酵素センサーによる魚の筋肉及び血中のグルコース計測

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Determination of Glucose in Fish Muscle and Serum with an Enzyme Sensor*1

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Glucose sensor was developed and applied to the quality control of fish products and the hemochemical health assessment of fish. Glucose sensor was prepared with the combination of a glucose membrane and an oxygen probe. Glucose oxidase was covalently immobilized on a membrane prepared from cellulose triacetate, 1,8-diamino-4-aminomethyloctane and glutaraldehyde. The optimum operating conditions of this sensor were determined as follows; pH 7.8, temperature: 30°C, flow rate of buffer solution: 0.65 ml/min.

One assay could be completed within 4 min. The enzyme sensor could be used for more than 100 assays without the decrease of output current.

The saline solution of fish serum (3–16 times dilution) was suitable for the determination of its glucose with this sensor. Furthermore, it was demonstrated that the amount of glucose in sardine meat (perchloric acid extract) could be rapidly measured by the glucose sensor developed in this study.

These results suggest that the sensor proposed is an economical and reliable method for analysis of glucose in the fish muscle and serum.

Recently, some enzyme sensors are very useful and practical, especially in the field of medical treatment. Thus many sensors as described below have been developed: glucose,[1–3] sucrose,[4] maltose,[5] cholesterol,[6] uric acid,[7] monoamines,[8] ascorbic acid,[9] lactic acid,[10] pyruvic acid,[11] AMP,[12] IMP,[13] inosine[14] and hypoxanthine.[15] The analytical method using an enzyme sensor is simple, rapid and economical compared with the conventional methods. Therefore, an application of this method can be expected for the quality control of fish products and the hemochemical health assessment of fish. The authors have developed enzyme sensors for nucleotides such as hypoxanthine, inosine, inosine-5′-monophosphate and adenosine-5′-monophosphate, and applied these sensors to the determination of fish freshness.[12–19] On the other hand, glucose content can be used as an indicator for fish freshness[20] and hemochemical health assessment of fish.[21]

In this study, glucose sensor was prepared with the combination of a glucose oxidase membrane and an oxygen probe for the determination of glucose in fish muscle and serum. The operating conditions of this sensor system were first of all examined and the contents of glucose in fish serum (carp and rainbow trout) and sardine muscles were determined using the sensor developed in this study.

Materials and Methods

Materials

Glucose oxidase (E.C. 1.1.3.4 from *Aspergillus niger* type II) was obtained from Sigma Co. Glucose and 50% glutaraldehyde were purchased from Tokyo Kasei Co. Dichloromethane, 1,8-diamino-4-aminomethyloctane and cellulose triacetate were obtained from Kokusan Kagaku Co., Asahi Kasei Co. and Eastman Kodak Co., respectively.

Rainbow trout *Salmo gairdneri* and carp *Cyprinus carpio* were reared at 10°C and 20°C respectively, in our laboratory. Sardines *Sardinops melanosticta* were purchased from a fish market and stored in ice.

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Preparation of Samples

Glucose in sardine was extracted from 2 g of the muscle with 10% perchloric acid (PCA). The PCA extract was neutralized with 10 N KOH and adjusted to 20 m*l* with a 10% neutralized PCA solution.

The bloods of rainbow trout and carp were collected from the caudal vessels by the injector containing heparin. The blood was centrifuged at 3,000 rpm for 10 min. The supernatant (serum) was used directly or as a saline solution for the determination of glucose in fish blood.

Preparation of Immobilized Enzyme Membrane

The membranes prepared from cellulose triacetate, glutaraldehyde and 1,8-diamino-4-aminomethyloctane were immersed in a 0.1% (w/v) glutaraldehyde solution (0.1 M Tris-HCl buffer, pH 8.4) for 2 h at 30°C.[15] The membranes were washed with an adequate quantity of 0.05 M phosphate buffer solution (pH 7.8) and ten sheets (0.7×0.7 cm) of the membrane were immersed in 3 m*l* of a 0.05 M phosphate buffer solution (pH 7.8) containing glucose oxidase for 3 h at room temperature. The membranes were washed with a phosphate buffer solution (0.05 M, pH 7.8) and kept in the same buffer solution. The immobilized enzyme membranes were stored at 5°C and examined for their relative activity every 24 h.

