4) and the indicated concentration of gallium chloride, indium chloride or sodium arsenite.

Measurement of lactate dehydrogenase (LDH) activity

After chemical exposure, 150 µl of the medium was removed from the cultures and 150 µl of 10% (v/v) Triton X-100 was added to lyse the cells. LDH activity in the culture medium (released LDH) and in the cell lysate (total LDH) was assayed by the method of Wróblewski and LaDue.[13] The percentage release of LDH was expressed as 100 × (released LDH activity)/(total LDH activity).

Labeling of newly synthesized proteins

After cultures had been exposed to the chemicals, the culture medium in each well was replaced by 0.5 cm^3 of methionine-deficient Eagle's minimum essential medium containing 100 µCi cm^{-3} of [^{35}S]methionine, 10 mM Hepes/NaOH buffer (pH 7.4) and the same chemical for 1 h. All cells in the well were lysed with 0.2 cm^3 of a lysis solution (1% Nonidet P-40 containing 0.1% SDS and 1 mM phenylmethylsulfonyl fluoride). The resulting lysates were subjected to electrophoresis.

Table 2 Effects of gallium, indium and arsenite on the release of LDH from rat kidney proximal epithelial tubule cells

Material	Concn. (µM)	LDH released (%)
Gallium	300	10.7 ± 2.7
	100	9.7 ± 0.9
	30	11.7 ± 1.0
Indium	300	10.3 ± 3.5
Control	0	10.1 ± 1.8
Arsenite	100	56.9 ± 5.2**
	30	49.3 ± 2.5**
	10	22.4 ± 0.5**
	3	15.9 ± 0.5
	1	14.7 ± 2.5
Control	0	12.9 ± 1.5

** $P < 0.05$ according to Welch's t-test ($n = 3$).

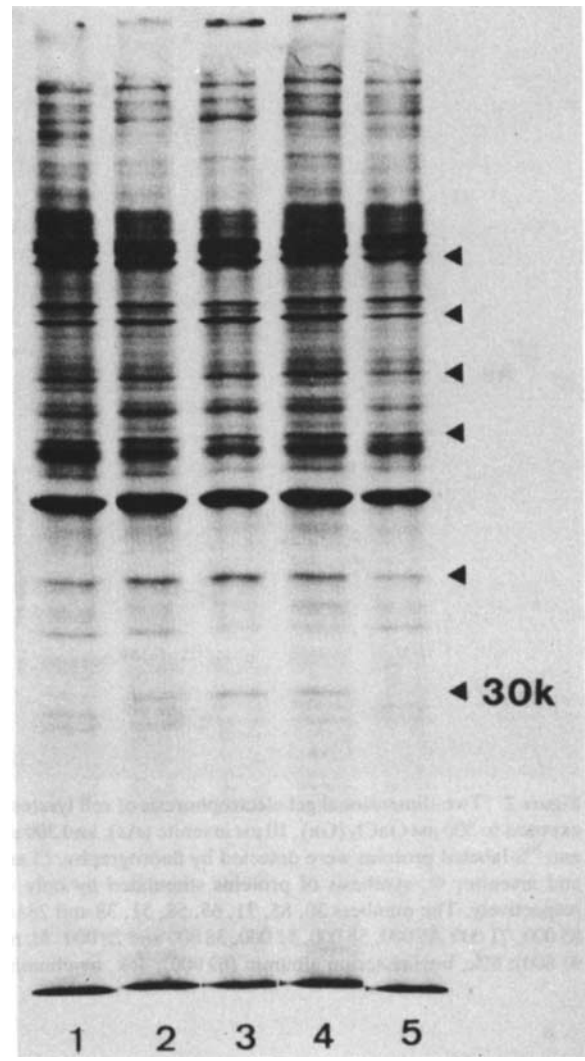

Figure 1 SDS–polyacrylamide gel electrophoresis of cell lysates prepared from cells exposed to arsenite and gallium(III) ions. Primary cultured rat kidney proximal tubule cells were exposed to the chemicals at the indicated concentrations for 20 h and the newly synthesized proteins were labeled with [^{35}S]methionine for 1 h. After the cell lysates had been separated on an SDS–polyacrylamide gel, ^{35}S-labeled proteins were detected by fluorography. Lane 1, 300 µM $InCl_3$; lane 2, 3 µM arsenite; lane 3, 10 µM arsenite; lane 4, 300 µM $GaCl_3$; lane 5, control. Arrows indicate the proteins induced by chemicals. 30k indicates the position of 30k protein.

