Bull. Environ. Contam. Toxicol. (1992) 49:98–104


Environmental Contamination and Toxicology

# Action of Acrolein on Rat Liver Membrane Proteins and Enzymes

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Acrolein and allyl alcohol are important synthetic intermediates in chemical industries. Acrolein is also a combustion product of petrol, coal, wood and plastics. Widespread exposure to acrolein is commonly due to the tobacco smoke and the burning of fat containing foods. Acrolein is the most toxic member of the class of 2-alkenals. Many of the biochemical and toxic effects of acrolein are caused by reaction with critical sulfhydryl groups. Based on human experiments, acrolein is approximately two to three times more irritating than formaldehyde. Acrolein has been reported to be highly toxic by all routes of exposures (Beauchamp et al, 1985). The extent of toxicological concerns for acrolein exposure particularly by the inhalation rout has been extensively reviewed(IARC, 1979).

When a chemical comes in contact with a cell, its first site of attack is the plasma membrane. A chemical thus, can damage the cell surface membrane in many ways, it either increases permeability of the cell, inhibits membrane enzymes and transport of nutrients, or acts on the control mechanisms. NADH-ferricyanide reductase activity has been found in many plasma membrane preparations. This activity measures the transmembrane NADH dehydrogenase as well as any internal NADH dehydrogenase such as NADH cytochrome $b_5$ reductase(Clark et al, 1982). It will also measure the external NADH dehydrogenase activity. These enzymes are probably involved in transmembrane electron transport which stimulate growth of the animal cell, ion transport and maintenance of membrane potential (Crane and Low, 1976). In the present study liver plasma membrane was used as a model for testing the extent of damage by acrolein on NADH-dehydrogenase, $Na^+ + K^+$-ATPase, 5'-nucleotidase and alkaline phosphatase. The membrane proteins were also analysed by SDS-PAGE.

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## MATERIALS AND METHODS

All the chemicals used were of analytical grade. Bovine serum albumin and ouabain were obtained from Sigma Chemical Co. U.S.A. and acrolein from E.Merck. Other chemicals and reagents were purchased from BDH or E.Merck. Male albino rats weighing 150 g were obtained from Industrial Toxicology Research Centre, Animal Colony, fed ad libitum on a pellet diet (Hindustan Liver Laboratory Feeds,India), and maintained under standard laboratory conditions. Plasma membrane of rat liver was prepared according to the method described by Evans (1970). Protein was determined according to the method of Lowry et al (1951) using bovine serum albumin as standard, and inorganic phosphate was measured by the method of Fiske and Subbarow (1925). Using different concentrations of acrolein, the enzyme NADH-$K_3Fe(CN)_6$ -reductase was measured by the method of Zamudio and Canessa (1966). 5'-nucleotidase and alkaline phosphatase were determined as mentioned by Kidwai et al (1971). $Na^+ + K^+$ $Mg^{+2}$-ATPase was determined using 100 mM NaCl, 10 mM KCl, 3 mM $MgCl_2$ and 3 mM ATP in 100 mM Tris-HCl buffer (pH 7.5) and 3 mM ouabain. Inorganic phosphate was measured after 15 min of incubation at 37°C. Enzyme activities are expressed in terms of specific activity as standard units/mg protein.

SDS-polyacrylamide gel electrophoresis of proteins was carried out according to the method of Laemmli (1970) as modified by Blackshear (1984) after treating the membrane with 15 uM acrolein for 15 min at 37°C and compared with controls. Membrane samples for electrophoresis were prepared by solubilizing the preparation in 0.5 M Tris HCl buffer pH 6.8 containing 8 M urea, 1% SDS, 5.0 mM EDTA and 1% $\beta$ -mercaptoethanol at room temperature for 10-12 hour. Following electrophoresis, gels were fixed in 10% TCA and stained with 0.25% coomassie-brilliant blue in 5% methanol and 10% acetic acid for sixteen hours. Destaining of gel was done in methanol: acetic acid: water (35:7:58). Gels were scanned using EC-gel scanner model 910 attached with Hewlett-Pakard 3390 A reporting integrator.

