Amperometric determination of phosphoglucomutase activity with a bienzyme screen-printed biosensor

Yue Cui,* John P. Barford, and Reinhard Renneberg

Department of Chemical Engineering and Department of Chemistry, Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong

*Corresponding author. Email: kecuiyue@ust.hk

Phosphoglucomutase (PGM) is a ubiquitous enzyme that is expressed in all organisms from bacteria to plants to animals and controls a key branch point for carbohydrate metabolism. PGM catalyzes the interconversion of glucose-1-phosphate and glucose-6-phosphate. In this process, the enzyme links various catabolic pathways to yield energy ATP or reducing power NAD(P)H, and several anabolic pathways, such as to lead to the synthesis of polysaccharides [1-4].

Due to its importance, measurements for the enzymatic activity of PGM have been widely performed, by the use of optical methods with coupled enzyme system [3-11], or the use of a combination of ion/molecule reactions and FT-ICR mass spectrometry [12]. However, these measurements either result in a long detection time, a large amount of enzyme consumption, or complex procedures which need to be performed by skilled personnel. The importance of amperometric enzyme-based biosensors has increased considerably in recent years thanks to the advantages of being highly sensitive, rapid, accurate, economical and easy-to-handle for specific measurement of target analyte in complex matrices such as blood, food product and

environmental sample. To the best of our knowledge, there is no report for the determination of PGM activity using an amperometric biosensor.

In this work, we present an amperometric determination of PGM activity with a bienzyme screen-printed biosensor. As shown in Fig.1, the principle is as follows: Phosphoglucomutase (PGM, EC 5.4.2.2, from rabbit muscle, Sigma) converts glucose-1-phosphate to glucose-6-phosphate. Glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49, from *Leuconostoc mesenteroides*, Sigma) catalyzes the specific dehydrogenation of glucose-6-phosphate by consuming NAD^+ . The product, NADH, initiates the irreversible decarboxylation and the hydroxylation of salicylate by salicylate hydroxylase (SHL, EC 1.14.13.1, from *Pseudomonas* sp., GDS Technology Inc., USA) in the presence of oxygen to produce catechol, which results in a detectable signal due to its oxidation at the working electrode.

The bienzyme electrode for the measurement of PGM activity is based on the detection of glucose-6-phosphate, the product of the PGM-catalyzed reaction. From literature, glucose-6-phosphate biosensors have been developed by several methods, including the combinations of G6PDH and various mediators, or the combination of phosphatase and glucose oxidase [13-15]. In this work, the successful coupling of SHL and G6PDH on a screen-printed electrode can also serve as a glucose-6-phosphate biosensor.

A potentiostat EP30 (Biometria, Germany) and a computer installed with the software FIABOLO were used. The screen-printed electrode (BioSensorTrend, Germany) with a 2-electrode configuration was composed of a platinum (Pt) working electrode (diameter: 1 mm) and an Ag/AgCl reference/counter electrode. A measuring cell (volume: 1 ml), connected to a syringe and installed with two connectors (cathode and anode) and an electrode-fitting site, was

constructed for experiments.

The screen-printed electrode was covered with a mixture of SHL, G6PDH and glutaraldehyde, followed by drying and storing at 4 °C overnight. The biosensor was then screwed into the measuring cell, which was filled with buffer solution, and rehydrated for around 1 h at room temperature to allow the enzyme matrix swelling before usage. Experiments were performed by applying the specific potential for this type of screen-printed electrode at 0.42 V and magnetically stirring the solution at 300 rpm to obtain a uniform distribution of PGM. The measurement was started by adding PGM into the buffer solution contained in the measuring cell, and the current velocity (nA min^{-1}) was recorded for plotting calibration curve. A syringe was used between each measurement for sucking the buffer solution out of the measuring cell to remove PGM in the solution, and thus to remove catechol on the electrode surface.

A two-steps optimization was needed before the sensor calibration in order to improve the sensor performance for the determination of PGM activity, which was shown in Table 1.

The first step is the optimization of the enzyme matrix, including the enzyme loadings and the immobilization agent concentrations. Various loadings of SHL, G6PDH and glutaraldehyde on screen-printed electrodes were investigated to obtain the maximum current velocity, as shown from Test 1 to Test 7 in Table 1. Based on the optimization, a mixture containing 0.33 U SHL and 1.88 U G6PDH with 1% glutaraldehyde in 0.5 μl of enzyme matrix was used for further experiments.

The second step was the optimization of the working condition, including pH value, substrate and cofactor concentrations in the buffer solution. The effects of glucose-1, 6-

diphosphate and MgCl_2 were also studied due to being the activating cofactor and metal cofactor for the PGM-catalyzed reaction. To improve the sensor performance, various loadings of glucose-1-phosphate, salicylate, NAD^+ , glucose-1, 6-diphosphate, MgCl_2 , and buffer pH were investigated, as shown from Test 7 to Test 24 in Table 1. In order to obtain the maximum current velocity for the measurement of PGM activity, the substrate and cofactor concentrations in the buffer solution should be sufficient to avoid the signal saturation due to their inadequate loadings. The optimized working condition obtained was 100 mM Tris-HCl buffer solution containing 5 mM glucose-1-phosphate, 5 mM salicylate, 5 mM NAD^+ , 50 μM glucose-1, 6-diphosphate and 5 mM MgCl_2 at pH 8.0.