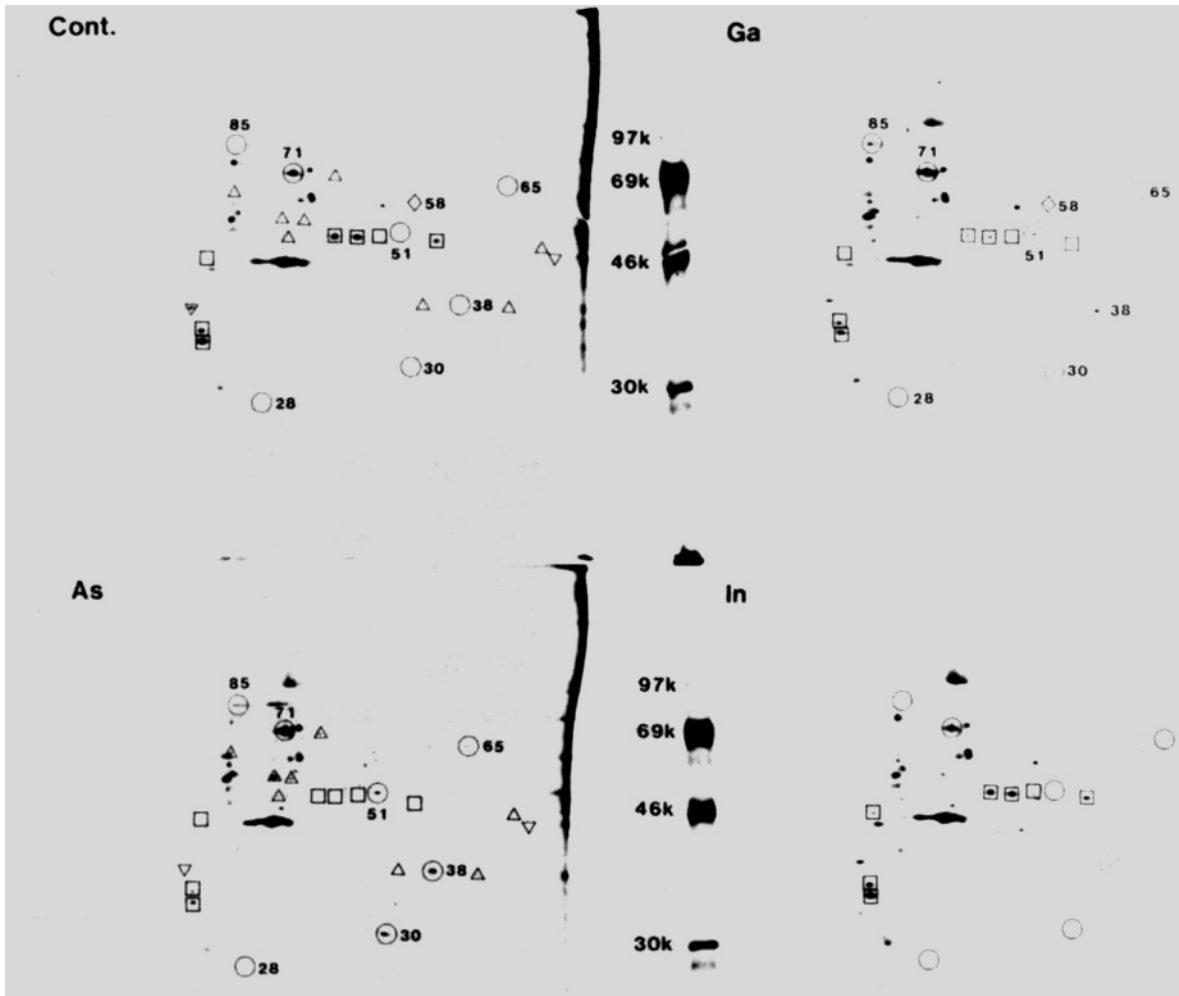

Figure 2 Two-dimensional gel electrophoresis of cell lysates. ^{35}S-labeled cell lysates from the control cells (Cont.) and the cells exposed to 300 μM $GaCl_3$ (Ga), 10 μM arsenite (As), and 300 μM $InCl_3$ (In) were subjected to two-dimensional gel electrophoresis and ^{35}S-labeled proteins were detected by fluorography. ○ and □, Synthesis of proteins stimulated and inhibited by both Ga^{3+} and arsenite; ◇, synthesis of proteins stimulated by only Ga^{3+}; △ and ▽, protein induced and reduced by only arsenite respectively. The numbers 30, 85, 71, 65, 58, 51, 38 and 28 indicate the positions of 30k protein and proteins with M_r values of 85 000, 71 000, 65 000, 58 000, 51 000, 38 000 and 28 000. M_r markers (methylated ^{14}C-labeled protein) 97k, phosphorylase *b* (M_r 97 000); 69k, bovine serum albumin (69 000); 46k, ovalbumin (46 000); 30k, carbonic anhydrase (30 000).

