Continuous Monitoring of Subcutaneous Glucose and Lactate Using Microdialysis with On-Line Enzyme Electrodes

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A microdialysis system coupled with an electrochemical flow cell was constructed for the continuous monitoring of subcutaneous glucose and lactate in a rat. The working electrode of the flow cell was prepared with either glucose oxidase or lactate oxidase immobilized in a redox polymer film on the electrode surface. Coupling an electrochemical glucose or lactate sensor directly with microdialysis eliminates the need to transfer sample aliquots to an LC system with a post-column oxidase enzyme reactor. Glucose and lactate in the dialysate from the microdialysis probe were selectively detected at the enzyme electrodes without any significant interferences from other oxidizable species. Calibrations of the enzyme sensor were easily performed by using a liquid switch in the system.

Microdialysis samples are often assayed by liquid chromatography, immunoassay, or capillary electrophoresis. The separation systems offer selective detection of analytes, but in many cases, the temporal resolution of the assay is not sufficient for continuous real-time monitoring of the microdialysate. These techniques are limited by the minimum sample volume required for practical sample handling and reliable measurements.

The perfusion rates for microdialysis are typically between 1 and 5 µL/min, so the amount of time required to collect an adequate sample volume limits the temporal resolution of the assay. The use of an on-line injector can minimize the sample volume requirement; however, this can be problematic when the time required for all peaks in a chromatogram to elute is greater than the period for sample collection.

In recent years, there have been attempts to make continuous measurements of substances in microdialysate by coupling the microdialysis probe to an on-line electrochemical enzyme sensor (1–4). The use of enzyme electrodes with high selectivity and sensitivity can circumvent the inconvenience and cost of chromatography separations. Compared with implantable enzyme sensors for real-time in vivo analyses, the coupling of microdialysis sampling with external enzyme sensors is shown to be less invasive, more stable, and easier to sterilize.

In this article, we show a complete assay system for the continuous subcutaneous monitoring of glucose and lactate obtained by coupling the microdialysis effluent on-line with glucose and lactate enzyme electrochemical sensors in a simple configuration.

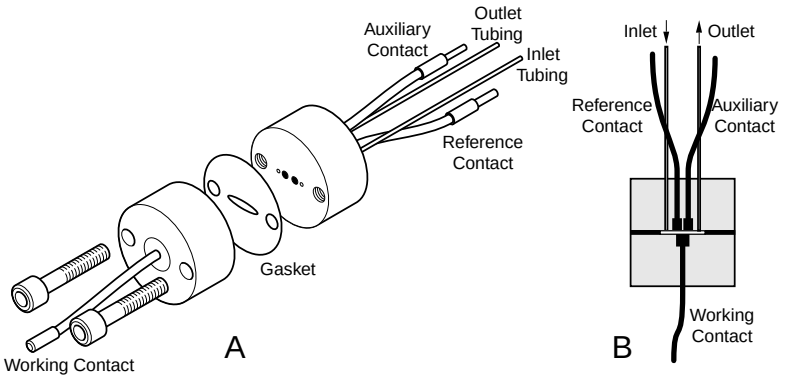
Reagents and Methods

Materials

Glucose oxidase (GOX) from Aspergillus niger (Type X-S, EC 1.1.3.4) and lactate oxidase (LOX) from Pediococcus species (EC 1.1.3.2) were purchased from

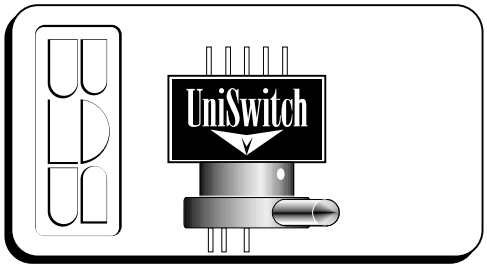
F1

(A) Exploded view of the flow cell; (B) side view of the flow cell.



F2

The BAS UniSwitch syringe selector.



Sigma. The enzyme "wiring" redox polymer (PVI_{15}-dmeOs) was synthesized as previously described (5). Nafion solution was purchased from BAS (CF-1049) and diluted with 95% ethanol (1:1) before use. L-(+)-lactic acid, lithium salt was purchased from Calbiochem Corporation. All other chemicals were analytical reagent grade and from Sigma or Aldrich. Artificial CSF solution was prepared as follows: 126 mM NaCl, 27.5 mM $NaHCO_3$, 2.4 mM KCl, 0.5 mM KH_2PO_4, 1.1 mM $CaCl_2$, 0.85 mM $MgCl_2$, 0.5 mM Na_2SO_4, pH 7.5. All standard glucose and lactate solutions were prepared with the artificial CSF solution.

