Fiber-optic glucose biosensor based on glucose oxidase (GOD) immobilised in a silica gel matrix

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Intrinsic fluorescence of silica gel immobilised glucose oxidase (GOD) was investigated in the UV and visible range by performing steady-state and time-course measurements. A glucose optical biosensor was developed and characterised using as catalytic element GOD immobilised on a gel disk bound to a bundle of optical fibres.

1 Introduction

In this paper, we report results obtained from a biosensor comprised of a monolithic silica sol–gel entrapping glucose oxidase (GOD) as sensing element coupled to a fibre-optic transducer element. GOD intrinsic fluorescence spectra were analysed in the UV and visible region in presence of different glucose concentrations, and the corresponding calibration curves were obtained. In addition, time course measurements were acquired following the procedures presented in previous works [1, 2]. Physicochemical and biochemical characterizations of our sol–gel are also reported.

2. Materials and methods

GOD is a typical flavoprotein. GOD from Aspergillus Niger is a dimer with two tightly bound flavoadenine (FAD) molecules per dimer. In the UV region, GOD displays a maximum absorption at 275 nm and a maximum intrinsic fluorescence emission at 340 nm due to the tryptophan residues. As all flavoproteins do, GOD displays absorption maxima in the visible region at about 380 and 450 nm and an intrinsic fluorescence emission maximum at about 530 nm at pH 6.5. As previously reported [1,2] changes in the UV and visible fluorescence of free and immobilised GOD have been found during its interaction with glucose because oxidised and reduced flavines exhibit different fluorescence intensities. Silica gel matrices were prepared as reported in ref. 3. For spectrofluorimeter measurements the resulting gel was a 8x35x3 mm parallelepiped in size, while a circular mould (3 mm in radius and 3 mm in thickness) was used as the bioactive element. The biosensor was constituted by a 0.5 m long Y-bundle (CeramOptec, GmbH, Germany) with 120 silica fibres in a circular arrangement. The bundle was split into two arms 0.2 m from one end, each containing half of fibres. All the ends were polished, and the bundle was covered with a flexible black insulator.The emission fluorescence spectra were collected with a spectrofluorimeter (Perkin–Elmer, model LS55). In the UV range, samples were excited at 275 nm, while the emission spectrum was recorded between 310 and 410 nm. In the visible range, samples were excited at 450 nm and emission spectra were considered between 500 and 580 nm. For analytical purposes, the emission fluorescence spectrum was treated as the size of the peak at 330 nm (530 nm) or as size of the integral area under the spectrum in the region 310–410 nm (500–580 nm) for the UV (visible) range. UV fluorescence time course measurements were also performed following changes in fluorescence measurements during the enzymatic reaction [3]. As a baseline, the initial fluorescence emission of immobilised GOD at 330 nm was measured (excitation wavelength equal to 275 nm). After the addition of glucose solution at different concentrations, temporal changes in fluorescence intensity were monitored. The glucose concentration was determined by t_{app} and the linear slope of the intensity signal rise (dI/dt). To test biosensor performance, we used a Xenon lamp source (Mod. PX2-Ocean Optics Ltd), a monochrometer (Mod. USB 2000, Ocean Optics Ltd.) for selecting appropriate wavelength and a photomultiplier tube (Mod.R298, Hamamatsu, Japan).

3 Results and discussion

In Fig. 1a the peak intensities of the emission spectra of entrapped glucose oxidase in the UV region are reported as a function of glucose concentration. In fig.1b the integral area values of the intrinsic fluorescence emission in the range 300 – 380 nm are reported as a function of glucose concentration. The data show a Michealis-Menten type behaviour and optokinetic parameters can be extracted as in ref. 3. Similar results are obtained for emission fluorescence in the visible range.

