Simultaneous Real-Time Monitoring of Glucose and Cholesterol Levels in Fish Using Wireless Biosensor System

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Abstract

Fish health condition can be evaluated by monitoring some changes of blood indicators. For example, blood glucose levels are closely correlated with stress levels and blood cholesterol levels are closely correlated with immune system function in fish. We developed a wireless enzyme sensor system to simultaneously monitor blood glucose and cholesterol levels in fish using separate electrodes in real-time. The electrodes were constructed with Pt-Ir wire as the working electrode and Ag/AgCl paste as the reference electrode. Glucose oxidase or a mixture of cholesterol oxidase and cholesterol esterase was immobilized on the working electrode using glutaraldehyde. Serval functional polymers were used to coat each sensor to control the adsorption of the protein on the enzyme sensor surface. The characteristics of each sensor were examined by testing their output current change in biometric sample. For in vivo measurement, we inserted the sensor in the eyeball interstitial sclera fluid (EISF) in fish. The glucose and cholesterol concentrations in the plasma, measured using standard assays, and in the EISF, measured using our sensors, were well correlated. When sensors coated with functional polymers were then implanted into the EISF of free-swimming Nile tilapia, we were able to successfully simultaneous measure glucose and cholesterol levels in the EISF of free-swimming fish for 180h.

Keywords: Biosensor; Glucose; Cholesterol; Simultaneous monitoring; Real-time monitoring; Fish

Abbreviations

EISF: Eyeball Interstitial Sclera Fluid;
MPC: 2-Methacryloyloxyethylphosphorylcholine;

Introduction

With the continued growth of the aquaculture industry, fish farmer always preserves a high density of aquaculture for increasing production efficiency. As a result, a lot of stress applied to the fish, starting with immunity decrease, deterioration of water quality environment due to the accumulation feed residual and excretions, fish were often placed in an easy state to prone to illness. Although fish farmers administer use medicine to fish for disease prevention, there are misgivings about the effects of the medicine that remain in the fish. For preventing this, fish health should be monitored daily to provide consumers with healthy fish. Blood tests are often performed as a method of monitoring fish health. The plasma chemical composition is an index of various biologic reactions. It was reported that the concentration of certain components can be used to monitor fish health [1]. In stress response, stress hormones activate a number of metabolic pathways that alter blood chemistry and hematology, such as carbohydrate metabolism by stimulating glucose production through gluconeogenesis, resulting in an elevation of plasma glucose, which is a very important index for stress evaluation [2-5] On the other hand, total cholesterol in blood is an index of immune system activation against bacterial infectious disease and reproduction of fish [6-8]. Understanding the implication of changes of these blood components may help in the development of methods to monitor the progression of the fish disease and stress states.

Capturing fish to collect blood, however, is time-consuming and complicated. Moreover, capturing will further stress the fish, and thus, it is difficult to determine the true level of stress in the cultivation environment. To address this issue, Endo et al. developed several biosensor systems to monitor the levels of glucose, cholesterol, and lactic acid in free-swimming fish [9-13]. Since these biosensors were well performed, they can only express specific fish physiological and their life could not be so satisfying. While the progress of the technology and society, people have been not satisfied with the monitoring of a single parameter. Rather than single component measurement, simultaneous monitoring of multi-component is ideal and more correct to understand a fish health condition. Since some studies of in vivo simultaneous measurement have been reported in another animal (human, rats, etc.) [14-16], not to mention the biosensor, no one has yet performed simultaneous real-time monitoring for in free-swimming fish. We believe that if multiple blood compounds can be monitored, it can help people understanding on fish health comprehensibly.

On the other hand, monitoring these compounds directly from the blood is also problematic, biological components especially protein in serum [17], bind to and block the biosensor. Therefore, an alternate body compartment is required. EISF contains similar components as the blood but does not contain the components that degrade sensor function. The total blood cholesterol concentration correlates with that in the EISF [10]. We used EISF as the substance from which to measure glucose and cholesterol in free-swimming fish.

