A PRELIMINARY INVESTIGATION ON A HISTAMINE BIOSENSOR CONSTRUCTED FROM DIAMINE OXIDASE IMMOBILISED ONTO AN OXYGEN PROBE

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Abstract

A histamine biosensor consisting of diamine oxidase (DAO) and a conventional oxygen electrode transducer was developed and applied for the determination of standard histamine solutions. Two different approaches for the histamine biosensor design were studied, i.e. the enzyme DAO was directly immobilized on the surface of the oxygen electrode membrane using glutaraldehyde or entrapped in a hydrogel film. For immobilisation with glutaraldehyde, the enzyme was cross-linked with glutaraldehyde as a bifunctional reagent on the electrode surface. For entrapment, DAO was entrapped in a polymeric hydrogel film, i.e. poly(hydroxyl ethyl methacrylate) (pHEMA) polymer and deposited onto the teflon membrane of the oxygen electrode. The response of the histamine biosensors with immobilized DAO showed good linear correlation between the changes of oxygen level with changes in concentration of histamine at both high concentration ranges (200-1000 mg/L) and low concentrations (20-100 mg/L). However, at high concentration range of histamine, the sensitivity of the biosensor response decreased for the direct DAO immobilisation with glutaraldehyde.

Abstrak

Biosensor untuk histamin mengandungi enzim diamin oxidase (DAO) dan elektrod oksigen biasa sebagai transduser telah dikembang serta digunakan untuk penentuan larutan piawai histamin. Dua rekabentuk biosensor histamin telah dikaji, iaitu enzim DAO dipegunkan secara langsung di permukaan membran elektrod oksigen menggunakan glutaraldehid atau ia diperangkap dalam filem hidrogel. Untuk pemegunan secara glutaldehid, enzim ditaut silang melalui kumpulan dwifungsi reagen glutaraldehid pada permukaan membrane elektrod. Untuk cara pemerangkapan, DAO dipegunkan dalam filem polimer hidrogel, iaitu poli(hidroksi etil metakrilat) (pHEMA) yang disalutkan di atas membran teflon elektrod oksigen. Rangsangan biosensor histamin dengan DAO terpegun menunjukkan korelasi linear yang baik antara perubahan oksigen yang diukur dengan perubahan kepekatan histamin pada kepekatan tinggi (200-1000 mg/L) dan kepekatan rendah (20-100 mg/L). Walaupun demikian, pada kepekatan histamin yang tinggi, kepekaan terhadap histamin menurun untuk biosensor yang melibatkan pemegunan yang terus DAO dengan glutaraldehid.

Introduction

Biogenic amines are aliphatic, alicylic and heterocylic organic bases of low molecular weight. These substances are ubiquitous in biological matrices. They are not only biosynthesized in animal and plant cells but also produced by microbial decarboxylation of amino acids. The amount and type of amine formed is therefore strongly influenced by the food composition, microbial flora and by several parameters, which allow bacterial growth during food storage, such as food treatment prior to storage, food additives, temperature, moisture, ripening and packaging [1]. To estimate bacterial spoilage, biogenic amines, especially putrescine, cadaverine and histamine, have been confirmed as useful chemical indicators [2]. The

most frequent intoxication caused by biogenic amines involves histamine. Histamine is formed mainly through decarboxylation of histidine by exogenous decarboxylase released by microflora associated with the fish or salt-water environment. Histamine poisoning is known to be associated with the consumption of fish such as tuna and sardines [1].

Analytical determination of biogenic amines is usually carried out by HPLC methods. Almost all proposed methods use indirect detection following precolumn derivatisation to form dansyl (DNS) derivatives [2,3,4], benzoyl derivatives [5] or o-phthalaldehyde (OPT) derivatives. Recently a method based on ion exchange chromatography with integrated pulsed amperometric detection (IC-IPAD) for simultaneous determination of underivatized biogenic amines was reported [6]. However, chromatographic methods are generally complicated and require long analysis time and expensive instrumentation. Biosensors such as electrochemical enzyme probes, based on oxygen electrodes using monoamine oxidase (MAO) and diamine oxidase (DAO) were developed for biogenic amine detection [7,8]. Other biosensors that utilized plant tissue was also reported [9].

