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Volatile hydrocarbons from hydrogen peroxide-induced lipid peroxidation of erythrocytes and their cell components

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The release of volatile alkanes, such as pentane and ethane, has recently been proposed as a reliable and sensitive index for lipid peroxidation [1–5]. It is generally accepted that these alkanes originate from the decomposition of lipid hydroperoxides [6]. In erythrocytes, lipid peroxidation is currently studied by measuring the formation of thiobarbituric acid-reactive material and fluorescent chromolipids. In the present study we describe the release of short-chain volatile hydrocarbons formed during hydrogen peroxide-induced peroxidation of the various cell components of erythrocytes.

Materials and methods

Red cells were isolated from the blood of healthy donors, as described by Beutler *et al.* [7], with microcristalline cellulose and α-cellulose (1:1, w/w). As anticoagulant, sodium citrate (3.13%) was used (1:5, v/v). The analysis of hydrocarbons in the head-space vials, used for the incubation of red cells with peroxidizing agents, was performed as previously described [4, 8]. Ghost membranes were isolated as described by Burton *et al.* [9]. Haemolysates were prepared by the lysis of erythrocytes with phosphate buffer (5 mM, pH 8.0) followed by centrifugation at 22,000 *g* for 10 min at 4° to remove the membranes. Haemoglobin was purchased from Sigma (human type IV). Experiments with erythrocytes, ghost membranes, ghost-free haemolysates and haemoglobin were performed with 10 mM hydrogen peroxide as peroxidizing agent. Erythrocytes and haemolysates were incubated with 0.25 mM sodium azide for the inhibition of catalase. Lipid peroxidation of ghost membranes was initiated in the presence of 2.0 mM $FeSO_4$ and 4.0 mM EDTA. Erythrocyte suspension (haematocrit 2.5%) was treated with hydrogen peroxide for 2 hr in a shaking water bath at 37°. Haemolysates and haemoglobin were incubated similarly, using equal concentrations of haemoglobin (0.8 g/dl) as used for the red cell incubation. Ghost membranes were treated with iron for 2 hr in the head-space vials shaken in a water bath at 37°. Protein content of ghost membranes was estimated by the method of Lowry *et al.* [10].

Results and discussion

The mean amounts (± S.E.M.) of hydrocarbon gases produced from the erythrocytes, ghost-free haemolysates and haemoglobin are shown in Table 1. Pentane and ethane were not obtained in the absence of erythrocyte membranes, indicating that they originate from the peroxidation of polyunsaturated fatty acids of membrane lipids. Ethylene, *n*-butane and *iso*-butane, formed during hydrogen peroxide-catalysed peroxidation of haemolysates and haemoglobin, were produced in concentrations comparable to those measured in the erythrocytes. The amount of propane produced by haemolysates and haemoglobin was less than that produced by erythrocytes.

Incubation of ghost membranes with Fe^{2+}-EDTA yielded mainly pentane, ethylene and ethane, but also small amounts of propane and *n*-butane (Table 2). The release of the latter gases could be particularly explained by the degradation of haemoglobin residues in the ghost membrane fraction. The high amount of ethylene could derive principally from the β-scission of lipid peroxides from ω3- and ω6- fatty acids. On the other hand, ethylene is known to be a degradation product from methionine [11] and could derive from haemoglobin residues and membrane proteins. *Iso*-butane was not observed with Fe^{2+}-EDTA, indicating that this gas may originate from amino acid

Table 1. Production of volatile hydrocarbons from erythrocytes, haemolysates and haemoglobin

Source	Ethane	Ethylene	Propane	*n*-Butane	*i*-Butane	Pentane
Erythrocytes $n=38$	0.19 ± 0.01	0.45 ± 0.01	0.41 ± 0.01	0.47 ± 0.03	0.18 ± 0.01	0.79 ± 0.02
Haemolysates $n=5$	0	0.42 ± 0.06	0.20 ± 0.04	0.40 ± 0.04	0.19 ± 0.01	0
Haemoglobin $n=3$	0	0.31 ± 0.01	0.34 ± 0.02	0.45 ± 0.01	0.18 ± 0.001	0

Incubation with 10 mM hydrogen peroxide for 2 hr at 37°. Erythrocytes and haemolysates were incubated with 0.25 mM sodium azide for inhibition of the catalase. Mean values ± S.E.M. Hydrocarbons are expressed as nmole/gHb per 2 hr. Experiments with erythrocytes and haemolysates were performed with blood from different donors.

Table 2. Production of volatile hydrocarbons from erythrocyte ghost membranes

Ethane	Ethylene	Propane	*n*-Butane	*i*-Butane	Pentane
0.21 ± 0.02	0.63 ± 0.07	0.11 ± 0.02	0.11 ± 0.03	0	1.08 ± 0.01

Incubation of ghost membranes with 10 mM hydrogen peroxide, 2.0 mM $FeSO_4$ and 4.0 mM EDTA for 2 hr at 37° in a shaking water bath. Mean values ± S.E.M. Alkanes are expressed as nmole/mg membrane protein. Experiments with ghosts isolated from four different donors.

degradation, probably from leucine, as recently shown in model experiments in our laboratory (W. Kessler and M. R. Clemens, unpublished data).

The present data show that incubation of red cells with hydrogen peroxide resulted in the production of a number of volatile hydrocarbons, but not all the gases were released during red cell membrane lipid peroxidation. The alkanes pentane and ethane seem to be the most reliable gases for monitoring the red cell lipid peroxidation, since neither was obtained from haemolysates or haemoglobin. The other volatile hydrocarbons, such as *n*-butane, propane and ethylene, are not principally derived from lipid peroxidation. We have evidence that the latter hydrocarbons produced during erythrocyte peroxidation derive particularly from proteins and amino acids. On the other hand, the formation of pentane and ethane from erythrocytes can yield valuable information regarding the toxic effects of foreign compounds, e.g. phenylhydrazine as a typical haemolytic agent.

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