When monitoring blood components, the length of the measurement time is relatively important. Because the enzyme layer of current sensors directly touches the sample, components in vivo adhere to the detection part of the sensor, which eventually degrades sensor function. Consequently, in the development of a biosensor system enabling the real-time measurement of both blood glucose and cholesterol, we applied a functional polymer to the detection part of the sensor. The functional polymers used in this actual experiment were 2-methacyloyloxyethylphosphorylcholine (MPC), polypyrrole (PPy), and hydrophilic polyurethane (HPU). The MPC polymer is a biocompatible polymer membrane that has attracted a great deal of attention in the medical field. It holds molecular configuration, which is similar to a cell membrane. Utilizing this sort of a polymer as a contacting part to measuring the site, the biocompatible sensor can be achieved. The biocompatibility and conductive properties of PPy make it an attractive substrate for construction on electrodes and devices [18, 19]. HPU, acting as a protective layer for the enzyme, does not come in direct contact with the body and is used to limit diffusion speed.

In this study, we use sensor output current decrease to assess their applicability on biocompatibility to find the best functional polymer for in vivo measurement. For the examination on the durability of each polymer-functionalized sensor, we measured the attenuation of output current value of the sensors in the biometric sample (EISF) through a long period of time. After that, a real-time monitoring of glucose and cholesterol was performed by the most durability polymer-functionalized sensor to evaluate durability and practicability of the sensor in free-swimming fish.

Material and Methods

Reagents and preparation

Glucose oxidase (GOx, 197 Units mg⁻¹, E.C.1.1.3.4, from Aspergillus niger), cholesterol esterase (Cest, 10 Units
cholesterol oxidase (Cox, 54 Units mg\(^{-1}\), E.C.232.842.1, from *Streptomyces* species) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Bovine serum albumin (BSA), 2-phenox ethanol, Nafion® (5%), 25% glutaraldehyde, and pyrrole were purchased from Wako Pure Chemical Industries (Tokyo, Japan). MPC polymer (LPIDURE-BL206) was purchased from Nippon Oil Factory (Tokyo, Japan). HPU (Kaiser OH-1X) was purchased from Wako Pure Chemical Industries (Tokyo, Japan). All other reagents used for the experiments were commercial or laboratory grade.

**Fabrication of a glucose and cholesterol-sensing enzyme electrodes using different functional polymers**

A needle-type electrode was prepared for EISF glucose and cholesterol monitoring in fish. The working electrode was made using an 18 mm length of Teflon-coated platinum iridium (Pt-Ir) wire. The Teflon was stripped at one end to expose 1 mm of Pt-Ir wire as the sensing area. Copper wire was wrapped around the Teflon-coated surface. An Ag/AgCl paste (BAS, Tokyo, Japan) wire was used as a reference electrode/counter electrode. The electrode was dipped in a 5% Nafion® solution and air-dried for 10 min before combining with the functional polymers.

**MPC-type enzyme biosensor**

For the glucose-sensing electrode, an enzyme solution containing 2.0 mg (197 U mg\(^{-1}\)) Cox and 6.0 mg BSA dissolved in 0.1 M phosphate-buffered saline (PBS, pH 7.8) was freshly prepared. For the cholesterol-sensing electrode, we used a solution containing 1:1 Cest (168 U mg\(^{-1}\)) and Cox (67.2 U mg\(^{-1}\)). The enzyme solution and BSA (20 mg) were then dissolved in 0.1 ml PBS (0.1 M, pH 7.8) and mixed just before the experiment. The Nafion®-coated electrode was dipped in the enzyme solution and air-dried for 10 min. This procedure was repeated twice. The electrode was kept in a petri dish sealed with Parafilm®, and 50 μl glutaraldehyde (25%) was added to induce cross-linking between the enzyme and BSA. The electrode was stored at 35°C for 6 h. The electrode was stored in 0.1 M PBS (pH 7.8) overnight. The electrode was dipped in the MPC polymer for 1 min and dried at 35°C for 20 min. This procedure was repeated twice to completely coat the electrode with the MPC polymer. The electrode was stored in 0.1 M PBS (pH 7.8). The electrode was called a glucose/MPC sensor or cholesterol/MPC sensor.