Biosensor provides a rapid and simple means of biogenic amine detection without the need of complicated sample pretreatment procedures. However, the successful of a biosensor construction depends heavily on the enzyme immobilisation technology [10]. Immobilisation not only allows the enzyme to be in close contact with the transducer but also helps in stabilising the enzyme for repeating usage. Enzyme immobilisation can be performed directly on the transducer or in most cases, via a membrane, which can subsequently be attached on the transducer. Enzymes can be immobilised either through adsorption, entrapment, covalent binding, cross-linking or a combination of all these techniques [6, 11, 12].

DAO is a deaminating and copper containing enzyme (oxidoreductase, EC 1.4.3.6), which consumes oxygen during reaction with amines. This enzyme has been found in a variety of microorganisms, plants and animals [13]. In the presence of dioxygen, DAO catalyses the oxidative deamination of primary amines, diamines and substituted amines to produce aldehyde, ammonia and hydrogen peroxide [14]. The enzyme is a homodimer of 60-105 kDa containing tightly bound copper and 6-hydroxy dopa (TOPA), a carbonyl cofactor [15].

In this paper, we have explored the used of DAO coupled with oxygen electrode to construct biosensors for the rapid detection of histamine, i.e. a biogenic amine. Two approaches for the construction of biosensors were attempted: DAO was either immobilised in a polymeric membrane via entrapment or direct cross-linking with a bifunctional reagent onto the Teflon membrane of an oxygen electrode. A brief evaluation of the response of these biosensors to histamine solutions was carried out and the results were compared with that of free or non-immobilized DAO.

Materials and Methods

Reagents

The reagents used in the experiment were as follows: Histamine dihydrochloride (Sigma, H7250), diamine oxidase (DAO) (Sigma D7876), phosphate buffer (0.1M, pH 7.0), poly(hydroxyl ethyl methacrylate) (PolyHEMA), glycine and glutaraldehyde (Sigma-Aldrich).

Procedures

Reaction of free (non-immobilised DAO) with histamine. Aqueous standard histamine solutions of histamine dihydrochloride in deionised water were prepared at concentrations of 0-1000 mg/L for the evaluation of the enzyme response. In the reaction chamber, 6mg/mL of DAO was added to a 0.1M phosphate buffer solution (pH 7.0) and various concentrations of histamine standard solutions were then added and allowed to mix. The change of oxygen was recorded after 10 min with a Clark oxygen electrode system consisted of accessories such as a reaction chamber and a chart recorder (Lab-Environment Instruments).

*Biosensor with DAO immobilisation by cross-linking method***.** The immobilization procedure was based on Tombelli and Mascini [16] with slight modification. DAO (3mg) was dissolved in 50μL of 0.1M phosphate buffer and 5μL of this solution was mixed in 3μL of 2.5% glutaraldehyde solution. The solution was then coated on the teflon membrane of the oxygen electrode and dried at room temperature for 30 min. It was then washed with 0.1M glycine solution for 30 min. The oxygen electrode was then exposed to various concentrations of histamine (0-1000 mg/L) and any change in oxygen level was then measured.

*Biosensor with DAO immobilisation by entrapment in pHEMA membrane***.** A volume of 50µl DAO enzyme solution (0.5mg DAO in 0.1M phosphate pH 7.0) was mixed with a 1 mL of 1% solution of pHEMA in a 4:1 water:dioxane mixture. And 20µL of this solution was then coated onto the oxygen electrode membrane and allowed to dry at 4°C for 24 h. After the pHEMA membrane formed a film, the oxygen electrode was then immersed in histamine standard solutions of range from 0-1000 mg/L. The changes of oxygen level of the solution were then recorded.

Results and Discussion

In the presence of DAO enzyme, histamine is converted to various products with the consumption of oxygen according to the following equation:

$$RCH_2NH_2 + O_2 + H_2O \xrightarrow{(DAO)} RCHO + NH_3 + H_2O_2$$

When the concentration of histamine increases, the amount of oxygen consumes will be higher and thus a larger difference in the reduction of oxygen (in arbitrary unit) will be observed. This is observed and shown in Figures 1 and 2, where the increase in the level of histamine leads to a larger difference in oxygen change because of higher consumption of oxygen has taken place.