Microdialysis

A rat was anesthetized with an intraperitoneal injection of 1 mL/kg of KX (10 mL ketamine (100 mg/mL) + 1 mL xylazine (100 mg/mL)). The microdialysis probe (DL-5, MF-7051, BAS) was placed in an introducer cannula (MF-7021, BAS). The animal's fur was clipped from the insertion site on the back at the base of the neck, and from the exit site 7 cm distal to the insertion point. Small incisions were made at the insertion and exit sites. The cannula was inserted under the skin at the neck and advanced to the distal incision. The cannula was pulled through the exit site, leaving the fiber portion of the probe under the skin. The probe was sutured in place and perfused with artificial CSF solution at 5 µL/min with a BAS Bee syringe pump (MD-1001). During microdialysis sampling, the awake unrestrained rat was housed in the BAS "Beekeeper Rodent Residence" containment and swivel system (MD-1575). All experimental protocols were approved by the BAS Animal Care and Use Committee.

Electrochemistry

The amperometric flow cell (UniCell, MF-2085, BAS) was constructed as previously described (6). ***F1*** shows the scheme of the flow cell. The glassy carbon enzyme working electrode (2 mm in diameter) was prepared as described earlier (5), where the redox polymer (PVI_{15}-dmeOs), the enzymes GOX or LOX, and the cross linker PEG were coated on the electrode surface and cured for 24 hours followed by a Nafion overcoating. The outlet of the microdialysis probe and the syringes with standard solutions were connected to the inlet of the flow cell through a UniSwitch syringe selector (***F2***, MD-1508, BAS). The working electrode was poised at +200 mV vs. Ag/AgCl for both glucose and lactate measurements. The currents were measured using either the Petit Ampère™ LC-3D (EF-1201, BAS) or the CV-1B (EF-1011, BAS) potentiostats. The potentiostat was connected to a dual-pen strip chart recorder (MF-8125, BAS). ***F3*** illustrates the complete system. To test the temperature dependence of the glucose sensor, a BAS LC-22C (EF-1043) temperature controller and a prototype UniCell heater block were used.

Chromatography

Liquid chromatography/electrochemical (LCEC) determination of glucose concentration in microdialysate samples was performed as described previously (7) with a BAS 480 liquid chromatography system (MF-480), a BAS glucose application kit (MF-8925), and a peroxidase enzyme electrode (MF-2095, BAS).

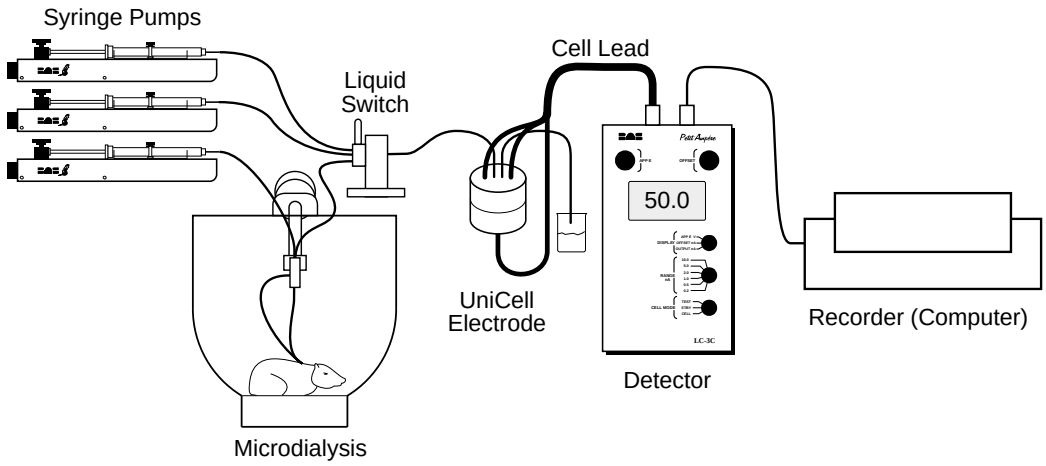
Unless stated otherwise, all experiments were carried out at room temperature.