**PPy-type enzyme biosensor**

We used an electrolytic polymerization method to synthesize the PPy. For the glucose-sensing electrode, an enzyme solution containing 2.0 mg Cox dissolved in 0.125 ml PBS (0.1 M, pH 7.0) in an Eppendorf tube was prepared and mixed. For the cholesterol sensor, an enzyme solution containing 2.5 μl Cox and 2.5 μl Cest dissolved in 0.5 ml PBS (0.1 M, pH 7.8) in an Eppendorf tube was prepared and mixed. Nitrogen bubbling was performed in the enzyme solutions for 5 min and the tubes were sealed until electrolytic polymerization. The electrode was connected to the potentiostat, and the applied voltage was set to 0.75 V. The electrode was then dipped in a mixed solution of enzyme and pyrrole for 3 min. The electrode was washed with distilled water and stored in PBS (pH 7.0 for the glucose-sensing electrode, pH 7.8 for the cholesterol-sensing electrode). These steps completely coated the electrode with PPy. The electrode was called a glucose/PPy sensor or cholesterol/PPy sensor.

**HPU-type enzyme biosensor**

A 5% HPU solution containing 50 μl distilled water and hydrophilic HPU was mixed and stored before use. The electrode was dipped in the solution after enzyme immobilization and air-dried for 10 min. The electrode was stored in PBS (pH 7.0 for the glucose-sensing electrode, pH 7.8 for the cholesterol-sensing electrode). The electrode was called a glucose/HPU sensor or cholesterol /HPU sensor.

**Evaluation of the needle-type glucose and cholesterol biosensors**

A dual sensor electrochemical system using both the glucose sensor and cholesterol sensor was used. A +650mV potential was applied to the Pt-Ir working electrode for the amperometric glucose and cholesterol measurements. The sensors were connected to a multi-channel potentiostat (model 13102BP, Pinnacle Technology, Lawrence, KS, USA). A handmade wireless potentiostat and receiver were used for wireless real-time monitoring. The output current was recorded via data acquisition software (PAL, Pinnacle Technology).

For the measurements, calibration curves were prepared in either phosphate buffer (0.1 M) or EISF as follows. While the calibration curve and optimal conditions for glucose measurement were used the data from our previous work [9]. For cholesterol sensor, 300 μl of EISF was extracted from Nile tilapia (*Oreochromis niloticus*) using a heparinized syringe fitted with a 27G needle. The EISF was mixed with 0.3 mg sodium azide as a preservative and transferred into a small vial. The biosensors were then placed into the vial to immerse with stirring for 1 h to allow the background output current to stabilize. An aliquot of a standard solution of cholesterol-olein (20 μl, 200 mg dl\(^{-1}\)) was added to the vial. When the sensor outputs had stabilized, another aliquot was added until the sensor output no longer increased.

**Sensor implantation and device immobilization**

A schematic diagram of the wireless monitoring system is shown in Figure 1. The system was constructed using a
glucose–sensing electrode, a cholesterol-sensing electrode, a multi-channel potentiostat, a receiver, and a personal computer. Sensor implantation and fixation were performed using the following procedure. Nile tilapia was food deprived for 24 h before the real-time monitoring. First, the fish were anesthetized in a water bath containing 0.1% 2-phenoxyl ethanol for 5 min. A 22G catheter consisting of an outer Teflon layer and inner puncture needle (23 mm, Serflow™, Terumo Co, Tokyo, Japan) was inserted into the interstitial fluid of the sclera of each eyeball for sensor implantation. The inner puncture needle was removed and the excess exposed catheter on the skin was trimmed off. The electrodes were then inserted into the outer Teflon layer and fixed in place using biomedical adhesive (Aronalpha-A Sankyo, Toagosei, Tokyo, Japan), which contained an ethyl cyanoacrylate monomer as the major ingredient. The potentiostat was covered using a waterproof polypropylene sheet (Honda Motor Co. Ltd., Tokyo, Japan) and sealed with a thermo-compression bonding device (NL-101J, Ishizaki Electric Mfg Co. Ltd., Tokyo, Japan).

The waterproofed wireless potentiostat was attached to the dorsal and pectoral fins of the fish using nylon threads and then connected to the sensor. The wireless potentiostat in this system transmits 512 MHz radio waves to the receiver and the output current of each sensor was recorded via PAL.

