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Zinc finger peptide based optic sensor for detection of zinc ions

Neelam Verma*, Gagandeep Kaur

Biosensor Technology Laboratory, Department of Biotechnology, Punjabi University, Patiala, Punjab, India

A R T I C L E I N F O

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A B S T R A C T

In the present work, polyacrylamide gel has been used as a matrix for the immobilization of zinc finger peptide and fluorescent dye acrydine orange on the micro well plate to fabricate the fluorescence based biosensor for the detection of zinc ions in milk samples. The fluorescent dye moves in the hydrophobic groove formed after folding of the peptide in the presence of zinc ions. Under optimized conditions, linear range was observed between 0.001 μg/l to 10 μg/l of Zinc ions, with a lowest detection limit of 0.001 μg/l and response time of 5 min. Presented biosensor has shown 20% decrease in fluorescent intensity values after 5 regenerations and stable for more than one month, stored at 4 °C. Interference study with other metal ions like lead, cadmium and copper showed a negligible change in fluorescence intensity in comparison to zinc ions. Developed bio sensing system was found to be novel, quick, reliable, miniaturized, stable, reproducible and repeatable and specific for zinc ion, which has been applied to various milk samples.

1. Introduction

Zinc (Zn) metal acts as a micronutrient which is essential to our body and has a range of biological roles but when its amount exceeds in the body than normal, this metal proves to be lethal. Both acute and chronic forms of zinc can be toxic (Osredkar and Sustar, 2011). Increase in zinc level in the body than normal, results in severe health damages like anemia, nausea, vomiting, skin problems, and stomach aches. Higher zinc content, destructs the pancreas by disturbing the protein metabolism causing arteriosclerosis. Exposure to the air containing zinc metal can result in to a metal fever by causing flu like symptoms (Moyo, 2014). Higher concentration of unnatural zinc in the environment is due to industrial sources such as mining, coal and waste combustion, steel processing, toxic waste sites, and other human activities. Symptoms of airway irritation and inflammation are caused by airway exposure to zinc dust and zinc-containing ambient particulates (Gu and Lin, 2010). In drinking water, zinc concentration is magnified, when it is stored in metal tanks. In natural surface water, concentration of zinc is usually below 10 μg/l. In ground water, its concentration is 10–40 μg/l. In tap water, the zinc level can be much higher as a result of the leaching of zinc from piping and fittings (World Health Organization guidelines for drinking water quality 2013).

Zinc metal can also enter in to dairy and milk products through grazing of dairy animals in the soil containing un-natural zinc, which could affect the health of infants and adults. Mother's milk containing large amount of zinc can be a lethal to unborn and newly born children (Moyo, 2014). Due to 150–450 mg of zinc per day intake, altered iron function, reduced immune function, genitourinary defects, copper deficiency and reduced levels of high-density lipoproteins can occur (Johnson et al., 2007).

Zinc concentration should not exceed more than 20 mg/day in healthy individuals (Maret and Sandstead, 2006). The Recommended Dietary Allowance (RDA) for women is 8 mg/day and for men it is 11 mg/day (Connie and Christine, 2009). The Permissible limit for Zinc in milk is 0.3–1 mg/l (According to Codex Alimentarius Commision).

Due to the above mentioned problems, there is a foremost requirement to develop greatly selective, rapid, sensitive and reliable method to analyse the concentration of zinc metal ions in various samples which are causing severe problems by entering in to the ecosystem more than the required level through different sources. As milk is the main source of daily diet for infants and adults, so milk was selected for this particular study. Zinc can be transferred from animal feed to food produced by animals like milk and other dairy products and enters the food chain results in severe health effects to the consumers.

Analysis of a the metals could be done by using several conventional techniques like Atomic absorption spectrophotometry (AAS), Differential pulse polarography, Electrothermal AAS (ETAAS), Differential pulse cathodic stripping voltammetry

* Corresponding author.
E-mail addresses: Verma.neelam2@gmal.com (N. Verma), anandgagandeep@yahoo.in (G. Kaur).

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(DPCSV), Inductively coupled plasma mass spectrometry (ICP-MS), Dynamic reaction cell inductively coupled plasma mass spectrometry (DRC-ICP-MS) and Electrochemical metal analyzer (Verma and Singh, 2005). To perform these techniques, there is a requirement of highly experienced and trained lab operators. Sample pre- treatment causing the process to be very much expensive and time consuming (Moyo, 2014). Biosensors are gaining importance and popularity over conventional analytical techniques because of specificity, low cost, miniaturization of sample, fast response time, portable ease of use. Biosensors are analytical tools consisting of an immobilized biological material (enzyme, antibody, whole cell and organelle) in intimate contact with transducing material which converts biochemical signal in to quantifiable electronic signal proportional to the concentration of the analyte present. These are considered to be useful and reliable tools for clinical and food analysis of compounds within short time (Verma et al., 2007).

