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Whole cell immobilized amperometric biosensor based on *Saccharomyces cerevisiae* for selective determination of vitamin B_1 (thiamine)

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Abstract

A new amperometric whole cell biosensor based on *Saccharomyces cerevisiae* immobilized in gelatin was developed for selective determination of vitamin B_1 (thiamine). The biosensor was constructed by using gelatin and crosslinking agent glutaraldehyde to immobilize *S. cerevisiae* cells on the Teflon membrane of dissolved oxygen (DO) probe used as the basic electrode system combined with a digital oxygen meter. The cells were induced by vitamin B_1 in the culture medium, and the cells used it as a carbon source in the absence of glucose. So, when the vitamin B_1 solution is injected into the whole cell biosensor system, an increase in respiration activity of the cells results from the metabolic activity and causes a decrease in the DO concentration of interval surface of DO probe related to vitamin B_1 concentration. The response time of the biosensor is 3 min, and the optimal working conditions of the biosensor were carried out as pH 7.0, 50 mM Tris–HCl, and 30 °C. A linear relationship was obtained between the DO concentration decrease and vitamin B_1 concentration between 5.0×10^{-3} and 10^{-1} μM. In the application studies of the biosensor, sensitive determination of vitamin B_1 in the vitamin tablets was investigated.

Keywords: *S. cerevisiae*; Whole cell biosensor; Vitamin B_1; Thiamine; Microbial biosensor; Biosensor

Vitamin B_1, also called thiamine, is one of the eight vitamins that make up the powerful group called the vitamin B complex. Like all of the B vitamins, this nutrient plays a major role in the good health of the body as well as in sound mental health. Vitamin B_1 serves many purposes in the body. It is an essential part of converting carbohydrates to energy and is necessary for the proper functioning of the nervous system, the heart, and the musculature system. Thiamine is very important to the brain, particularly in terms of emotional health and well-being, and also is useful for focus and concentration. It plays an important role in Wernicke–Korsakoff syndrome, a form of amnesia caused by brain damage occurring in long-term alcoholics who rely mainly on alcohol for nutrition. The acute syndrome normally is reversible but may proceed to profound dementia, although its progress can be stopped by a timely injection of a large dose of thiamine. There have been suggestions that thiamine may have a beneficial effect in treating Alzheimer's disease [1–4].

There are a variety of physical conditions and diseases associated with deficiencies in vitamin B_1. Symptoms include pain, numbness and tingling in the extremities, muscle weakness, and a lack of physical coordination, particularly in the larger muscle masses that make up the leg muscles. A deficiency in thiamine can cause enlargement of the heart, and this can lead to congestive heart failure and lung congestion. A severe deficiency in vitamin B_1 can lead to nerve damage, brain damage, and even death. Mental symptoms associated with a serious lack of vitamin B_1 include fatigue, psychosis, and confusion [5–7]. Thiamine is

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also used as a supplement in the vitamin complexes and in some food products, especially those for babies. Dietary supplements are an affordable, safe, and effective way to consistently meet the recommended daily intake levels of vitamin B_1 [8,9]. It is obvious that thiamine is an important factor for body functions and good health and also is a necessary supplement for the food products and vitamin complexes, but in contrast to these advantages, it can also be a risk factor when it is used in large doses, so determining vitamin B_1 is very important in clinical analysis, food processing, and pharmaceutical and biotechnological processes [10–12]. There are only a few basic methods to determine vitamin B_1. Some of these methods are based on chromatography [13–18], spectrophotometry [16–18], voltammetry [19], and biosensors [20,21]. In the development of biosensors, some biologically active materials such as enzymes, cells, and plant and animal tissues have been used mostly. Enzymes are used mostly in biosensor construction due to their high specific activities and analyte sensitivities. Therefore, most of the enzymes used in biosensor applications are unstable and so are expensive for routine analysis of the target analytes. To solve this problem, the whole cell biosensors have been developed [22–26]. In the literature, there is only one microbial biosensor study based on *Lactobacillus fermenti* bacteria for the determination of vitamin B_1 developed by Karube and coworkers [26]. So, in the current study for the determination of vitamin B_1, a new whole cell microbial biosensor based on *Saccharomyces cerevisiae* was developed. There are some advantages to using yeasts in the biosensor construction, including speed of growth, easy manipulation, and growth of a variety of carbon sources. Yeasts are particularly robust with a wide physicochemical tolerance (e.g., pH, temperature, ionic strength, tough cell walls).

