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Quantitative Measurement of General Odorant Using Electroantennogram of Male Silkworm Moth, *Bombyx mori*

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Abstract The investigation of electroantennogram (EAG) using insect antennae has been primarily focused on the measurement of insect pheromone. Insect has highly specialized olfactory receptors inside their antennae. In this paper, EAG was applied to detect general odorants and the feasibility of this system for the olfactory biosensor was investigated. Electroantennogram measurement was carried out using the antennae of male silkworm moth, *Bombyx mori,* and ammonia gas as the model odorant. EAG parameters including peak amplitude, decay, and level were analyzed for the quantitative measurement. The peak amplitude increased linearly with the ammonia concentration and the reproducible electrical signals were generated at least for 2 hrs after the antenna was cut off from the silkworm moth.

Keywords: electroantennogram, *Bombyx mori,* ammonia, olfactory sensor

The olfaction is one of the most important and critical senses in animals. Most animals depend on the olfaction to identify food, predators and mating partners. Although humans have a poorly developed olfactory sense, they can detect some odorants at concentrations as low as 10^{-3} ppb and discriminate 10,000 distinct odors [1]. However, they rarely rely on the olfactory sense rather than other senses in daily life and humans often use it as an aesthetic sense Recently, the interest in the olfaction research increases for its industrial applications. The flavor and fragrance market is estimated at $10-12 billion for 1997 [2].

An electric nose has been developed by mimicking the biological olfactory system. The principle of the electric nose is a statistical mapping technique based on the fundamental olfactory mechanism. It consists of various gas sensor arrays Gas sensors used in the electrical nose are metal oxide, piezoelectric device, conducting polymer, and optical fiber [3]. The electric nose depends on the electrical signals generated from the various odorant-binding materials. Therefore, many different sensing materials are necessary to discriminate among a wide range of odor molecules. A biological olfactory system has a thousand of different receptor cells in it If we use this biological system instead of chemical materials, we can jump over the hurdle presented in the electric nose [4]

Compared with a mammalian olfactory system, an insect system has some features. The anatomy is convenient for isolating the antenna which is the olfactory organ. Electrical signal recording techniques are well established. Electroantennogram (EAG) signals are recorded from the whole antenna as small voltage shifts between two electrodes inserted at the base and tip of the antenna. EAG was first used by Schneider to record mechano- and chemo-reception on the antenna of *Bombyx mori* [5], and has been widespread. The EAG technique has been used to characterize the activity of various insect pheromones and plant volatiles [6] EAG with aliphatic alcohol and acetate was also reported [7-9] In this study, the feasibility of insect antennae as the olfactory biosensor for the quantitative measurement of general odorant was investigated using NH_3 as the model odorant.

An antenna was cut off from an adult male silkworm moth, *Bombyx mori,* using microscissors It was then placed on the antenna holder which consists of two 1.7 mm diameter wells as shown in Fig. 1. The wells were filled with 0.1 M KCl solution. The recording electrode was put in the well where the base of antenna was submerged while the reference electrode was put in the other well where the tip of antenna was submerged. The antenna holder containing two wells was placed in the cylindrical plastic casing whose diameter and length are 60 mm and 250 mm, respectively. Air flow rate was constantly maintained at 30 liter/min by a suction pump and various concentrations of NH_3 gas as the model odorant were injected into the air stream using a syringe.

The electrical signal was preamplified (IX1, Dagan Co., USA), displayed on an oscilloscope (TDS 220, Tektronix Inc , USA), and stored on a computer hard disk for later analysis and plotting. Intensity of the amplifier was measured using an 0 0672 V step input. The amplified output was 7 39 V. This output value is 110 fold higher than the input signal, and this information was used for the calculation of original electrical signal generated from the insect antenna.

The standard NH_3 gas was taken from the gas phase in the equilibrium with 30% NH_3 solution at 25°C. By

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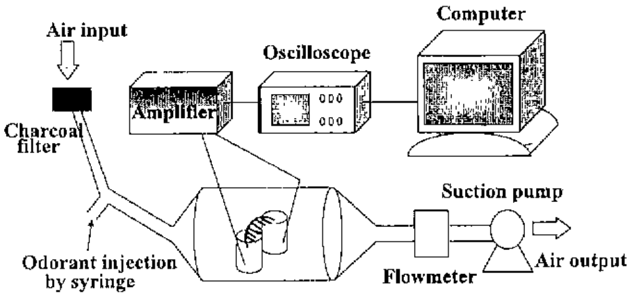

Fig. 1. Experimental apparatus.