**In vivo measurement and calibration**

Simultaneous real-time measurement of glucose and cholesterol was realized using a wireless system. To measure the changes in glucose and cholesterol concentrations in the blood that correlate with the output current value of the sensor under a normal breeding environment, real-time monitoring of fish in the free swimming state was performed using a wireless system. Because proposed sensor provides a less stress condition for fish, all the fish were kept alive after the experiment. Insertion of the glucose and cholesterol sensors in the film outside eyeball required around 20 min, which was twice as long as usually required to insert one biosensor. After confirming the recovery of the tilapia and a stable output current, the tilapia was anesthetized and the first blood sample was obtained. The blood was sampled 5 times in the measurement and the output current of the sensor was long-term recorded. Glucose and cholesterol levels were separately estimated using a one-point calibration method. For the one-point calibration method, the sensor sensitivity “S” was determined from a single measurement as the ratio between the sensor output current “I” and the tested substance level “G”. The sampled substance levels were then estimated from current “I” using the equation G (t) =I (t)/S. The sensor output current was first allowed to stabilize after the sensor was implanted into the sclera. Each fish was netted from the preserve and anesthetized with 400ppm 2-phenoxyl ethanol by bath exposure. Blood samples were collected from the caudal vein along the backbone by inserting a heparinized syringe fitted with a 23G needle (0.65mm×25mm) into the basal anal fin. The sampled substance level “G” was measured. These parameters (I, G) were used as the calibration reference.

**Experimental conditions**

Nile tilapia was transferred to a water tank (60 cm×30 cm×36 cm). Dissolved oxygen in the water tank was saturated using an air pump. Continuous glucose and cholesterol monitoring of EISF was performed under the above-described experimental conditions using our sensors.

**Results**

**Durability of cholesterol sensors coated with various functional polymers**

The results of durability testing are shown in Figure 2. The cholesterol concentrations in EISF were measured for the three types of polymer-functionalized cholesterol sensors and control sensor (polymer unfixed). The time at which the output current had stabilized was set as 0 h. Output current at 0 h was defined as 100% and the decrease in the sensor output current over time was expressed as a percentage of the output current at 0 h. The output current of the control sensor began to decrease within 2 h and had decreased to 85% by 6 h. The cholesterol/HPU sensor holds a 91% output for 6 h. The cholesterol/PPy sensor had a similar decrease in 10% at 6 h. On the other hand, the cholesterol/MPC sensor output current decrease rate stabilized within 2 hours and maintained an
output current at 97% for 6 h. Based on these results, the cholesterol/MPC sensor was the most durable sensor in EISF.

Figure 2. Durability testing of materials for the cholesterol biosensor in EISF.

Calibration curve of the biosensor

The calibration curve of cholesterol/MPC sensor was generated using sequentially added aliquots of a cholesterol standard solution in phosphate buffer (Figure 3). The sensor output current increased and well correlated with the increase in cholesterol concentration. Good linearity was observed in the concentration range of 0 to 100 mg dl⁻¹ (y = 0.14564 + 0.056551x, R=0.9988). To evaluate the potential use of the sensor for in vivo applications, the linearity of the sensor was also investigated in EISF (Figure 4). On the other hand, the calibration curve of the glucose sensor and optimize conditions were used that of the data we got in our previous study [13]. The sensor was placed in the EISF (300 μl) which was obtained from Nile tilapia. The original cholesterol concentration of the EISF was 67.6 mg dl⁻¹ and the sensor was first stabilized in that solution. After that, a 200 mg dl⁻¹ cholesterol standard solution was sequentially added into the EISF sample. It took approximately 15 min for the output current to stabilize after each addition of cholesterol. The sensor output current and cholesterol concentration correlated well in a concentration range of 67.6 to 76 mg dl⁻¹ (y=-13.807+0.23713x, R=0.9829).

Effect of environmental conditions on sensor output current

The response of the cholesterol sensor system is easily influenced by analytical conditions. Temperature and pH are two of the factors that affect sensor function, so the effects of these factors on sensor output were analyzed prior to the in vivo experiment (Figure 5a). The sensor was placed in a standard 200 mg dl⁻¹ cholesterol solution (pH 6.5). The temperature was altered in the range of 10 to 60°C in 10°C increments. The increase in the sensor output current correlated with the increase in the temperature up to 50°C and then began to decrease. To analyze the effect of pH, the sensor was immersed into standard 200 mg dl⁻¹ cholesterol solutions with pH values ranging from 5.5 to 8.0 (Figure 5b). Sensor output increased with the increase in pH from a pH of 5.5 to pH 7.5, and then began to decrease.