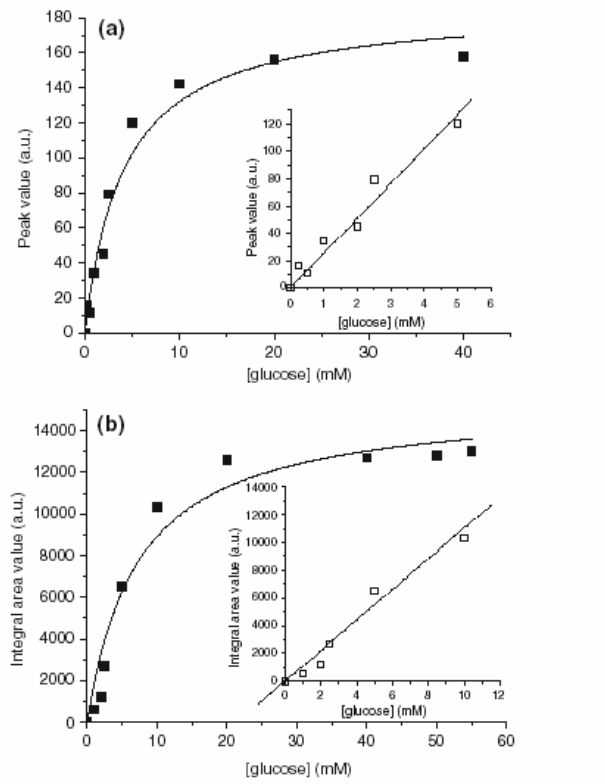

Fig. 1 *UV range:* ***a*** *Fluorescence emission peak (****b*** *Fluorescence emission integral area) as a function of glucose concentration. Insest: glucose calibration curve.*

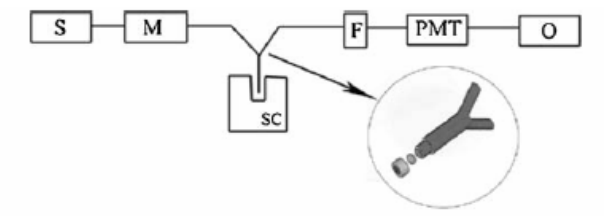

Fig. 2 *Fluorescence apparatus for fiber optic biosensor testing (S source, M monochromator, F optical filter, PMT photomultiplier, O oscilloscope, SC sample cell). Inset: biosensor schematic*

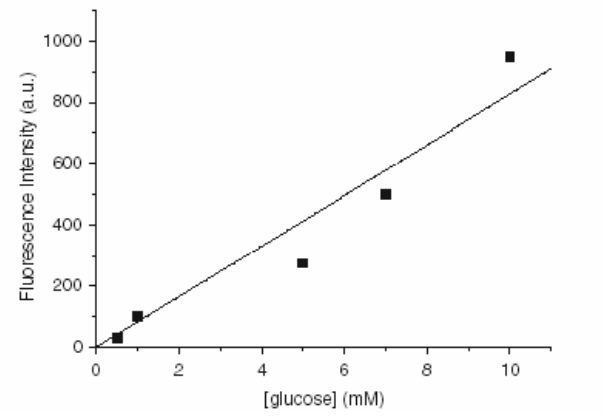

Fig. 3 *Glucose calibration curve obtained with the biosensor represented in this figure.*

Using time course measurements [2,3] the dynamic change in fluorescence can be used to get large linear calibration range. This preliminary investigation made us confident that a useful biosensor could be constructed using optical fibres interfaced with a properly designed catalytic matrix. Figure 3 portrays a schematic of the set-up for testing the optical fiber biosensor utilising our catalytic silica matrix. The bioactive element contained a disk of our catalytic gel (6 mm in diameter and 3 mm in thickness) bound to a bundle of optical fibres with a screw cup. One end of the Y bundle was connected to the excitation source equipped with a monochromator, and the other end was connected to the detector. An optical filter (Melles-Griot, France) and a lens focusing system were placed between the end connected to the detector and the entrance to the PMT housing. The optical fibre bundle and detector allowed us to perform fluorescence measurements in the visible region. We excited the system at 450 nm, and the filter allowed us to detect fluorescence in the 500–580 nm range. The sensing end of the biosensor was in contact with a cuvette containing glucose solutions placed in a modified holder (Mod.CUV-ALLUV 4-way, Ocean Optics-Ltd). The detector output signals were sent to an oscilloscope.

4 Conclusions

Changes in intrinsic UV and visible fluorescence were used to obtain linear calibration curves for glucose concentration determination. The same approach was used for time course measurements. When time course measurements were considered, the silica support allowed us to obtain larger linear calibration ranges and higher sensitivity than those reported for other supports. Using the results of these investigations, a glucose optical biosensor was designed and tested.

4 References

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