Bull. Environm. Contam. Toxicol. 29, 562–565 (1982)

Enzymatic Formation of an Olefin in the Metabolism of 1,1,2,2-Tetrachloroethane: An *in vitro* Study

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Several products are detected in urine when halogenated alkanes are inhaled by humans or animals. This indicates that there are several mechanisms involved in their biotransformation. Of these reactions, oxidative or reductive dehalogenation is assumed as a major pathway (VANDYKE & GANDOLFI 1975). In these reactions, it is considered highly probable that olefins would be formed enzymatically from saturated hydrocarbons (VANDYKE 1977). The metabolism of 1,1,2,2-tetrachloroethane is complex. Although dichloroacetic acid is the major metabolite (YLLNER 1971), trichloroethanol and trichloroacetic acid are also detected in urine when 1,1,2,2-tetrachloroethane is administered to rats (IKEDA & OHTSUJI 1972). This suggests an in vivo formation of olefins such as trichloroethylene (TRI) and tetrachloroethylene (TETRA). A non-enzymatic formation of these two olefins, however, had been assumed since these two compounds are formed from 1,1,2,2-tetrachloroethane in aqueous solution (YLLNER 1971). In the present communication, evidence for the enzymatic formation of an olefin from 1,1,2,2-tetrachloroethane will be presented and discussed in relation to the detoxification mechanism of halogenated alkanes.

MATERIALS AND METHODS

Animals and Phenobarbital Pretreatment. Rats weighing ca. 200g were used. Sodium phenobarbital in saline solution was administered to two rats (75 mg/kg, ip) daily for 4 days (the PB group). Same number of rats were treated with the same volume of saline ip simultaneously (the control group).

In Vitro Assay System. On the morning of the 5th-day, rats were stunned. Then, the livers were immediately irrigated from the abdominal aorta with isotonic KCl, removed, washed in chilled KCl, weighed, and homogenized in 3 volumes of isotonic KCl with a Potter-Elvehjem homogenizer with a Teflon pestle. The supernatant fraction after centrifugation at 10,000xg at 4°C for 10 min was employed as the enzyme source.

0007-4861/82/0029-0562 $01.00
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The assay mixture contained, in a final volume of 5mL, 2.5 mg of enzyme protein (0.1 mL as volume), 0.2 mmol sodium phosphate buffer (pH7.4), 7.5 μmol nicotinamide, 2.5 μmol $MgCl_2$, 30 μmol G-6-P, 0.5 μmol NADPH, and 60 μmol 1,1,2,2-tetrachloroethane. The incubation was conducted for a given duration from 15 to 60 min and terminated by the addition of 1 mL of 0.75 % (W/V) tungstic acid in 1N H_2SO_4. When indicated, the 10,000xg supernatant fraction from the PB group was boiled for 5 min at 100°C (the boiled enzyme) and used in place of the active enzyme to estimate the non-enzymatic conversion of 1,1,2,2-tetrachloroethane to TRI or TETRA. The formation of TRI and TETRA was studied with a gas chromatograph (GC) and the amount produced was determined as the increment over zero time control.

GC Condition. A gas chromatograph equipped with flame ionization detectors (FID) was used. The stainless steel columns (3 mm x 2 m) were packed with 25 % PEG-1000 on 80-100 mesh Celite 545. The temparature of the injection port and the columns was 140° and 110°C, respectively. The flow of N_2 was 40 mL/min. The calibration curve (concentration vs. peak height) was prepared using an authentic TRI or TETRA in aqueous solution. For identification purpose, a glass column (3 mm x 2 m) packed with 10 % Reoplex 400 on 80-100 mesh Chromosorb W was employed with an electron-capture detector.

RESULTS AND DISCUSSION

When assay mixtures after 60 min of incubation in the presence of the liver preparation from the PB group were analyzed under the standard GC condition, peaks were detected that had the same retention time with the authentic TRI on the PEG-1000 column. The formation of TRI was further confirmed by analysis of this incubation mixture on the Reoplex 400 column. The retention time was again the same with the authentic TRI and the same amount was detected on the two columns studied. The time course of the TRI formation in the mixture with the three enzyme preparations is illustrated in Fig. 1. Although TRI was formed even with the boiled enzyme in agreement with the report by YLLNER (1971), the formation of TRI was enhanced by phenobarbital pretreatment. Based on this finding, it is concluded that TRI is formed both enzymatically and non-enzymatically. For TETRA, however, in vitro formation was not detected. In the present communication, enzymatic conversion of alkane to alkene was proven and supports the hypothesis (HENSCHLER 1977) that, in the metabolism of halogenated alkane, destabilization of molecular conformation would occur after dehalogena-

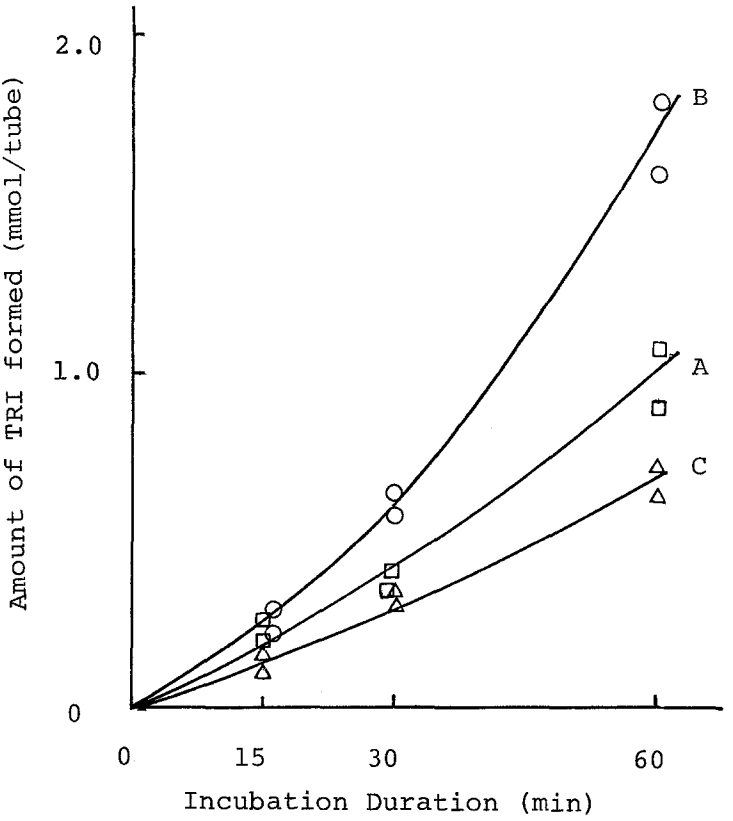

Fig. 1 Formation of TRI from 1,1,2,2-Tetrachloroethane

A: (squares) With control liver preparations

B: (circles) With PB-pretreated liver preparations

C: (triangles) With boiled enzyme preparations

tion or dehydrohalogenation to result in formation of olefins. It was also proven that the activity of the metabolic pathway concerned is low under physiological condition but high after phenobarbital pretreatment. Along this line, it is possible to explain why hepatotoxicity of alkanes such as halothane is enhanced by phenobarbital pretreatment (CASCORBI & SINGH-AMARANATH 1970), when the reactive metabolite formation from resultant alkene is assumed. The fact that TRI but not TETRA was formed enzymatically further suggests that the mechanism of olefin formation be closely related to the dehalogenation or dehydrohalogenation. The study on the metabolism of halogenated alkanes

under various conditions is ongoing in our laboratory.

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Accepted August 30, 1982