A Trial of Fluorine Determination Using the Exoskeleton of Antarctic Krill *Euphausia superba*

Kazue Mogami,* Tomohiko Kitazawa,* Takeaki Kikuchi,* and Etsuo Watanabe*

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A sensor for the determination of fluorine was constructed using an Ag-electrode and the exoskeleton of Antarctic krill from which endogenous fluorine had been previously removed. The principle of the proposed sensor is based on the membrane potential difference being produced by the recombination of fluorine on the exoskeleton of Antarctic krill. A linear relationship was observed between the rate of the increase in the output of the sensor and the concentration of fluorine (0.5~5 ppm) when the sample solution was stirred at 1000 rpm and 25°C. Fluorine contents in raw and dried leaves of Japanese teas were determined by the proposed sensor and a conventional method. Good comparative results were obtained between the values determined by both methods. One assay could be completed within about 30 min.

Materials and Methods

**Removal of Fluorine from the Exoskeleton of Antarctic Krill**

Frozen raw Antarctic krills were obtained from Taiyo Fishery Co. Ltd. As shown in Fig. 1, the exoskeleton covering the cephalothorax of krill was collected by hand and washed with distilled water. The exoskeleton was dipped in a 2 N HClO₄ solution for 2 h to remove endogenous fluorine.

**Preparation of Fluorine Electrode**

A fluorine electrode was assembled by fitting the exoskeleton on a tip of Ag-electrode and covering with a dialysis membrane.

**Apparatus and Assay Procedure**

The proposed fluorine biosensor using the exoskeleton as a receptor is shown in Fig. 2. After the fluorine electrode was washed with 2 N HClO₄ and its output was stabilized in distilled water, the electrode was transferred to the sample solution (0.05 N HClO₄) and the membrane potential difference generated was recorded. Fluorine concentration of the sample was calculated from the rate of the increase in potential difference of the sensor. The new exoskeleton should be used for the next analysis.

* Department of Food Science and Technology, Tokyo University of Fisheries, Konan, Minato, Tokyo 108, Japan (対魚育英，北海道，野田武昭，渡辺悦生：名古屋大学食総学部)
Determination of Fluorine in Japanese Green Teas

After the raw and dried leaves of Japanese teas (about 5 g each) were freeze-dried and ground into powder, fluorine was extracted by shaking for 1 h with 100 ml of 0.05 N HClO₄. The extract (0.05 N HClO₄ solution) was centrifuged at 15000 rpm for 10 min at 5°C. The supernatant was collected as a sample for measuring fluorine contents with the biosensor. For the measurement by the conventional method, 5 ml of total ionic strength adjustment buffer was added to 10 ml of the supernatant and total volume was made to 50 ml with distilled water.

**Preparation of Total Ionic Strength Adjustment Buffer**

Sodium chloride (58 g), 1,2-cyclohexanediamine tetraacetic acid (4 g) and acetic acid (57 ml) were dissolved in approximately 500 ml of distilled water, adjusted to pH 5.3 with 5 N NaOH and the total volume was brought to 1000 ml with distilled water.

**Results and Discussion**

**Membrane Potential of Fluorine-free Exoskeleton of Krill**

The potential difference between two reference electrodes immersed in two solutions of 0.5 N NaCl and 0.05 N HClO₄ was measured with the following electrochemical cell: Ag/AgCl/NaCl 0.5 N; HClO₄ 0.5 N//membrane of krill exoskeleton //HClO₄ 0.05 N; NaCl 0.5 N AgCl/Ag. This cell should have a membrane potential difference of 59 mV at 25°C if the transport of H⁺ or ClO₄⁻ through the exoskeleton of krill is selective. Although this cell gave a membrane potential difference of 44 mV, the membrane difference was changed to 26 mV when 0.1 ml of 0.05% fluorine was added to the side of 0.05 N HClO₄. Therefore, fluorine could be determined by the membrane potential difference of fluorine-free exoskeleton of krill.
Removal of Fluorine from the Exoskeleton of Krill