Figure 3. Calibration curve using cholesterol standard solution with an Nafion®/COx/Cest/MPC sensor

Aliquots of cholesterol standard solution were added to phosphate buffer (0.1 M, pH 7.0) and the calibration curve was drawn based on the change in output current in response to each addition of standard solution. Applied voltage was +650 mV.

Figure 4. Calibration curve of Nafion®/COx/Cest/MPC sensor in EISF.

After the sensor output current had stabilized in EISF, aliquots of a
cholesterol standard solution (200 mg dl⁻¹, pH 8.0) were sequentially added to create the calibration curve.

Figure 5. Analysis of the effect of temperature (a) and pH (b) on sensor output current.

(a): Phosphate buffer (0.1 M, pH 7.0) was used; cholesterol concentration was 200 mg dl⁻¹; applied voltage was +650 mV. (b): Temperature was 25°C; cholesterol concentration was 200 mg dl⁻¹; applied voltage was +650 mV.

Real-time in vivo measurement of glucose and cholesterol levels using the functional polymer-coated biosensors

Long-term and real-time monitoring of glucose and cholesterol levels in fish using the glucose/MPC and cholesterol/MPC sensors and wireless potentiostat are shown in Figure 6. Figure 6 shows the calculation of estimated glucose and cholesterol levels from the sensor output current using the one-point calibration method and actual blood glucose and cholesterol levels. The sensors were inserted into the EISF of living fish and then the output current was allowed to stabilize. Blood was sampled for the first time after the output current stabilized, and real-time monitoring began after the first blood sample. The one-point calibration method was used to calculate estimated glucose and cholesterol levels. Blood and cholesterol levels were successfully measured for 180 h.

Figure 6. Real-time monitoring of blood glucose and cholesterol concentrations, which were calculated from the output current of each biosensor using a one-point calibration method.

Blood was sampled to measure glucose (open circles) and cholesterol (filled circles) at the indicated times.

Discussion

The living body does not adapt very well to long-term insertion of a biosensor because the material for the sensor is made of metal. The surface of the sensor adsorbs impurities such as protein in vivo. The buildup of adsorbed impurities overtime results in a decrease in output current. To examine the durability of the biosensor, we measured the change of the output current value of the sensors over a long period. The sensors were inserted behind the fish eye, thus immersing the glucose and cholesterol sensors in EISF, and durability of the cholesterol sensor was evaluated. The high durability of biosensors made using each functional polymer was confirmed. While, the parameters of glucose biosensor (selected polymer, temperature, pH etc.) were used those introduced in our published paper [20]. The output of the biosensors made with the MPC polymer maintained the
highest level while the output current of the electrodes made with the other functional polymers decreased over time. The MPC polymer is biocompatible, and it had only a small effect on the enzyme function. The Nafton®/Cox/Cest/MPC electrode creates a film on the enzyme layer when the electrode is placed in the MPC polymer solution and then dried. It is assumed that the biocompatibility layer protects the enzyme layer and decreases protein adsorption. The result of our experiment clearly showed that MPC-type sensor had excellent durability when placed in the EISF of Nile tilapia. A similar experiment using Gox to measure glucose was conducted in which it the glucose/MPC electrodes were determined to be durable and the optimal calibration conditions were identified [20].

For use in testing, the biosensor output must be linear within the physiologic range of the substance being measured. The biosensor output current was confirmed to correspond to the cholesterol concentration and was linear in the range from 0 to 100 mg dl⁻¹. Because the cholesterol concentration in tilapia EISF ranged from ~20 to ~100 mg dl⁻¹, these sensors could be applied to the measurement of cholesterol in tilapia blood. To simulate in vivo conditions, we also evaluated the response of the biosensor output current to cholesterol in EISF. Though this experiment was conducted in vitro, the output current decreased compared with those of measured by using standard solution due to a non-specific sorption. In the EISF, the biosensor output was linear within a range of cholesterol of 67 to 76 mg dl⁻¹, which is within the range of cholesterol measured in tilapia’s blood.

