ANALYTICAL SCIENCES JUNE 2005, VOL. 21

Identification of Gas Emanated from Human Skin: Methane, Ethylene, and Ethane

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We investigated whether methane, ethylene and ethane gas can be detected in gas emanating from human skin, which is called skin gas. Skin gas was collected with a homemade stainless-steel trap system, which was cooled with liquid nitrogen, and analyzed with a gas chromatograph fitted with a flame ionization detector (FID). Skin-gas samples were obtained by covering a hand for 30 min with a polyfluorovinyl bag in which pure helium gas was introduced. The bag, the trap system and GC were set up online to avoid any contamination by air. Methane, ethylene and ethane in skin gas were successfully collected at an average amount emanated for 30 min (from ten subjects) of 150 ± 63, 20 ± 11 and 17 ± 8 [mean ± SD] pg/cm^2, respectively.

(Received March 7, 2005; Accepted May 2, 2005)

Introduction

Breath,[1,2] sweat,[3] and saliva[4] are all human excretions/secretions available for non-invasive analytical tests.[5] Glucose,[6] ethyl alcohol,[3] lactate,[7] caffeine[8] and alkali metals[9] have been detected in sweat or inner-body fluid under the skin,[10] and the concentrations of some of these chemical substances in sweat are found to correspond closely to their concentrations in blood.[3,6]

Such chemical substrates generally rise to the skin surface both with perspiration and through the skin, itself.[3,5-10] Since there is a network of blood capillaries under the skin, it is possible that some chemical substrates in blood travel directly to the skin's surface.

Volatile gases emanating from human skin are rarely detected, owing to the difficulty of collecting them and their low concentrations, which fall below the detection limit of conventional analytical methods.

We have already discovered hydrogen gas and acetone vapor in skin gas,[11,12] and the relationship between the emanated amount of hydrogen in skin gas and its concentration in breath has been well established.[11] The acetone vapor in skin gas was found to increase according to the length of fasting, having a confirmed relationship with the acetone concentration in breath.[12] In the present study, we present proof of the existence of methane, ethylene, and ethane in gas emanated from human skin (skin gas).

Methane, as well as hydrogen gas, is produced in the human body by anaerobic bacteria, which exist in the large intestine, and is generated when humans cannot utilize carbohydrates and dietary fiber as energy sources. Methane produced in the large intestine by the fermentation of intestinal flora is absorbed through the intestinal wall and intervened circulation of blood, carried to the lungs, from which it is exhaled as a component of breath.[13] As mentioned above, hydrogen and acetone have already been detected emanating from the skin, carried to the surface *via* the network of sub-cutaneous blood capillaries; therefore, we assume that it is also possible for methane to do the same. One potential use of methane skin gas could be as a marker for indigestion.

Ethane is generated as the final metabolite of n-3 polyunsaturated fatty acid, and the measurement of breath ethane has been used as an *in vivo* index of lipid peroxidation that is caused by reactions with free radicals.[14,15] However, it is difficult to measure even a trace of ethane in the breath due to its low concentration, and due to the difficulty of avoiding contamination with air, also in which there is ethane. Therefore, breathing with hydrocarbon-free gas would be required in order to accurately determine the ethane concentration in breath. An alterative approach is to detect ethane in skin gas, which is described in this report.

In addition to methane and ethane, the processes of absorption, metabolism, distribution and excretion have also been investigated for ethylene gas.[16]

In this study, we successfully detected methane, ethylene and ethane gas emanated from human skin using a six-way valve assembly that features a stainless-steel sample loop and a water bath to avoid air contamination.

Experimental

Materials

A polyfluorovinyl bag (Tedlar® bag) obtained from Sanplatec (Tokyo, Japan) was modified for sampling skin gas and room air. The stainless-steel tubing (0.5 mm inner diameter, 1.7 m

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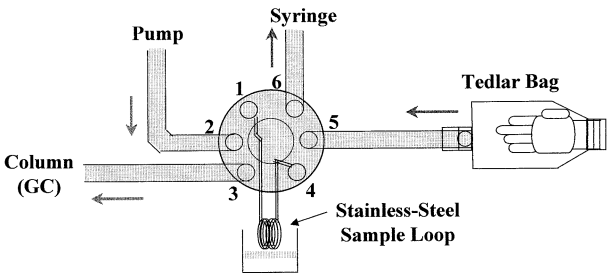

Fig. 1 Schematic diagram of the analysis system for human skin gas. The apparatus comprises the following parts: a modified polyfluorovinyl bag, a trap system with a sample loop, which is cooled with liquid nitrogen.

long, inner volume 300 μl, in which the inner wall was coated with 5% diphenyl 95% dimethylpolysiloxane) was kindly donated by Frontier Laboratories Ltd. The tubing was used for a sample loop in the trap-system. Methane, ethylene and ethane gas were obtained from GL Sciences (Tokyo, Japan). The diluted gases were used for calibration.