Results and Discussion

For in vivo determinations using enzyme sensors, regular calibration of the sensor is crucial. In this study, the on-line system for monitoring microdialysate was designed to enable easy calibration of the enzyme electrode during the assay. As shown in ***F3***, the outlet of the microdialysis probe and two syringes mounted on syringe pumps were connected to the inlet of the electrochemical cell through a liquid switch. The liquid switch allowed a

F3

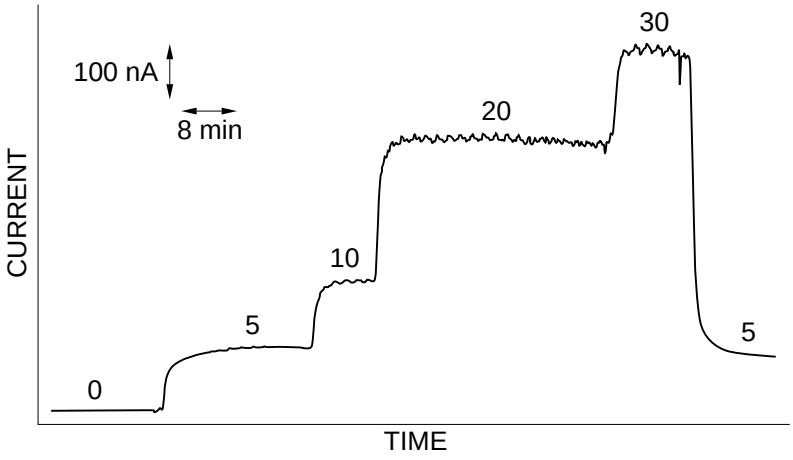
On-line system for monitoring micro-dialysate.



change of the perfusion solution through the cell without interrupting flow or introducing air bubbles into the system. The two syringes connected to the liquid switch were filled with different standard glucose or lactate solutions, allowing simple calibrations by using the liquid switch to select different standard solutions. While one syringe was selected and its solution perfused through the cell, the other syringe could be refilled manually with new standard solution and returned to the syringe pump without affecting the perfusion; therefore, the number of the calibration points was unlimited. ***F4*** shows the response of the glucose sensing electrode to some standard glucose solutions perfused at 5 µL/min. The system showed an average 0–90% rise time of 2 min when the concentration of glucose was changed by using the liquid switch. The steady-state current response was linearly proportional to the glucose concentration from 0.1 to 20 mM. The electrode response was found to be flow rate dependent. At a constant glucose concentration, when the flow rate was changed within the range of 1–5 µL/min, the current decreased as the flow rate increased (e.g., when the flow rate was changed from 1 µL/min to 5 µL/min, there was a decrease of 10% in response). When the flow rate was increased further above 5 µL/min, there was hardly any change in the current response. When the glucose-sensing electrode was tested with a series of standard glucose solutions perfused at 2 µL/min, the linear range of the response was about the same as that at 5 µL/min. The 0–90% rise time of the system, however, increased to 3 min at 2 µL/min.

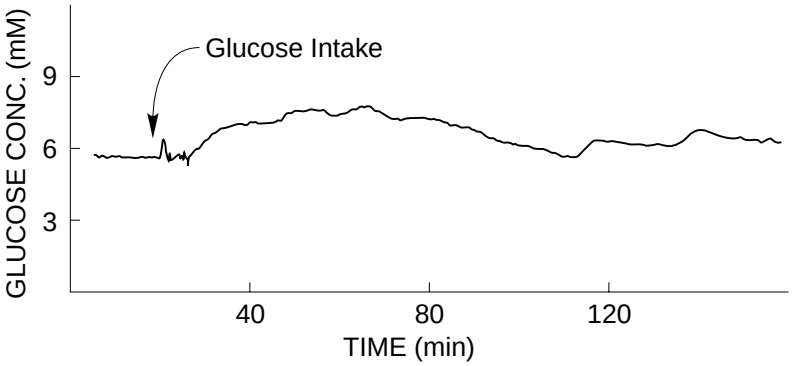
F4

Current-time response of the glucose sensor at different glucose concentrations. Numbers on each current plateau indicate the glucose concentration (mM). Flow rate = 5 µL/min.