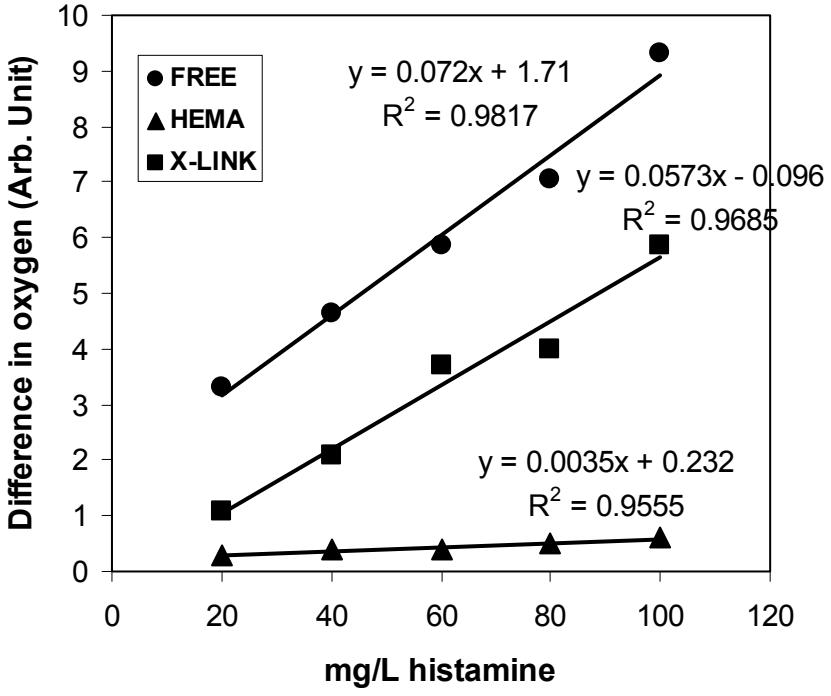

Figure 1. The changes of oxygen level for free DAO in solution and immobilised by different procedures in the presence of a low range of histamine from 20-100 mg/L (in 0.1 M phosphate buffer, pH 7)

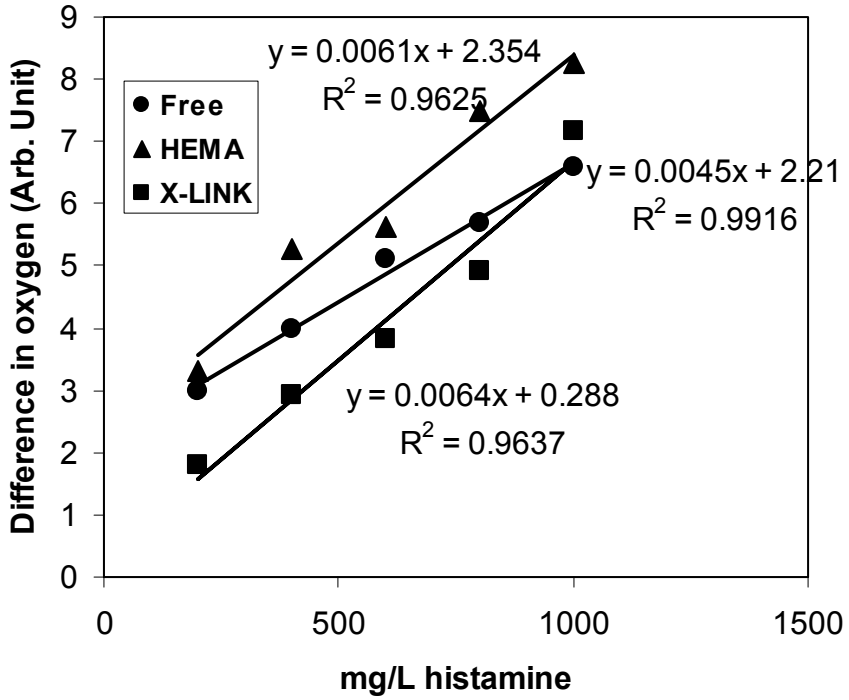

Figure 2. The changes of oxygen level for free DAO in solution and immobilised by different procedures in the presence of histamine at a high concentration range of 200-1000 mg/L (in 0.1 M phosphate buffer, pH 7)