Trapping procedure

Figure 1 shows a schematic diagram of the apparatus for determining the components of skin gas. The apparatus comprised the following parts: a modified polyfluorovinyl bag (part of the Tedlar® bag) in which the left hand was inserted, a trap system with a sample loop for trapping sample components that was cooled with liquid nitrogen, and a gas chromatograph with a flame ionization detector (FID; Model GC-14A, Shimadzu, Kyoto, Japan).

The procedure for trapping skin gas was as follows: A skin-gas sample was introduced into the stainless-steel tubing by manual suction with a syringe (20 mL, Top Surgical Manufacturing Co., Tokyo) at a flow rate of *ca*. 15 mL/min for 40 s. During this process, the skin-gas sample was fed into the stainless-steel sample loop cooled with liquid nitrogen. After the six-port valve (Model 7000, Six-port sample injector, Rheodyne, CA) was rotated from the trapping position to the injection position, the trap tubing was heated directly by a dryer to 70˚C to aid thermal desorption of the components in the skin gas sample.

Sampling procedure

Measurement of components in skin gas. Each skin-gas sample was collected from the left hand. The left hand was held under running tap water for 10 s and washed by spraying it with distilled water for 5 s. The hand was then wiped with paper, and left in room air for several minutes to dry. Next, the left hand was inserted into the modified bag, which was sealed around the wrist with Parafilm® (Parafilm® "M", American National Can™, Chicago). Following that, all other gases existing in the modified bag were replaced with helium gas at a flow rate of 1000 mL/min for 10 min; the modified bag was connected to an inlet of the trap system. The arm was dipped into a water bath to avoid air contamination, and the helium gas volume in the modified bag was adjusted to 200 mL by releasing it from a valve. The water bath was kept at about 38˚C. One cycle for sampling skin gas was 30 min, during which time the subjects had to keep the sampling bag on their hand.

Skin gas was analyzed before each measurement (at 0 min) and after 30 min of collection. A control test was simulated by using a glass bottle instead of a left hand, and the collected blank gas was measured under equivalent conditions to measurements of skin gas.

Measurement of components in room air. A room-air sample was diluted by 50% with helium gas in a Tedlar® bag. The diluting process was as follows: 200 mL of helium gas and 200 mL of room air were mixed in a Tedlar® bag, and then analyzed by GC.

Analytical conditions

The experimental conditions required for GC to determine the components of skin gas are as follows: a capillary column (wide-bore capillary glass column, 1.2 mm inner diameter and 40 m long, 2-μm film thickness in the stationary phase, coated with G-950; kindly donated by the Chemical Inspection and Testing Institute of Japan, Tokyo); injection and detection port temperature, 40˚C; column temperature, 30˚C. GC analysis took 15 min.

Subjects

Eight men and two women were involved in the present experiment under full agreement after receiving an explanation of the experimental aims. All subjects were in good health and none of them claimed any intake of medicine for their health care. Their ages ranged from 21 to 59.

Sampling bag

A modified bag for skin gas (shown in Fig. 1) was arranged by cutting one side of a Tedlar® bag equipped with two sleeves with valves (6 mm o.d.). A modified bag for sampling room air also had sleeves with valves (6 mm o.d.) and a connector (6 mm o.d.).

Calibrations

Dilute methane, ethylene and ethane gases were placed into a Tedlar® bag filled with 1 L of helium gas by directly introducing 500 μL, 50 μL and 50 μL of standard original gases. Then, 10 mL portions of methane, ethylene and ethane were again diluted into another Tedlar® bag with 1 L of helium gas. The final concentrations of the calibration gases were as follows: methane, 5.0 ppm; ethylene and ethane, 0.5 ppm. The methane was calibrated from 0.01 to 1.00 ppm; ethylene and ethane were calibrated from 0.01 to 0.20 ppm. These calibration curves had a good linear relationship, with correlation factors of 0.989, 0.986 and 0.989, respectively.

Calculating total weight of components in skin gas

The total weight of the components in skin gas is calculated by the equation following:

$$w = \frac{mVM}{22.4} \times 10^3 \quad (1)$$

The term w (pg) is the weight of a component in skin gas, m (ppm) is its concentration, V (ml) is the sampling volume of skin gas (V = 200 (ml) was used in the present study) and M (g/mol) is the mol weight of the component. In Eq. (1), the number 22.4 represents a conversion factor based on the ideal gas law.