Fig. 2 shows the calibration curve for PGM activity using the bienzyme screen-printed sensor. The measurements of PGM activities were performed by the biosensor with the optimal enzyme matrix and working condition. As the PGM activity was proportional to the rate of production of glucose-6-phosphate, it was further proportional to the rate of production of catechol and the current velocity, thus current velocity (nA min^{-1}) was used for the determination of PGM activity. As shown in the figure, a linear relationship was obtained between the current velocity and the PGM activity from 0.05 U ml^{-1} to 5 U ml^{-1} with a detection limit of 0.02 U ml^{-1} (slope: $76.36 (\text{nA min}^{-1})/(\text{U ml}^{-1})$, $R^2 = 0.9988$, $n=3$). The linear detection range and detection limit are decided by the recording method, and if the record of current velocity is changed from nA min^{-1} to nA (2 min)^{-1} or nA (3 min)^{-1} , the linear detection range and detection limit will be changed and improved.

Also, the sensor has a fast measuring time (1 min) and a short recovery time (2 min) with high reproducibility. Hence, the total measurement of PGM activity takes less than 4 min with

simple operations, which is more rapid and convenient than conventional methods.

In summary, we have demonstrated the measurement for the enzymatic activity of PGM using an amperometric screen-printed biosensor based on the co-immobilization of SHL and G6PDH. The sensor shows high performance characteristics with a broad detection range (0.05-5 U ml⁻¹) and a rapid measuring time (1 min). Thus, we provide a new analytical approach to the determination of PGM activity rapidly, sensitively, economically and easy to handle.

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Fig.1. Schematic illustration of the amperometric screen-printed biosensor for the determination of PGM activity (G1P: glucose-1-phosphate; G6P: glucose-6-phosphate; GLP: D-glucono-1, 5-lactone 6-phosphate; PGM: phosphoglucomutase; G6PDH: glucose-6-phosphate dehydrogenase; SHL: salicylate hydroxylase).

Fig.2. Calibration curve for PGM activity using the amperometric screen-printed biosensor (Sensor: 0.33 U SHL, 1.88 U G6PDH with 1% glutaraldehyde in 0.5 μ l of enzyme matrix. Buffer: 100 mM Tris-HCl buffer containing 5 mM glucose-1-phosphate, 5 mM salicylate, 5 mM NAD⁺, 50 μ M glucose-1, 6-diphosphate and 5 mM MgCl₂ at pH 8.0).

Table 1

Optimization of the enzyme matrix and working condition

Test	SHL	G6PDH	GA ^a	G1P	Salicylate	NAD	G1,6diP ^b	MgCl ₂	pH	Relative current velocity (%) ^c
	(U)	(U)	(%)	(mM)	(mM)	(mM)	(μ M)	(mM)		
1	0.33	0.94	1.00	5	5	5	50	5	8	68.6 $\pm$ 1.0
2	0.33	1.41	1.00	5	5	5	50	5	8	96.5 $\pm$ 2.0
3	0.17	1.88	1.00	5	5	5	50	5	8	73.0 $\pm$ 1.5
4	0.50	1.88	1.00	5	5	5	50	5	8	98.9 $\pm$ 1.8
5	0.33	1.88	0.67	5	5	5	50	5	8	98.1 $\pm$ 1.7
6	0.33	1.88	1.33	5	5	5	50	5	8	56.4 $\pm$ 0.3
7	0.33	1.88	1.00	5	5	5	50	5	8	100.0 $\pm$ 1.9
8	0.33	1.88	1.00	0	5	5	50	5	8	0.0 $\pm$ 0.0
9	0.33	1.88	1.00	2	5	5	50	5	8	99.8 $\pm$ 2.2
10	0.33	1.88	1.00	10	5	5	50	5	8	98.2 $\pm$ 3.2
11	0.33	1.88	1.00	5	0	5	50	5	8	9.8 $\pm$ 0.8
12	0.33	1.88	1.00	5	2	5	50	5	8	97.1 $\pm$ 3.0
13	0.33	1.88	1.00	5	10	5	50	5	8	99.9 $\pm$ 3.6
14	0.33	1.88	1.00	5	5	0	50	5	8	0.0 $\pm$ 0.0
15	0.33	1.88	1.00	5	5	2	50	5	8	97.5 $\pm$ 2.2
16	0.33	1.88	1.00	5	5	10	50	5	8	100.0 $\pm$ 3.6
17	0.33	1.88	1.00	5	5	5	0	5	8	20.2 $\pm$ 0.6
18	0.33	1.88	1.00	5	5	5	20	5	8	98.3 $\pm$ 1.6
19	0.33	1.88	1.00	5	5	5	100	5	8	96.7 $\pm$ 3.3
20	0.33	1.88	1.00	5	5	5	50	0	8	21.8 $\pm$ 1.2
21	0.33	1.88	1.00	5	5	5	50	2	8	76.4 $\pm$ 3.3
22	0.33	1.88	1.00	5	5	5	50	10	8	96.6 $\pm$ 2.9
23	0.33	1.88	1.00	5	5	5	50	5	7	73.9 $\pm$ 2.2
24	0.33	1.88	1.00	5	5	5	50	5	9	75.2 $\pm$ 1.6

^a GA: glutaraldehyde.^b G1, 6diP: glucose-1, 6-diphosphate.^c The relative current velocity (%) was calculated by normalizing the current velocity of the test to the maximum current velocity obtained from test 7 in measuring the PGM activity of 5 U ml⁻¹ (n=3).