Both approaches of biosensor construction yielded linear response ranges to histamine from 20-100 mg/L and 200-1000 mg/L. The linear response range of the biosensors is similar to that of the non-immobilized (free) DAO at all histamine concentrations investigated. For the direct immobilization procedure, although the responses of the biosensor are linear even at higher concentrations of histamine, i.e. from 200-1000 mg/L, the sensitivity has decreased considerably when compared to low histamine concentration range (Table 1). In comparison, the reduction in sensitivity at high levels of histamine is more pronounced for the free DAO. Such a reduction in the response of the biosensor at high level of histamine (>10,000 mg/L) is attributed to the inhibitory effect of the enzyme by histamine and this was reported for both free and immobilized DAO [17].

Table 1. A comparison of the sensitivity of DAO after various ways of immobilization and the non-immobilized (free DAO) when response to different ranges of histamine concentrations.

Enzyme immobilization	Sensitivity slope (ΔO_2/unit concentration)	
	20-100 mg/L	200-1000 mg/L
HEMA	0.0035	0.0061
Glutaraldehyde (X-LINK)	0.0573	0.0064
FREE	0.0720	0.0045

On the contrary, even though both free and direct immobilized DAO demonstrated reduction in sensitivity with higher concentrations of histamine but DAO immobilized in pHEMA membrane demonstrated an increase in sensitivity by almost two times at higher concentrations of histamine (from 200-1000 mg/L) (Table 1). The entrapment of DAO in the pHEMA hydrogel membrane may have limited the direct contact of the enzyme with bulk histamine concentration, thus less histamine in the solution is accessible to the entrapped DAO and this has avoided the inhibitory effect by histamine at higher concentration. In the case of DAO immobilized directly by glutaldehyde method, the enzyme is mostly covalently bound onto the surface of the teflon membrane and therefore more accessible to the bulk histamine concentration.

The direct immobilisation technique using glutaraldehyde gave higher sensitivity when compared to immobilisation in pHEMA membrane. The sensitivity of the direct immobilization procedure is similar to that of the free enzyme, which indicates that direct immobilisation procedure has no clear effect on the DAO activity. The reduced response of the histamine biosensor constructed from pHEMA membrane may be attributed to inaccessibility of histamine to the immobilized DAO because of diffusion barrier in the polymer matrix or it may be due to the slow leaching of the enzyme out of the membrane since DAO is not covalently attached to the matrix. Loses of the immobilised enzyme from pHEMA membrane will lead to lower response characteristics of the biosensor [18].

Direct immobilization of DAO using glutaraldehyde as activator on Immunodyne membrane [17] and glass beads [19] have been reported. Immobilization of DAO on glass beads with amperometric detection of hydrogen peroxide resulted from reaction with histamine demonstrated linear response range of 55 – 430 mg/L histamine. Above 500 mg/L of histamine, the sensitivity of the biosensor diminished [19]. This result is similar to the direct immobilization of DAO with glutaradehyde and oxygen detection adopted in this work.

Conclusion

This preliminary investigation on the use of immobilized DAO as a biosensor for histamine based on oxygen electrode transduction demonstrated that histamine can be detected after the enzyme was immobilised directly onto the teflon membrane of the oxygen probe or entrapped in a pHEMA hydrogel. The use of pHEMA hydrogel improved sensitivity at higher concentrations of histamine when compared to direct enzyme immobilization with glutaraldehyde, where the sensitivity had decreased when the histamine concentration increased. This work has shown that pHEMA can be a useful matrix for the analysis of histamine over a wide concentration range without a reduction in sensitivity. Research is currently in progress to improve the property of the pHEMA matrix so that the sensitivity of the biosensor to low level of histamine can be increased.

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