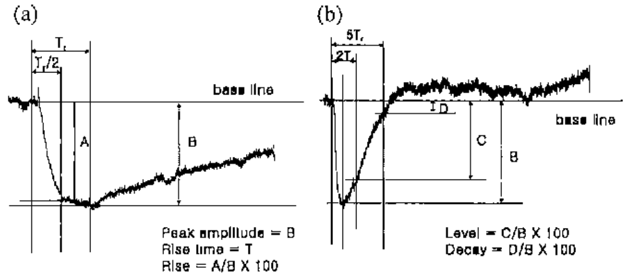

Fig. 2. Typical EAG generated by the NH_3 stimulation and parameter analysis. (a) type I, (b) type II The signal curves generated from a pair of antennae originated from the same silkworm moth are never identical, however, each curve shows the typical type I or type II characteristic curve pattern for every silkworm moth

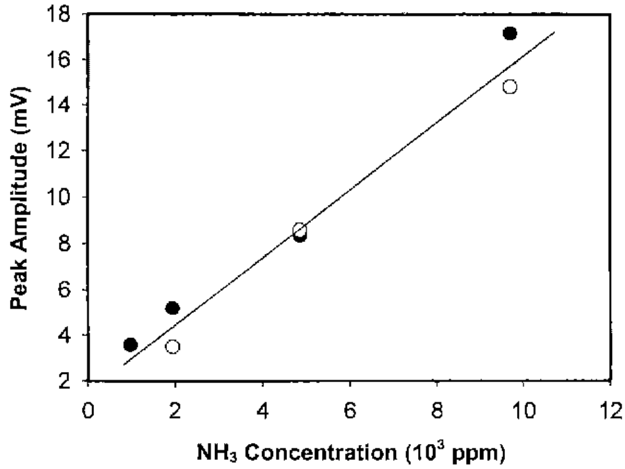

Fig 3 Correlation between the peak amplitude and NH_3 concentration. O· Experiment 1, ●. Experiment 2.

the thermodynamic calculation, the NH_3 concentration was determined to be 9.69×10^3 ppm The sampled NH_3 gas was diluted with air using a syringe to make various concentrations of NH_3 gas mixture

Fig. 2 shows the typical EAG responses stimulated by NH_3. Most previous EAG researches have been performed for the detection of insect pheromones or host plant volatiles; however, this work shows the possibility of general odorant measurement using EAG technique. The insect has two antennae and the electrical signals generated from the right and left antennae resulted in different shapes. One signal curve shows faster behavior than the other as shown in Fig 2(a) and (b) The fast behaving curve shows fast rise and decay, and the decayed signal usually passes the original base line in this type of curves. In some cases, the right antenna generates faster curve pattern than the left one, and vice versa in other cases. The curve pattern showing the slower behavior was denoted by type I and the other one was denoted by type II. The signal curves generated from a pair of antennae originated from the same silkworm moth are never identical; however, each curve shows the typical type I or type II characteristic curve pattern for every silkworm moth.

The original responses have much noise, and the noise in the response curve makes the parameter analysis difficult. Thus, the noise was removed to some extent by taking an average of seven data sets. Fig. 2 shows the typical EAG responses to the NH_3 stimulation The response curves are divided into two parts, i e. the first rising part and second decaying part. The first and second parts mean the regions before and after reaching the peak apex, respectively. The slow behaving curve (type I) is suitable for the analysis of the rising part since the rise in the fast behaving curve is too fast for the analysis. Whereas the fast behaving curve (type II) is suitable for the analysis of the decaying part since the decay is rather sharp and clear in this curve.