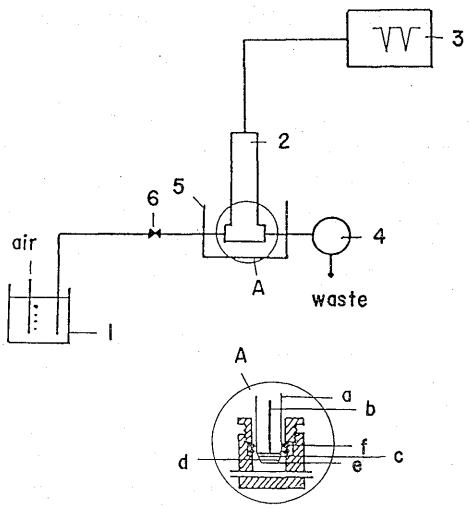

Fig. 1. Schematic diagram of the glucose sensor system. 1. Buffer tank, 2. Oxygen probe, 3. Recorder, 4. Peristaltic pump, 5. Thermostated bath, 6. Injection port, A: a. Oxygen probe, b. Platinum cathode, c. Teflon membrane, d. Enzyme membrane, e. Cellulose acetate membrane, f. Rubber ring.

Apparatus and Assay Procedures

Glucose sensor system consisted of enzyme membrane, oxygen probe, recorder and peristaltic pump as shown in Fig. 1. Phosphate buffer (0.05 M, pH 7.8) was transferred continuously to the sensor system by a peristaltic pump at a flow rate of 0.65 m*l*/min. After the output current became steady, a 50 μl aliquot of the glucose solution (2.5–10 mM), the PCA extract of fish muscle, fish serum or its saline solution was injected directly into the flow line. The temperature was controled at 30°C during the enzyme reaction. The amount of glucose was calculated from the current decrease of the enzyme sensor. As a conventional method, the Glucostat reagent (Worthington Chemical Corp.) was used to determine the glucose content.

Results and Discussion

Glucose sensor determines the amount of oxygen consumed by the enzyme reaction as a current decrease of oxygen probe (Fig. 2). As shown in Fig. 1, phosphate buffer solution saturated with oxygen was transferred continuously to the sensor system by a peristaltic pump. Therefore, in order to set up the optimum operating condition of the sensor, the flow rate of the buffer, temperature and pH of the enzyme reaction were first of all determined.

Response Properties of the Glucose Sensor

Fig. 3 shows the typical response curve of the glucose sensor. A 50 μl aliquot of 2.5 mM glucose solution was injected into the flow line at 30°C,

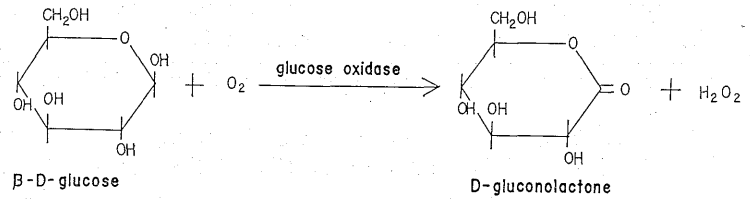

Fig. 2. Enzyme reaction of glucose.

pH 7.8 and the flow rate of 0.65 ml/min. After the injection of the sample, the output current began to decrease within 30 s, and then a minimum current was obtained within 130 s. One assay could be completed within 240 s. The current decrease between the initial and the minimum currents was used as the measure of glucose concentration.

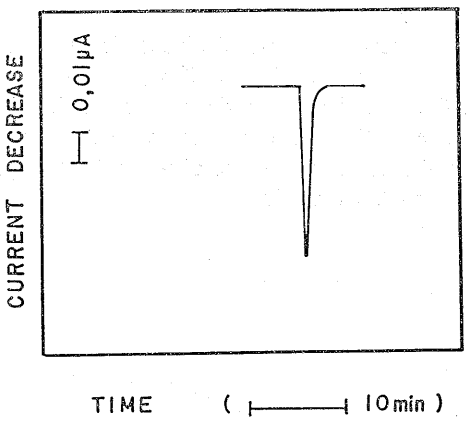

Fig. 3. Response of the enzyme sensor for glucose. A 50 μl aliquot of glucose (2.5 mM) was injected and the output current was recorded. The flow rate, temperature and pH were 0.65 ml/min, 30°C and 7.8, respectively.