F5

Glucose concentration in microdialysate calculated from the sensor output during the course of an in vivo experiment. Flow rate = 5 µL/min.

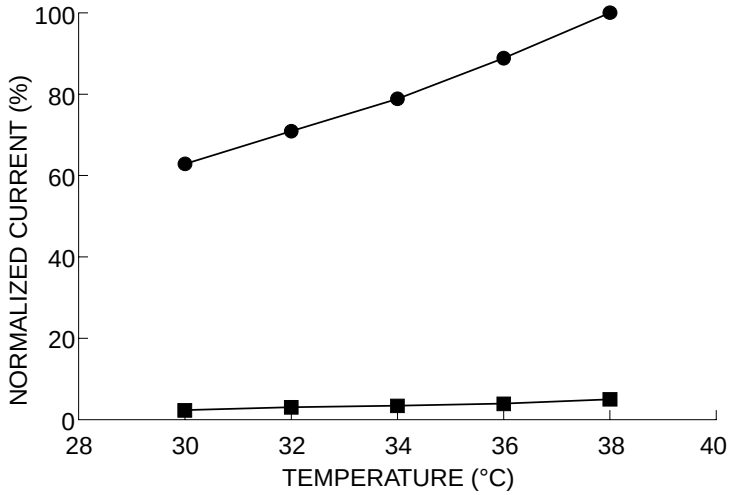


After calibrating the glucose-sensing electrode with standard glucose solutions, the rat subcutaneous dialysate from the microdialysis probe could be selected by the liquid switch and perfused through the flow cell. ***F5*** shows the variation of the apparent glucose concentration in the dialysate calculated from the sensor output during an in vivo assay. Upon an oral intake of a glucose solution (3 mL, 2.8 M) by the

F6

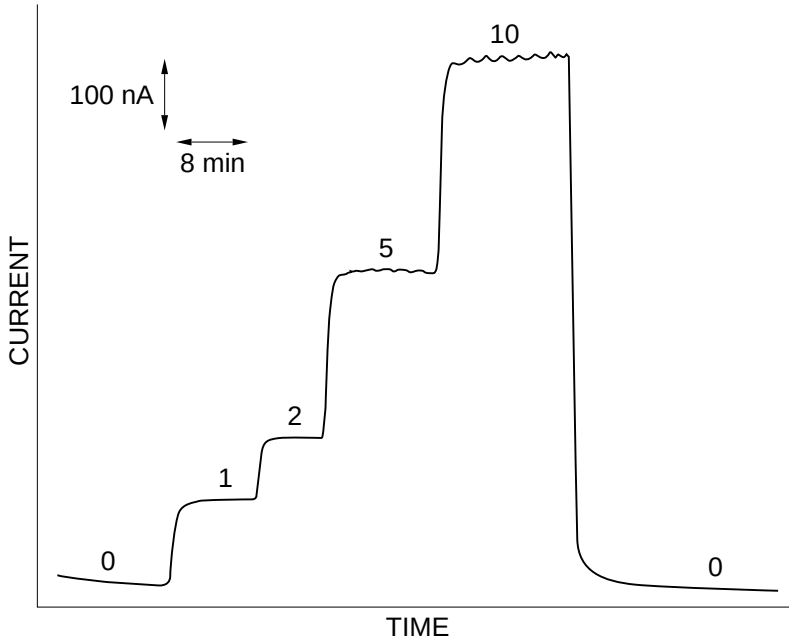
Effect of temperature on the glucose sensor output. Flow rate = 5 µL/min.

● *10 mM glucose*
■ *CSF solution*



F7

Current-time response of the lactate sensor at different lactate concentrations. Numbers on each current plateau indicate the lactate concentration (mM). Flow rate = 5 µL/min.