The output currents of fluorine electrodes with the exoskeleton of krill dipped in 0.05, 0.5, 1 and 2 N HClO₄ solutions for 2 h were examined with a 3 ppm fluorine solution. The results are shown in Fig. 3. The effect of HClO₄ on removing endogenous fluorine from the exoskeleton of Antarctic krill is dependent upon the HClO₄ concentration, i.e. less than 0.05 N. The output of the sensor was nevertheless increased, with an increase in concentration of HClO₄ for the extraction of fluorine from the exoskeleton in a range of 0.05 N to 2 N. The initial decrease of membrane potential appeared to be caused by the local change of fluorine concentration. Fluorine in the sample solution appeared not to be recombined to the same site as endogenous fluorine had been bound in the exoskeleton, because the chitin structure could be changed with the treatment of HClO₄. The new site for the recombination of fluorine may be formed as the result of change in the chitin structure. The exoskeleton was treated with 2 N HClO₄ in the following experiments, since the output of fluorine-electrode with exoskeleton treated by 2 N HClO₄ gave a maximum potential difference.

Response of the Fluorine Sensor

A typical response curve of the proposed sensor is depicted in Fig. 4. The output of the sensor increased with an increase in time and concentration of fluorine during about 60 min. Therefore, the concentration of fluorine can be determined from the rate of the increase (tan θ) in the output.

Effect of Temperature and Stirring Rate on the Response of the Sensor

Once the sensor was prepared, the establishment of assay conditions is necessary to enhance the sensitivity of the sensor. Therefore, influences of temperature and stirring rate of the sample solution on the output of the sensor were first examined. Since it was presumed that binding amounts of fluorine increased rapidly with an increase of temperature, fluorine solution (190 ppm) was used as a sample solution in the experiment pertaining to the effect of temperature on the output current. Results are shown in Figs. 5 and 6. A maximum response was obtained when the temperature and stirring rate were approximately 29°C and 1000 rpm, respectively; the sensor was found to be unstable at 29°C but stable at 25°C (data not shown). From these results, the optimum assay conditions for the sensor were established as follows; temperature: 25°C, stirring rate of sample solution: 1000 rpm.

Calibration Curve

A linear relationship existed between the rate of the increase in the output of the sensor and the concentration of fluorine in a range of 0.5 ppm to 200 ppm. However, the calibration curve of the biological tissue sensor for fluorine operating under the optimum conditions as mentioned above is presented in Fig. 7, because fluorine concentration of the sample solution was in a range of 0.5 ppm to 2 ppm.

Fluorine Contents in Japanese Green Teas

It is well known that fluorine contents in Japanese green teas are fairly high. Fluorine contents in green tea leaves harvested in 1988 and Japanese
green teas prepared from these leaves were determined by the proposed sensor and conventional methods. Results are given in Fig. 8. Good comparative results were obtained between the values determined by the sensor and conventional methods ($r=0.837$). Fluorine contents were $44\pm5.8$ ppm to $106\pm17.5$ ppm in raw tea leaves and $47\pm2.0$ ppm to $115.9\pm14.0$ ppm in dried tea leaves. One assay could be completed within about 30 min. Fluorine contents determined by the proposed sensor method were always lower than those by the conventional method. This phenomenon suggested the presence of compounds causing the decrease of membrane potential in the sample solution as well as the existence of another site to combine compounds on the membrane. Though it was confirmed that chloride was able to combine to the binding site for fluorine, the experimental values were smaller than those obtained by the conventional method. Therefore, it was considered that the influence of chlorine upon the membrane potential was at least negligible. The cause of the membrane potential decrease should be examined in further detail. Effect of individual membranes (for instance, thickness) on the output current of the sensor was scarcely observed.

**Reusability of the Sensor**

The output of the sensor was gradually reduced on repeated runs. This could be due to the incomplete removal of recombined fluorine after each assay, although the sensor was dipped in $2\,N\,\text{HClO}_4$ for 1 h prior to the next run to remove recombined fluorine. Therefore, the sensor should be dipped in $2\,N\,\text{HClO}_4$ for more than 2 h. In
this experiment, a new exoskeleton was placed on the tip of electrode for each assay.

In future, a possibility of continuous analysis of fluorine will be examined from more detailed studies on the removal and recombination of fluorine to the exoskeleton of Antarctic krill.

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References