Influence of pH and Temperature on the Current Decrease of the Sensor

Figs. 4 and 5 give the influence of pH and temperature on the output of the sensor, respectively. Fig. 4 indicates that the maximum response was obtained at pH near 9. Similarly, Fig. 5 shows that the output of sensor was reached to the maxi-

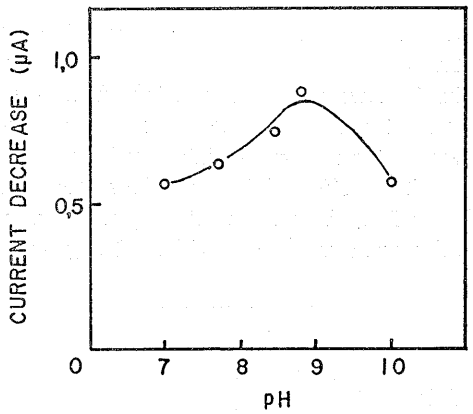

Fig. 4. Effect of pH on the current decrease of the sensor. Other conditions were the same as Fig. 3.

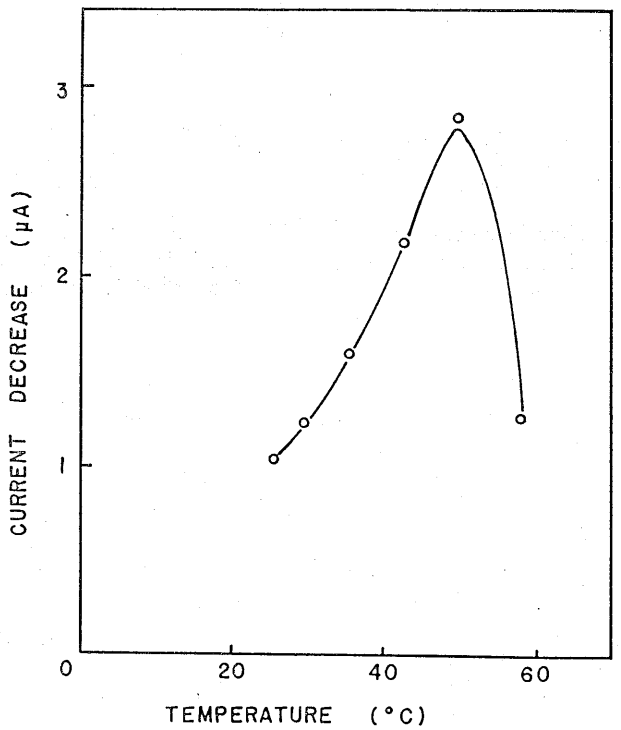

Fig. 5. Effect of temperature on the current decrease of the sensor. A 50 μl aliquot of glucose (5.0 mM) was injected. Other conditions were the same as Fig. 3.

mum at temperature near 55°C. For the operation of the sensor, it is preferred to choose the conditions that give the current decrease maximum. However, the conditions described above lead to the inactivation of the enzyme. The output of the sensor became unstable at higher temperatures, because this sensor system is based on the amount of dissolved oxygen. Therefore, the glucose sensor developed in this study was operated at pH 7.8 and 30°C.

Influence of the Flow Rate on the Output Current of the Sensor

Fig. 6 shows the influence of the flow rate on the output current of the sensor. The output current of the sensor was influenced by the flow rate of the buffer. When the flow rate of the buffer is fast, the sample might pass the immobilized enzyme membrane without being reacted. On the contrary, the slower flow rate results in a broadening of the peak because of the diffusion in the cell. Therefore, the sample solution should be injected into the flow line at the definite flow rate of the buffer solution. In this experiment, the flow rate of the buffer solution was set at 0.65 ml/min.

Calibration Curve

The calibration curve of glucose is depicted in Fig. 7. A sample solution (50 μl) was introduced into the flow line under the conditions described above and the current decrease was measured. Linearlity of the curve was obtained in the range of 2.5 to 8.0 mM of glucose.

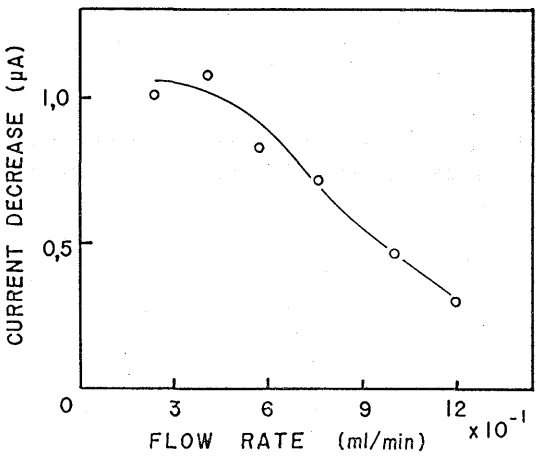

Fig. 6. Effect of flow rate on the current decrease of the sensor. Other conditions were the same as Fig. 5.