Several environmental factors can influence the output current of the biosensors. For example, the temperature alters the output of the sensor. An increase in the temperature resulted in an increase in the output current of the sensor up to ~50°C and then it subsequently decreased. In chemical reactions, reaction speed generally increases with increased temperature. The same applies to enzyme reactions; however, many enzymes lose their catalytic function at temperatures of 60°C or more. Because the enzyme is a protein, and heat denaturation occurs at high temperatures, the structure of the enzyme begins to change and its activity decreases. The decrease in the output at ~50°C was likely due to a decrease in the enzyme activity. Keeping the biosensor at 50°C for a long time might lead to degradation of the enzyme. And for normal species of fish, 50°C also is an intolerable temperature. Therefore, we thought that a temperature of ~25°C would be appropriate for maintaining enzyme stability. The MPC polymer used in this experiment is not heated sensitive. Thus, the sensor output can be measured sufficiently at the breeding temperature of tilapia, which is 20~30°C based on the present and previous experiments [20], we determined that the optimal temperature for simultaneous glucose and cholesterol measurements was 30°C.

The pH of the solution also influences biosensor output current. The output current value increased in the range of pH 5.5 to pH 7.5, and then decreased right around a neutral pH. The pH influences enzyme activity. In the range of the optimum pH, the catalyzed reaction was highly stable and the influence of pH was small. Examination of the optimum pH is important for determining the optimal reaction conditions for the enzyme. The optimum pH of Gox is 7.5 and Cest are 7.0 according to the product information provided by the manufacturer (Sigma-Aldrich). Therefore, we thought that the optimum pH of the enzyme-immobilized sensor used in this experiment would be in the vicinity of a neutral pH. Because sensors with or without MPC polymer had a similar response to pH, we thought that the MPC polymer did not influence the pH-responsive characteristics of the sensor. Moreover, the MPC polymer is a macro-molecule that has a polar phosphorylcholine group, therefore, it is inferred that changes in the pH would have little effect on the MPC polymer. The result of our experiment clearly demonstrated that the highest output current of each sensor was at a nearly neutral pH. In general, the inner body pH in fish, including tilapia, is about 6.0-7.0. Although minor deviations in pH influence sensor output, the output current was large enough that it was still possible to obtain enough data under this pH condition using our cholesterol biosensors. Previously, we determined that a pH of 7 is the optimal pH for glucose/MPC biosensor [20].

We used the one-point calibration method to calculate the cholesterol and glucose levels from the output current value of each sensor shows in Figure 6. The corresponding values of the calculated blood glucose and cholesterol levels and actual blood glucose and cholesterol levels showed similar temporal changes. Thus, we concluded that these compounds can be measured in real-time using these sensors. It is presumed that the output of the sensor was steady for a long period because the sensor was coated with the MPC polymer. This finding suggests that MPC polymer decreases the effect of the obstructing materials that bind to the sensor in vivo.

Moreover, the sensor made with the MPC polymer had ~1.5 times greater measurement durability using a similar method in flatfish (sea fish) [20]. These results indicate that the MPC polymer in EISF is useful in both freshwater fish and sea fish. Real-time measurements of cholesterol levels in tilapia blood using a sensor without MPC was performed for 14 h; in contrast, a sensor with MPC could measure cholesterol for approximately 13 times longer. These findings suggest that MPC decreased the influence of the adsorption of the protein on sensor function, just like the glucose sensor.

Conclusions

We constructed biosensors with a biocompatible polymer using MPC polymer with excellent durability. Two biosensors, one for glucose the other for cholesterol, were inserted into the EISF of free-swimming tilapia, and glucose and cholesterol concentrations were continuously measured for 1 week. We successfully developed simultaneous real-time monitoring of multiple compounds in free-swimming fish. In
the future, because not all kinds of fish hold EISF, determining the appropriate position for placing the sensor is urgently important. For fish that does not hold EISF, a biosensor that can measure whole blood samples would be ideal. Whole blood measurements with a sensor using a biocompatible layer, such as carbon nanotubes and MPC polymer, were reported in the medical treatment field [21,22]. While, in the present research, because two kinds of sensors need to be inserted in the EISF of each fish, the operation becomes complex and time-consuming. Moreover, inserting two sensors and the wireless potentiostat places a rather large load on the fish. In the future, an intelligent biosensor system which can measure two or more compounds in one sensor is desired and our proposed system will be lighter to reduce the load on the fish.

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References