## RESULTS AND DISCUSSION

Table 1 shows the ouabain sensitive $Na^+ + K^+$-ATPase activities in presence of 3 X $10^{-5}$ and 6 X $10^{-5}$ M acrolein. It was observed that ATPaseswere inhibited at a concentration of 6 X $10^{-5}$ M acrolein, and the

Table 1. Effect of acrolein on $Na^{+}$-$K^{+}$-$Mg^{+2}$-ATPase on rat liver plasma membrane

| Additions | specific activity (units/mg protein) |
|---|---|
| $Na^{+}+K^{+}+Mg^{+2}$ | 0.047 |
| $Na^{+}+K^{+}+Mg^{+2}$ + Ouabain | 0.035 |
| $Na^{+}+K^{+}+Mg^{+2}$ + Acrolein($1\times10^{-5}$ M) | 0.046(No change) |
| $Na^{+}+K^{+}+Mg^{+2}$ + Acrolein($3\times10^{-5}$ M) | 0.047(No change) |
| $Na^{+}+K^{+}+Mg^{+2}$ + Acrolein($6\times10^{-5}$ M) | 0.023(51%) |

Values are mean from five separate estimations and are within standard limits of 5% variation.

Table 2. Inhibition of rat liver plasma membrane enzymes by acrolein

| Concentration (M) | Specific activity(units/mg protein) | |
|---|---|---|
| | NADH-ferricyanide reductase | 5'-nucleotidase |
| 0.0 | 2.8 | 0.400 |
| $4.0\times10^{-6}$ | 2.4 (14%) | - |
| $7.0\times10^{-6}$ | 2.0 (30%) | - |
| $1.4\times10^{-5}$ | 1.2 (56%) | 0.300 (25%) |
| $2.8\times10^{-5}$ | 0.06(98%) | - |
| $3.0\times10^{-5}$ | - | 0.100 (75%) |
| $7.5\times10^{-5}$ | - | 0.058 (86%) |
| $2.3\times10^{-4}$ | - | 0.031 (92%) |

The values in parenthesis are per cent inhibition. Values are mean from 3-5 separate estimations with in standard limits of 5% variation.

activity obtained below the inhibition level of ouabain also indicated the inhibition of ATPase other than $Na^{+}+K^{+}$-ATPase as well. In this case much higher concentration of acrolein was needed compared to the amount required for dehydrogenases (Table 2). Acrolein has been shown to inhibit rabbit alveolar macrophages $Ca^{+2}$-ATPase activity *in vitro* (Low et

al, 1977). Acrolein is known to reduce pulmonary NAD in rats following inhalation exposure suggesting slowing of cellular oxidation processes (Alabert et al, 1971). The present in vitro study undertaken to visualize the direct impact of acrolein on plasma membrane enzymes further indicated that NADH-$K_3Fe(CN)_6$ reductase was inhibited about 56% by $1.4 \times 10^{-5}$M and 98% by $2.8 \times 10^{-5}$M acrolein. These concentrations of acrolein are below the levels reported for specific inhibitors N-ethylmelamide and p-chloromercurobenzoate in isolated rat liver plasma membrane using $K_3Fe(CN)_6$ as electron acceptor (Masuda et al, 1973). Therefore, small amount of acrolein inhibiting this enzyme can be of far reaching consequences to the the animal cell. Reduction of this enzyme due to low concentrations of acrolein suggests the toxic nature of this chemical on the exposed surface of plasma membrane resulting into the disturbances in growth, ion transport and membrane potential. In addition, as this enzyme assay measures the external NADH-dehydrogenase activity, it can also be used as a marker for external surface of membrane damage particularly in plasma membrane from liver and other tissues where the membranes are isolated in non-vesicular form (Morre et al, 1978). It is known that external NADH-oxidases are present on the surface of plasma membrane while $Na^+ + K^+$-ATPase is a transmembrane protein. It is, therefore, likely that NADH-oxidase is more prone to attack by the environmental chemicals compared to $Na^+ + K^+$-ATPase. 5'-nucleotidase was inhibited at a much lower concentration of acrolein compared to $Na^+ + K^+$-ATPase. This enzyme is also a transmembrane glycoprotein (Evans and Gurd 1973) and interacts with actin by its cytosolic domain and therefore is more protected compared to dehydrogenases but less so to $Na^+ + K^+$-ATPase. Alkaline phosphatase was inhibited 25% by $1 \times 10^{-5}$ M acrolein which was similar to the inhibition observed on 5'-nucleotidase.