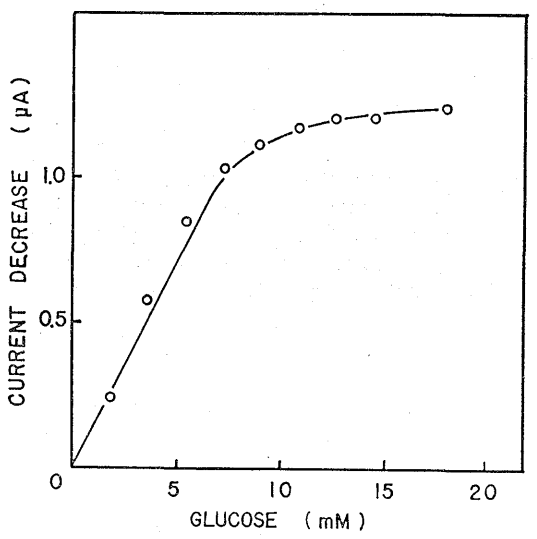

Fig. 7. Calibration curve of the glucose sensor. A 50 μl aliquot of glucose (2.5–8.0 mM) was injected. Other conditions were the same as Fig. 3.

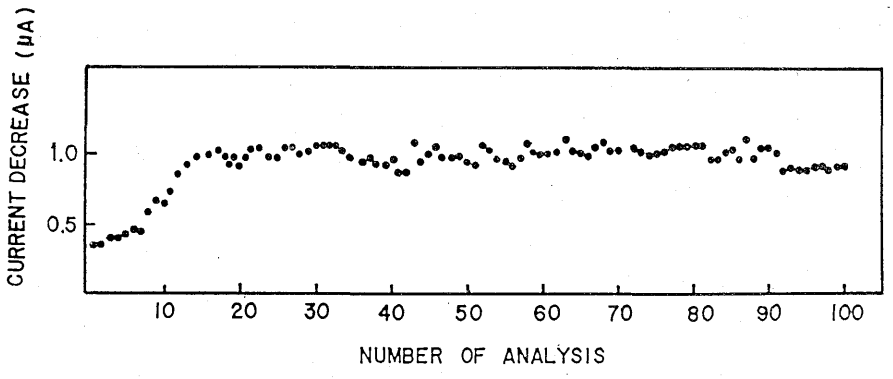

Fig. 8. Reprodusability of the sensor. Measurements were carried out at the conditions described in Fig. 5.

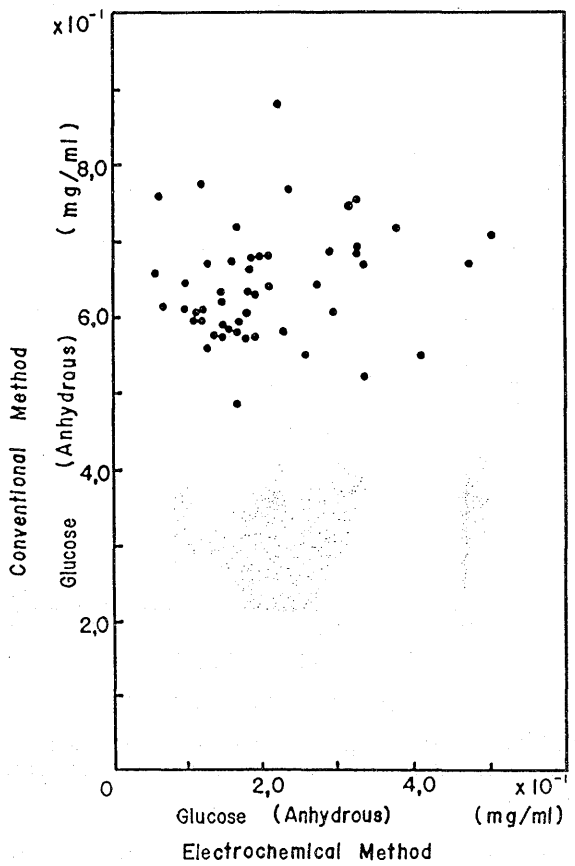

Fig. 9. Correlation between glucose content in the serum of rainbow trout determined by electrochemical and conventional methods. A 50 μl aliquot of the serum was injected. Operating conditions were as follows; pH: 7.8, temperature 30°C, flow rate: 0.65 ml/min.