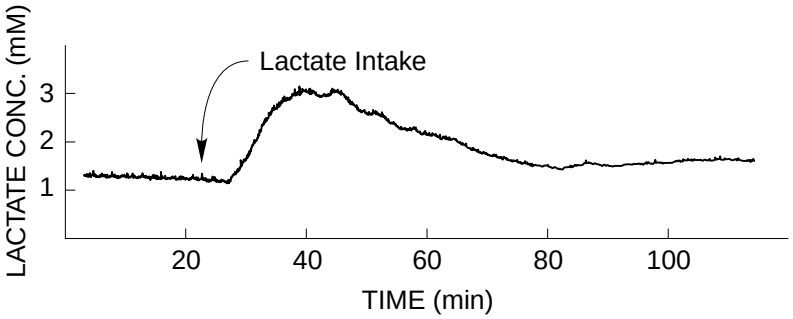


rat, there was an increase in the glucose response, which then returned to the original level after about 40 min. In microdialysate samples, the presence of oxidizable interferents is often a challenge for many anodic electrochemical sensors. In this experiment, due to the mild operational potential of the enzyme electrode (200 mV vs. Ag/AgCl), oxidation currents from the interferents ascorbic acid, uric acid, acetaminophen, and L-cysteine were negligible (5). In this study, we also collected some microdialysate samples and analyzed them with a BAS glucose applications kit and an LCEC system. This system uses chromatography to separate the interferents from glucose. The glucose concentration in the microdialysate sample determined by LCEC was found to be consistent with the on-line glucose-sensing electrode, indicating that there was no significant interference at the on-line glucose sensor. The average half-life of the glucose electrodes continuously operated with standard glucose solutions was 60 hours. The half-life of the glucose electrode operated with both standard glucose solutions and microdialysate was about 40 hours. Although the electrode lost half its sensitivity, the linearity of the response did not change and the electrode was still usable. During continuous operations, the loss of sensitivity was generally faster at the beginning of the experiment and slowed down with time. During all the tests, the electrode was calibrated with standard glucose solutions periodically every 2–3 hours, and the most recent standard was used to interpret the sensor output.

The glucose sensor response was found to be affected by temperature. In a comparison experiment with two on-line glucose assay systems operating simultaneously, the flow cell of one system was put in a temperature-controlled heater at 30 °C, and the cell of the other system was left out at room temperature. Glucose solutions of the same concentration were perfused through these two cells, and after 9 hours of continuous operation, it was found that the sensor without temperature control had an oscillating response typical of the room temperature drift. The maximum peak-to-peak oscillation observed was 20 nA, which was equivalent to a 1-mM variation in the glucose concentration calculated from the sensor output. On the other hand, the sensor in the heater showed a more stable response with no oscillation. During a continuous in vivo assay, the oscillation of the sensor response upon temperature variation may result in misleading information. Therefore, temperature control of the sensor should be necessary. ***F6*** shows the effect of temperature on the glucose sensor response to both a blank artificial CSF solution and a 10 mM glucose standard solution. As the temperature increased, the increase in the glucose response was more significant than that of the baseline response from the blank CSF solution. Although this result indicates that a higher sensitivity of the sensor may be obtained at a higher temperature, one also has to consider the temperature dependence of the stability of the immobilized enzyme in the sensor. More work

F8

Lactate concentration in microdialysate calculated from the sensor output during the course of an in vivo experiment. Flow rate = 5 µL/min.



needs to be done to define an optimal operational temperature for the enzyme sensor.

The lactate on-line assay system was tested in a manner similar to that for glucose. ***F7*** shows the response of the lactate sensor to lactate standard solutions perfused at 5 µL/min. Over the range of 1–5 mM lactate, the current increased linearly as the lactate concentration increased. As with the glucose sensor, the lactate assay system also showed an average 0–90% rise time of 2 min when the concentration of lactate was changed by using the liquid switch. ***F8*** shows the variations of the apparent lactate concentration in the rat subcutaneous microdialysate calculated from the sensor output during an in vivo assay. Upon an intraperitoneal injection of a lactate solution (1 mL, 2 M) to the rat, there was an increase in the lactate response, and after 60 min, the lactate concentration in the dialysate returned to the original level. The half-life of the lactate electrode operated with both standard lactate solutions and rat microdialysate was about 24 hours. As with the glucose sensor, the lactate sensor was also calibrated with standard lactate solutions periodically every 2–3 hours, and the most recent standard was used to interpret the sensor output.

Conclusions

The present work demonstrated a suitable system for on-line subcutaneous monitoring of glucose and lactate by coupling microdialysis with a "wired" glucose or lactate sensor. This system offers easy calibration, fast response, and satisfactory sensitivity and selectivity. The design of this system has the potential of being used with a number of other electrochemical sensors for continuous determinations of interesting analytes in microdialysates. The stability of the sensors used here is satisfactory for many research purposes; however, improvements are clearly needed for a totally satisfactory commercial implementation.

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