Polyacrylamide gel electrophoresis

The proteins in the cell lysates were separated by conventional SDS–polyacrylamide gel electrophoresis (SDS–PAGE) and two-dimensional gel electrophoresis [both 11% acrylamide (w/v)] according to the methods of Laemmli[14] and O'Farrell[15] respectively. ^{35}S-labeled proteins separated on the gels were detected by fluorography.

RESULTS

The cytotoxic effects of arsenite, gallium(III) and indium(III) on primary cultures of rat kidney proximal tubule epithelial cells were estimated from the release of LDH in the culture media. As shown in Table 2, no LDH release occurred at concentrations of 3 μM arsenite, 300 μM gallium(III) ions and 300 μM indium(III) ions. However, the LDH release was slightly increased

on exposure to 10 μM arsenite. This suggests that these chemicals might not show cytotoxicity at the concentrations examined.

The effects of arsenite, gallium(III) ions and indium(III) ions on protein synthesis were examined at non-cytotoxic concentrations. The newly synthesized proteins labeled with [^{35}S]methionine were detected by fluorography after SDS–PAGE (Fig. 1). Exposure to 300 μM gallium(III) ions and 3–10 μM arsenite stimulated the synthesis of six proteins in the kidney cells. A protein with an M_r of 30 000 (30k protein) was markedly induced by gallium(III) ions and arsenite. Levels of arsenite and gallium(III) ions at which no cytotoxicity was shown did not cause changes in incorporation of [^{35}S]methionine into cellular proteins in these cells (data not shown). No significant changes were observed upon exposure to indium(III) ions.

The effects of 10 μM arsenite, 300 μM gallium(III) and 300 μM indium(III) on protein synthesis were examined intensively using two-dimensional gel electrophoresis and fluorography. As expected from the results of SDS–PAGE analysis, a 30k protein was markedly induced upon exposure to either arsenite or gallium(III). The synthesis of six other proteins was also stimulated (Fig. 2). The approximate M_r values of these proteins were 85 000, 71 000, 65 000, 51 000, 38 000 and 28 000 (circles on Fig. 2). A concomitant decrease in the synthesis of seven proteins was induced by arsenite and gallium(III) ions (squares in Fig. 2). Some of the changes in protein synthesis were specific to arsenite exposure. The proteins whose synthesis was stimulated or inhibited are marked by triangles in Fig. 2. A protein with M_r 58 000 was induced slightly on exposure to gallium(III) ions. No significant changes in protein synthesis were ovserved after treatment with indium(III) ions as determined by two-dimensional gel electrophoresis.

DISCUSSION

With the dramatically increased use of elements such as gallium, arsenic and indium in the microelectronics industry, it is essential to develop biological indicators of early exposure to these toxic elements. Good markers must be easy to measure clinically; thus proteins which are released from cells and excreted in the urine after exposure to toxic compounds will be excellent candidates for markers.

The synthesis of several proteins in the proximal tubule epithelial cells of rat kidneys is specifically altered by *in vitro* exposure to arsenite and gallium(III) ions. Our results suggest that induction of the 30k protein as well as other proteins in the proximal tubule cells may be useful as an early indicator of exposure to arsenite and/or gallium prior to the onset of cell injury. If the 30k protein and other proteins are induced in the kidneys *in vivo* on exposure to arsenite or gallium, these proteins can be excreted in the urine and thus be useful early indicators of arsenite or gallium exposure. Although *in vivo* induction of these proteins in human by arsenite or gallium has not been examined, a heat-shock protein with an M_r of 74 000 was reported to be induced in the rabbit kidney afer arsenite administration.[15]

The identity of the proteins altered on exposure to arsenite or gallium(III) ions remains to be determined. However, the proteins with M_r 85 000 and 71 000 may be coincident with the 90k and 70k heat-shock proteins, respectively, since the relative positions of these proteins on two-dimensional gels are similar to those previously reported for the heat-shock proteins.[17,18] Our preliminary observations are that the expression of 90k and 70k heart-shock protein mRNAs was stimulated in cells exposed to 300 μM gallium (III) ions. The alterations in protein synthesis observed here may be related to the initiation of toxic effects of arsenite and gallium(III). Identification of the proteins altered by arsenite and gallium(III) ions will give an insight into the toxicity mechanisms of compounds containing these elements.

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