In the present work, we have made an effort to develop specific biosensors which can detect zinc ions in water and milk samples by using zinc fingers as a biocomponent, immobilized in a sort of semisolid matrix, which can provide a better environment for proper folding of the peptide in the presence of zinc ions. Keeping this view in mind, polyacrylamide gel along with a fluorescent indicator acridine orange has been used in the present study. Zinc finger peptide does not has inherent signal transduction machinery; hence, a hydrophobic fluorescent dye has been incorporated to get the signal, which fluoresces in the hydrophobic core formed after folding of the peptide in the presence of zinc ions. To best of our knowledge, following zinc finger with a specific sequence has not been used for the zinc biosensor. The strategy for immobilization in polyacrylamide gel matrix of this particular bio component along with a fluorescent dye has not been experimented yet for sensing of zinc ions in milk samples. The main purpose of our work is to fabricate a specific biosensor for zinc ion detection, with very low detection limits, longer storage stability and lower response time. This would further be useful to monitor zinc ions concentration in various food samples.

2. Experimental works

2.1. Material used

All the chemicals and reagents used in the study were of analytical grade. Zinc fingers peptide was procured from Link Biotech New Delhi, immobilized on to the micro well plates (48-wells) from Axygen. Fluorescent measurements were done by using mini-opticon with a fluorescent indicator (acridine orange) in a micro-well plate.

2.2. Biocomponent

Purified synthetic Cys_2His_2 Zinc Finger Peptide (Apo form) having sequence with 26 amino acids **(Ac-PYKCPECGKSFSQSSNLQKHQRTHTG-Am)** (Fig. 1A) is used as a bio component (Yana et al., 2010). Acetyl group is attached at N terminal of the sequence and amide group is attached to the C-terminal of the sequence. The main zinc-binding residues shown in green are conserved cysteines, which contain thiol, and in blue are conserved histidines, which contain imidazole. The orange color residues showing phenylalanine and leucine form a small hydrophobic core which coordinate Zinc metal ion (Zn^{2+}) and drive the folding of the polypeptide chain.

Bioassay principle relies on the change in fluorescent count as a result of folding of zinc finger peptide in the presence of zinc ions. Acridine orange, a hydrophobic dye, used in the assay, moves in to the vicinity of hydrophobic core of the peptide formed after folding and dye becomes strong emitter in the hydrophobic environment (Fig. 1B).

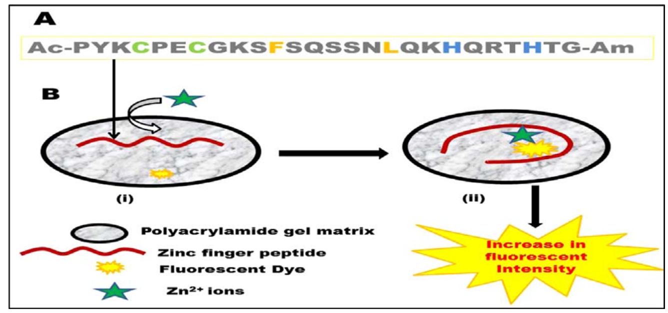

Fig. 1. (A) Zinc Finger Peptide sequence. (B) Schematic diagram showing the sensing mechanism of the presented biosensor (i) Unfolded Zinc finger peptide immobilized in polyacrylamide gel along with fluorescent dye. (ii) Folded zinc finger peptide in the presence of zinc ions forms a hydrophobic core and dye becomes a strong emitter causing increase in fluorescent intensity.

2.3. Association of reaction components

The reaction mixture was miniaturized to 40 μl, having 20 μl of acridine orange dye (0.2 μg/μl), 10 μl of Zinc finger peptide (0.1 μg/μl) and 10 μl of immobilized matrix (0.2 μg/μl) after optimization of experimental variables, (i) choice of suitable matrix, (ii) effect of concentration of matrix, (iii) incubation time, and effect of concentration of zinc ions were also investigated by incubating the reaction mixture at 30° C in the micro well plate and change in fluorescent intensity was measured by using mini opticon as a transducer.

3. Results and discussion

A fluorescent based biosensor has been developed for zinc ion detection, in the present study. Characterization of bioassay principle is explained in Section 3.1. Optimization of various parameters affecting the fluorescent response is discussed in Section 3.2. Regeneration properties and stability of the zinc finger peptide in the biosensing system is investigated in Section 3.3. Selectivity of biosensor over other metal ions has been checked in Section 3.4. and application of the developed biosensor in milk samples is described in Section 3.5.

3.1. Characterization of bioassay principle

In the microwell plate, the zinc finger peptide (0.1 ug/μl), acridine orange dye (0.2 ug/μl) and zinc ions (0.001–1 ug/l) were added in the ratio of 1:2:1. The fluorescent intensity was noted down at different concentration of zinc ions to confirm the bioassay principle. It was investigated that increase in fluorescent response is related to the increase in folding of zinc finger peptide, proportional to the zinc ion concentration from 0.001 to 1 ug/l (Fig. S1). Therefore, based on this characteristic, we were able to develop a fluorescent based biosensor. The folding of zinc finger peptide in the presence of zinc ions is well explained before (Yana et al., 2010; Jiang and Guo, 2004).

3.2. Optimization of variables affecting experimental parameters

Effect of different experimenting variables has been studied to optimize the reaction conditions and fluorescent response was measured.