cytochrome cd_1 함유 솔젤을 이용한 nitrite 이온 센서

sol-gel containing cytochrome cd_1 nitrite reductase for the optical nitrite sensor

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1. Introduction

Currently, nitrite is commonly measured spectrophotometrically using the Griess reaction which involves the production of an azo-dye by reacting by reacting sulfanilamide and *N*-(1-naphthyl)ethylenediamine with nitrite.[1] In addition to the Griess reaction, quantitative analysis of nitrite can be performed using UV/Visible spectrophotometry, fluorimetric assays, chromatography and electrochemistry.[2] However, all of the current methods require sample pretreatment, measurements are subject to interferences and the techniques used are not all conductive for *in situ* measurements. In this work, sol-gel glasses containing cytochrome cd_1 nitrite reductase were prepared and investigated their nitrite sensing properties.

2. Experimental, Results and Discussion

The cytochrome cd_1 was readily entrapped within a sol-gel monoliths. Different pore sized sol-gel were examined to established the degree of entrapment and the subsequent leaching of the enzyme from the monolith. The pore size of a sol-gel is dependent upon the water/silica sol ratio (*r*-value) used, with larger pores being obtained with increasing water concentration. It was established that a sol-gel with an *r*-value of 15 achieved the optimum entrapment of the cytochrome cd_1. Using such a sol-gel, it was determined that 79% of the initial concentration of the cytochrome cd_1 was entrapped while 13% of this enzyme concentration leached from the sol-gel during the ageing period of 1 week.

Redox spectra of the encapsulated cytochrome cd_1, obtained using dithionite as the reductant, were found to be identical to those obtained in solution, *i.e.* In order to establish the nitrite biosensing capabilities, the encapsulated cytochrome cd_1 was challenged to increasing concentrations of nitrite. Fig. 1 shows the changing absorption spectrum as the encapsulated cytochrome cd_1 is re-oxidized by the nitrite anion. These spectral changes are similar to those observed for the binding of nitrite ions to cytochrome cd1 in solution. In order to achieve the optimum nitrite detection limit for the encapsulated enzyme the changes of intensity of the absorption band due to the d1 haem, i.e. 460 nm, were monitored. A calibration curve for the decrease in absorption intensity, measured at 460 nm, as a function of nitrite concentration was linear between 0.075 - 1.250 μM. The calibration curve is represented by:

At 460 nm: A = 0.0363 - 0.0079 X $[NO_2^-]$ (r^2 = 0.920)

The limit of detection for nitrite was determined to be 0.075 μM with a sensitivity of 7.9 nM^{-1}. A potential limitation of the use of bulk sol-gel monoliths for the encapsulation of enzymes is the time taken for analytes to diffuse through the gel and for an equilibrium response to be achieved. In this study the monolith was *ca.* 1.5 mm thick and an equilibrium response took approximately 30 min. In order to overcome this possible limitation a sol-gel sandwich configured thin film was also investigated.

3. Conclusions

In this work, bulk sol-gel monoliths containing cytochrome cd1 nitrite reductase were prepared and investigated their nitrite sensing properties.

The cytochrome cd1 has been encapsulated in a bulk sol-gel monolith with no structural changes observed and retention of enzymatic activity. The most appropriate wavelength for the detection of nitrite was 460 nm. The detection of nitrite ions in the range 0.075 - 1.250 μM was achieved, with a limit of detection of 0.075 μM.

References

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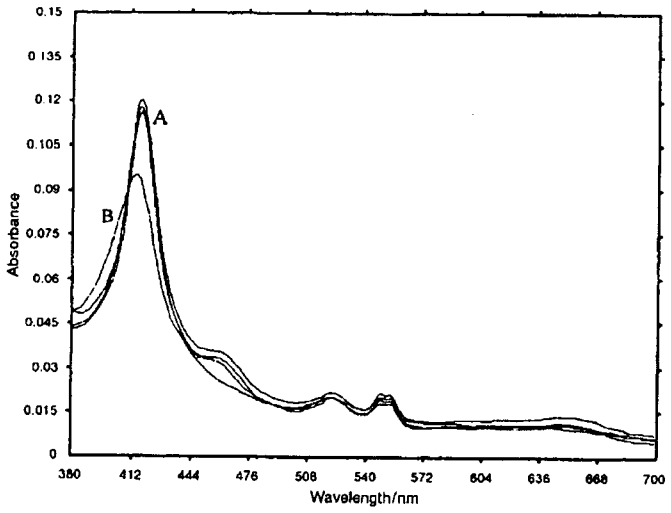

Fig. 1 UV/Visible absorption spectra of the (A) reduced and (B) reoxidized cytochrome cd1 nitrite reductase encapsulated in a bulk sol-gel monolith following the addition of nitrite ions.