The gel scan of control and treated membrane (Fig.1) was different in the region of spectrin like proteins also known as fodrin (Geiger 1983; Glenney and Glenney 1983). It is reported that rat liver plasma membrane contains a 240 KDa protein which is concentrated in the basolateral domain (Gloor and Gazzotti 1987). Our membrane preparation was a mixture of all the regions of plasma membrane and therefore, protein bands in the region of 240 KDa were also present. Acrolein treatment of plasma membrane preparation drastically affected the proteins in high molecular weight region. The gel-scan pattern of treated and untreated plasma membrane was different

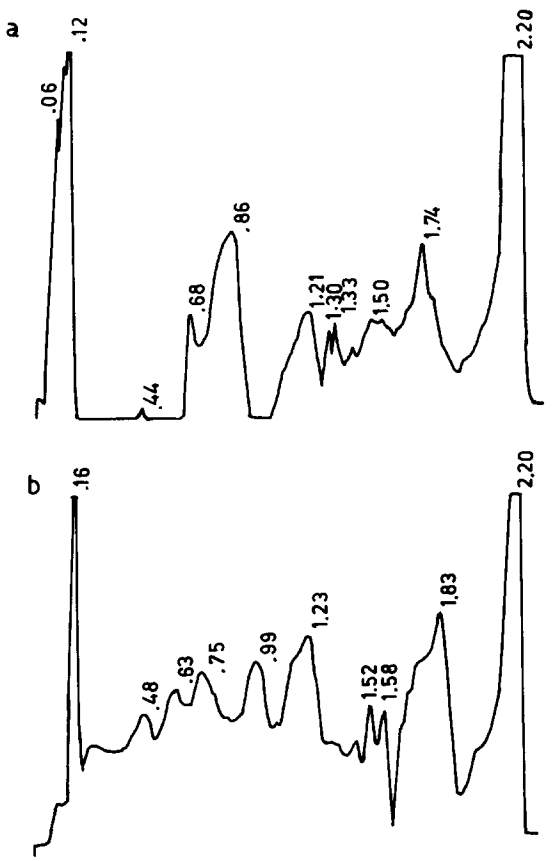


Figure 1. Densitometric profile of SDS-PAGE of (a) acrolein treated and (b) normal rat liver plasma membrane preparation.

in that the peaks at high molecular weight region either did not resolve significantly and showed broadening in the treated membrane or disappeared completely from the gel. In addition, there were some other minor alterations in the profile towards low molecular weight region(Table 3). The shifting of high molecular weight protein bands on SDS-PAGE due to acrolein treatment could be due to the polymerization of surface proteins which could not be dispersed by SDS. The results further indicate that acrolein at low concentrations does not penetrate the membrane lipid bilayer significantly and interacts more by attacking the membrane surface proteins.

Table 3. Analytical details of densitometric scanning of SDS-PAGE

| Control | | | Acrolein treated | | |
|---|---|---|---|---|---|
| Retention time (min.) | Area/ height | Area percent | Retention time (min.) | Area/ height | Area percent |
| 0.16 | 0.035 | 8.756 | 0.06 | 0.040 | 7.792 |
| 0.48 | 0.062 | 1.145 | 0.12 | 0.055 | 16.032 |
| 0.63 | 0.093 | 2.711 | 0.44 | 0.046 | 1.555 |
| 0.75 | 0.101 | 3.417 | 0.68 | 0.064 | 4.383 |
| 0.99 | 0.098 | 4.450 | 0.86 | 0.116 | 11.944 |
| 1.23 | 0.178 | 14.498 | 1.21 | 0.154 | 8.142 |
| 1.52 | 0.050 | 3.190 | 1.30 | 0.027 | 0.839 |
| 1.58 | 0.046 | 3.048 | 1.33 | 0.029 | 0.956 |
| 1.83 | 0.189 | 24.897 | 1.50 | 0.053 | 0.645 |
| 2.20 | 0.083 | 33.888 | 1.74 | 0.078 | 4.109 |
| | | | 2.20 | 0.077 | 43.605 |

The present *in vitro* study revealed that acrolein interaction at low concentration inhibited plasma membrane enzymes to varying degree and drastically changed the membrane protein profile suggesting atleast a superficial change which could be due to the polymerization of plasma membrane proteins. Our *in vitro* study correlates well with the results of various *in vivo* studies on acrolein, that the major toxic effects of acrolein exposure can be ascribed to its reactions with proteins. Alterations in cell membrane proteins including enzymes can influence the cellular homeostasis.

Acknowledgment. This work was supported by a grant from Indian Council of Medical Research, New Delhi, India.

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Received July 30, 1991; accepted January 6, 1992.