Several parameters can be defined for the analysis of the curve as shown in Fig 2 Peak amplitude (B) is the height of the peak from the base line, and it is the most widely used parameter. Parameters related to the rising part are rise and rise time, whereas parameters related to the decaying part are level and decay. The rise time (T_r) is the time at which the signal reaches the lowest point. The rise ($A/B \times 100$) is the ratio between the value (A) at the first half of the rise time and the peak amplitude (B) The level ($C/B \times 100$) is the ratio between the value (C) at 2 T_r and the peak amplitude (B) The decay ($D/B \times 100$) is the ratio between the value (D) at 5 T_r and the peak amplitude (B) [6,10].

The peak amplitude is the most important parameter which has been widely used in EAG researches for the detection of insect pheromones. Fig. 3 shows the correlation between the peak amplitude and NH_3 concentration in the case of type I antenna. The peak amplitude increases linearly with the NH_3 concentration. Similar results were obtained from the type II antenna (data not shown) However, any correlations could not be found between other parameters and NH_3 concentration. Although those parameters other than the peak amplitude can not be used for measuring the odorant concentration, it is expected that they can be used for discriminating different odorants.

Fig 4 shows the stability of parameter values when the type I antenna was used. This figure shows that the peak amplitude and other parameters are stably maintained at least for 2 h after the antenna was cut off from the silkworm moth. These results show the possibility of quantitative measurement of general odorant using EAG technique.

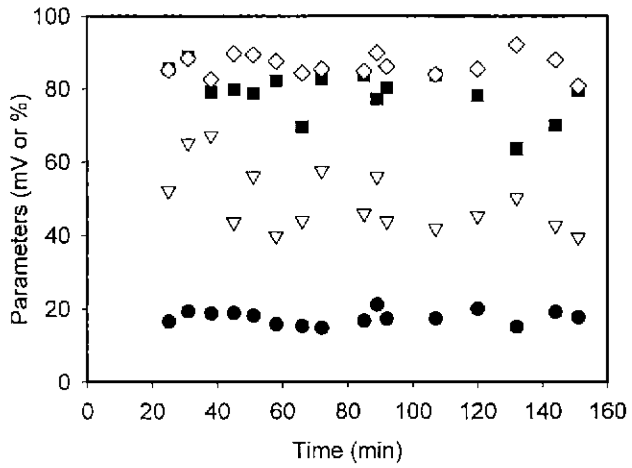

Fig 4. Stability of various parameters ●, Peak amplitude (mV), ■, rise (%), ◇, level (%); ▽, decay (%)

REFERENCES

[1] Axel, R. (1995) The molecular logic of smell. *Scientific American* 273. 54-159

[2] Strassburger, K J (1997) A better smelling technology *Chemtech*, December, 18-24

[3] Dickinson, T. A., J. White, J S Kauer, and D. R. Walt (1998) Current trends in 'artificial-nose' technology *Trends Biotechnol* 16. 250-258.

[4] Park, T. H. and E. S Yun (1998) Smell perception process and olfactory sensor. *Korean J. Biotechnol Bioeng* 13(6): 631-637.

[5] Schneider, D. (1957) Electrophysiological investigation on the antennal receptors of the silk moth during chemical and mechanical stimulation. *Experientia* 13. 89-91.

[6] Van der Pers, J N C. (1997) The insect antenna as biological sensor. *Proc. Int. Symp. Biologica Control of Insect Pests* 13-14: 97-107

[7] Schofield, S., A. Cork, and J Brady (1995) Electroantennogram responses of the stable fly, *Stomoxys calcitrans*, to components of host odour *Pysiol Ent* 20 273-280

[8] Sauer, A. E., G Karg, U T Koch, J J. Kramer, and R. Milli (1992) A portable EAG system for measurement of pheromones concentrations in the field. *Chem. Senses* 17: 543-553.

[9] Marion-Poll, F and D Thiery (1996) Dynamics of EAG responses to host-plant delivered by a gas chromatograph. *Entomologia Exp. Appl.* 80 120-123

[10] Dickens, J. C, J H. Visser, and J N. C. van der Pers (1993) Detection and Deactivation of pheromone and plant odor components by the beet armyworm, *Spodoptera exigua* (Hubner) (Lepidoptera Noctuidae). *J Insect Physiol.* 39: 503-516

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