Results and Discussion

Figure 2 shows typical chromatograms of gas that emanated from hands and of room air. After a modified Tedlar® bag was fastened to each subject's left hand, gas present in the Tedlar® bag was analyzed by gas chromatography. The chromatogram

Table 1 Amount emanated from skin for 30 min for methane, ethylene and ethane (pg/cm^2)

Subject	1	2	3	4	5	6	7	8	9	10	Average	Standard deviation
Surface area of the hand	429	468	408	384	448	420	344	385	443	420	415	36
Methane	84	69	171	225	228	162	93	111	234	126	150	63
Ethylene	15	6	39	27	36	9	18	12	18	21	20	11
Ethane	15	3	30	21	18	15	—	—	15	18	17	8

The average values of the amount emanated for 30 min for methane, ethylene and ethane for the ten subjects were 150 ± 63, 20 ± 11 and 17 ± 8 pg/cm^2, respectively. Methane and ethylene were detected for all subjects, and ethane for eight subjects.

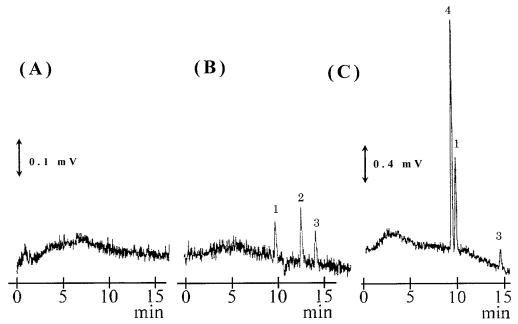

Fig. 2 Typical chromatograms of gas emanated from the hand (A, B) and room air (C). A, Skin gas collected at the zero period; B) skin gas collected at 30 min; C, room air. Peaks: (1) methane, (2) ethylene, (3) ethane, (4) unknown.

obtained at the zero period is shown in Fig. 2(A), while the typical chromatogram of skin gas is shown in Fig. 2(B). The peak retention times were 9.9 min (methane), 12.6 min (ethylene) and 14.2 min (ethane). These peaks were identified chromatographically by the addition of standard gases into the sample gas.

Methane and ethane were present in abundance in room air (C in Fig. 2), and their concentrations were 3.7 and 0.1 ppm, respectively.

Figure 2(A) shows no distinctive peak, whereas in Fig. 2(B), the concentrations of methane (1), ethylene (2) and ethane (3) are clearly visible. By dipping their arm into a water bath during sampling, the subjects could avoid any contamination from room air; thus, gases emanating from human skin could be detected. Further proof of no contamination is that peak 4 in chromatogram C is not present in A and B in Fig. 2, but is highest in C. Therefore, in this experiment, we confirmed the existence of methane, ethylene, and ethane in skin gas.

The emanated amounts of methane, ethylene and ethane in skin gas for the ten subjects are listed in Table 1. The experimental results, concentrations of the components, were processed by Eq. (1) and are listed in Table 1. The values of the surface area of the hand are total value of the skin surfaces of the palm, the back of the hand and the fingers. Paper patterns of the palm and fingers were taken, and the areas were measured. Also, it was decided that the skin surface area of the back of the hand was the same as the palm.

In Table 1, the average values of the amount emanated for 30 min of methane, ethylene and ethane for ten subjects are 150 ± 63, 20 ± 11 and 17 ± 8 pg/cm^2, respectively. Methane and ethylene were detected for all subjects, and ethane for eight subjects.

Conclusion

It has already been shown that methane gas exists in the breath at concentrations greater than 1 ppm.[13] Methane produced in the intestines is transported to the lungs *via* the bloodstream. However, some of that methane in the peripheral vessels present just under the skin surface can be released through the skin. In this study, the methane, ethylene and ethane in skin gas were firstly identified. A sampling procedure for skin gas was also established. The bag, a cold-trap system and GC were set up online to avoid any contamination by air, since there is generally 3.7 ppm of methane and 0.09 ppm of ethane in room air. These concentrations are much higher than those measured in skin gas. This indicated that the carefully trialed sampling procedure was effective.

This collection procedure for skin gas is non-invasive and places minimal stress on subjects. The practice of analyzing skin gas shows promise in easily determining a person's health status, and as such, could be applied in the future for health care.

Acknowledgements

This work was partly supported by the Regional Science Promotion Program, Japan Science and Technology Agency. The authors are thankful for the technical support of the Chemicals Evaluation and Research Institute and Frontier Laboratories Ltd.

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