Stability and Reusability of the Sensor

As shown in Fig. 8, the sensor could be used for more than 70 assays under the same conditions stated above. However, this sensor required about 1 h till the output current became steady. As far as the enzyme membrane was stored at 5°C, it was stable even after 30 days of storage.

Application for the Determination of Glucose in Fish Muscle and Serum

For the purpose of hemochemical health assessment of fish, the glucose sensor developed in this study was applied to the estimation of glucose content in the serum of rainbow trout. The amount of glucose in the serum of rainbow trout was determined by both sensor and conventional assay methods (Fig. 9).

As presented in Fig. 9, there is no correlation between the results obtained by the sensor and conventional assay methods. It seems because high viscosity of the serum caused the inhibition of the enzyme reaction. Therefore, the serum of carp blood was diluted 3–16 times with the saline solution prior to the injection into the flow line of the enzyme sensor system. As a result of pretreatment, a linear relationship was obtained between the results by the sensor and conventional methods (Fig. 10).

Further, glucose content of sardine meat was determine by the sensor from the standpoint of quality control. The usefulness of glucose content in fish meat as the possible indicator of fish freshness has been reported.[20] Fig. 11 gives the amounts of glucose in sardine meat determined by the sensor and conventional assay methods. An approximately linear correlation existed between results obtained by both methods.

As a conclusion, the glucose sensor consisted of the immobilized glucose oxidase membrane and oxygen probe was developed and applied to the determination of glucose in fish muscle and serum. It was demonstrated that the glucose sensor was

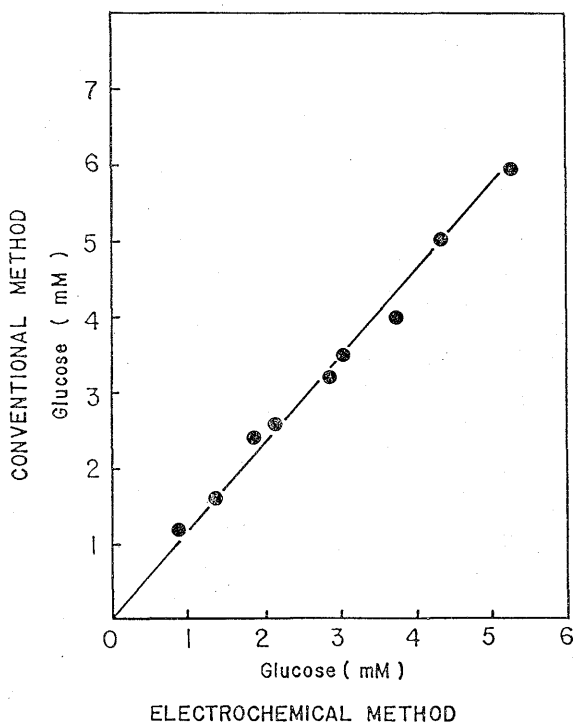

Fig. 10. Correlation between glucose content in the serum saline solution of carp determined by electrochemical and conventional methods. Measurements were done at the conditions stated in Fig. 9.

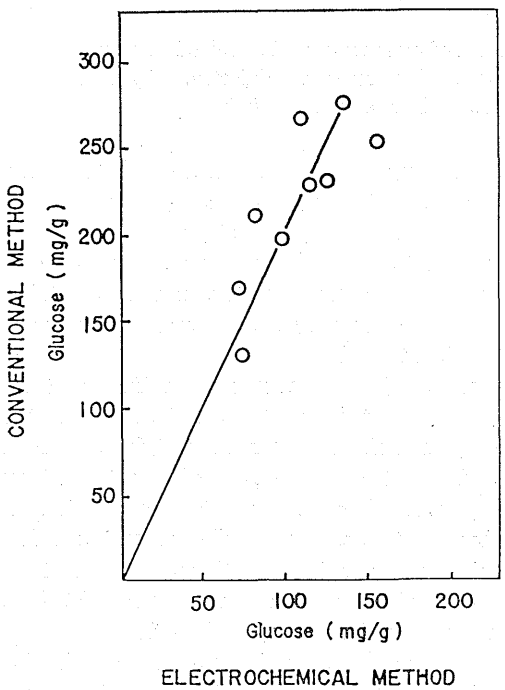

Fig. 11. Correlation between glucose content of sardine meat determined by electrochemical and conventional methods. Operating conditions were the same as Fig. 9.

suitable for the determination of glucose contents in sardine meat and serum of carp for the evaluation of quality and hemochemical health assessment of fish.

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