This article describes a whole cell biosensor for selective and non-time-consuming vitamin B_1 determination based on *S. cerevisiae* immobilized in gelatin by using a crosslinking agent glutaraldehyde on a Clark-type dissolved oxygen (DO)[1] probe. Measurements were carried out by standard curves that were obtained by the determination of respiration activity of microorganism related to its metabolic activity or consumed DO level related to vitamin B_1 concentration injected into the reaction medium.

Materials and methods

Microorganism and chemicals used in the experiments

In the experiments, a strain of *S. cerevisiae* NRRL-12632 was used after being obtained from the National Center for Agricultural Utilization Research Laboratory (Peoria, IL, USA). Thiamine hydrochloride (vitamin B_1), pyridoxine (vitamin B_6), pantothenic acid (vitamin B_5), nicotinic acid, calf skin gelatin (225 bloom), glutaraldehyde (25%), and all other chemicals used in the experiments were purchased from Sigma Chemical (St. Louis, MO, USA). All solutions used in the experiments were prepared just before their use.

Apparatus

A YSI 58 digital oxygen meter with 0.01-mg/L DO concentration sensitivity, YSI 5700 DO probes (with YSI 5740 cable), highly sensitive Teflon membranes (0.0005 inch thick) for oxygen (YSI, Yellow Springs, OH, USA), a Hettich Universal 30 RF centrifuge (Germany), a Stuart Scientific linear shaker bath (SBS 35, UK), a Sonifier B-12 (Bronson Sonic Power, Danbury, CT, USA), a Pharmacia LKB Novaspec II spectrophotometer (UK), and a Nuve thermostat (Turkey) were used.

Culture medium of S. cerevisiae cells

S. cerevisiae NRRL 12632 was grown in potato dextrose broth (PDB) at 30 °C for 16 h. This active culture was used as inoculums. The yeast strain was cultured in 500-ml Erlenmeyer flasks containing of 100 ml fermentation medium (PDB) consisting of 4 g/L potato extract and 20 g/L dextrose (pH 5.6). Thiamine–HCl was added to the fermentation medium at the concentration of 10 μg/ml after membrane filter sterilization. The Erlenmeyer flasks were inoculated with 1% (v/v) of 16-h-old *S. cerevisiae* NRRL 12632, and flasks were kept on a rotary incubator shaker at 30 °C with agitation (150 rpm) for 20 h. The culture medium contained yeast extract (3.0 g L^{-1}), $(NH_4)_2SO_4$ (10.0 g L^{-1}), $MgSO_4 \cdot 7H_2O$ (0.5 g L^{-1}), $CaCl_2$ (0.1 g L^{-1}), peptone (0.5 g L^{-1}), KCl (1.0 g L^{-1}), and thiamine–HCl (20 mg L^{-1}). After a 12-h fermentation period (i.e., the log phase of cell growing), culture medium was centrifuged at 6000*g* for 10 min. The cells were resuspended with physiological saline water twice and were recentrifuged with same condition. At the end of the process, the cells of *S. cerevisiae* were lyophilized and used for the preparation of whole cell amperometric biosensor for vitamin B_1 determination.

To obtain the growth curve of this strain, 0.5 ml of the samples from culture broth were withdrawn at selected times and the colony-forming units/ml were calculated by using the pour plate method on potato dextrose agar (PDA).

Preparation of whole cell amperometric biosensor

The specific growth rate of the cells is maximal and constant in the logarithmic phase, so *S. cerevisiae* cells in the log phase were removed from the culture medium and lyophilized to use whole cell biosensor construction. For this purpose, the cells were removed from the culture medium by centrifugation at 6000*g* for 10 min at 4 °C. The cells were washed with physiological saline water and centrifuged again at 6000*g* for 10 min at 4 °C. Finally, the cells were placed in 5.0 ml of physiological saline water and lyophilized.

[1] *Abbreviations used:* DO, dissolved oxygen; PDB, potato dextrose broth; PDA, potato dextrose agar.