

Enzyme-Based Fiber Optic Sensor

Sir: Fiber optic chemical sensors have been reported for numerous ionic and nonionic substances. Reviews by Peterson (1) and Seitz (2) have summarized the recent advancements in fiber optic sensor development. For the most part, fiber optic sensors reported to date are based on light intensity changes owing to alterations in an immobilized indicator material. The purpose of this brief correspondence is to report a novel type of fiber optic chemical sensor in which an isolated enzyme is immobilized at the surface of a bifurcated optical fiber bundle. Response of the resulting biosensor is based on directly measuring the enzymatic generation of a spectrophotometrically detectable product. The feasibility of this approach to biosensors is demonstrated with a model system which employs alkaline phosphatase (EC 3.1.3.1) as the immobilized enzyme, *p*-nitrophenyl phosphate as the substrate, and *p*-nitrophenoxide as the detected product.

EXPERIMENTAL SECTION

Apparatus and Reagents. Figure 1 shows a block diagram of the optical fiber biosensor arrangement. The light source is a 100-W quartz halogen tungsten lamp in conjunction with a constant voltage transformer, Oriol Model 6393. Light from this source passes through a neutral density filter to cut down the incident intensity before being focused onto the input fiber bundle using an Oriol Model 77800 fiber optic input assembly. The incident radiation is transported through one arm of the bifurcated bundle (Oriol Model 77533). Back scattered radiation is then transported through the other arm of the cable to a detecting system which is composed of a collimating beam probe (Oriol Model 77652), a narrow band-pass filter, and a UV-visible photomultiplier tube (Oriol Model 77761). A constant voltage of 502 V is supplied to the photomultiplier by an Oriol Model 7070 photomultiplier readout device which also measures the intensity current. The readout device is equipped with an ambient light suppressor; however, measurements described here were performed with a lighttight housing around the probe and sample cell in order to avoid problems of ambient light intensity changes. The output from the readout device is displayed on a strip chart recorder. The common end of the bifurcated optical fiber bundle has a bundle diameter of 4.5 mm and an overall probe diameter of 6.4 mm. Finally, the bifurcated cable is composed of glass fibers which are randomly distributed at the common end of the bifurcated bundle.

All solutions were prepared with distilled, deionized water which was obtained by treating house distilled water with a Milli-Q three house water purification unit. Alkaline phosphatase (type VII-S), *p*-nitrophenoxide, *p*-nitrophenyl phosphate, and 2-amino-2-methyl-1-propanol (AMP) were purchased from Sigma Chemical Co., St. Louis, MO. The nylon mesh was from Small Parts, Inc., Miami, FL.

Procedures. Figure 2 shows a schematic diagram of the optical fiber biosensor configuration. The sensor is composed of two sheets of nylon mesh held at the common end of the fiber bundle. The enzyme is immobilized on the inner nylon membrane and the outer membrane is present to scatter the incident radiation.

Alkaline phosphatase has been immobilized by covalent attachment to the nylon. The nylon was altered to provide a suitable functional group using the procedure of Hornby and Morris (3) as adapted by Mascini and co-workers (4). In our experiments, a small piece of nylon webbing with a pore size of 5 μm was secured around a glass rod using nylon thread. This nylon net was placed in a simmering dimethyl sulfate solution and was allowed to sit for 5 min. The treated nylon net was then immersed into a test tube which contained freshly distilled, anhydrous methanol for 30-40 s followed by a second immersion into fresh anhydrous methanol for 1 min. Following this treatment, the nylon was released from the rod, and placed into a 50-mL solution of 0.5 M lysine, pH 9.0, for 2 h. The net was then washed completely with a 1.0 M sodium chloride solution. At this point a small portion of the membrane was tested for the presence of an amine group by placing a small amount of 2,4,6-trinitrobenzenesulfonic acid on the membrane and observing the formation of the

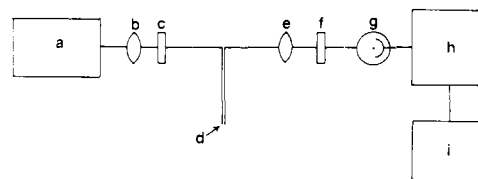


Figure 1. Block diagram of optical fiber biosensor arrangement: (a) light source; (b) focusing lens; (c) neutral density filter; (d) biosensing tip; (e) collimating lens; (f) narrow-band-pass filter; (g) photomultiplier tube; (h) photomultiplier readout; and (i) strip chart recorder.

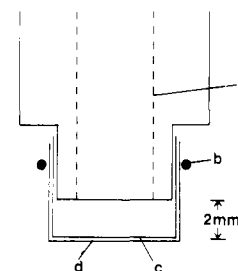


Figure 2. Configuration of *p*-nitrophenyl phosphate biosensor: (a) common end of bifurcated bundle; (b) retaining O-ring; (c) inner nylon mesh with enzyme; (d) outer nylon mesh (not drawn to scale).

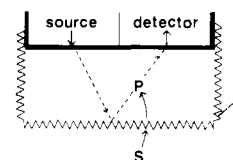


Figure 3. Schematic of processes occurring at biosensing tip: (a) enzyme/scatter layer; (S) enzymatic substrate; (P) light absorbing product.

characteristic yellow-orange color. Next the entire membrane was immersed for 45 min in a 12.5% glutaraldehyde solution which contained 0.1 M borate, pH 8.5. Following a second wash with 0.1 M sodium chloride, the treated net was placed in an alkaline phosphatase solution which was composed of 0.1 M phosphate, pH 7.0, with approximately 100 IU of enzyme overnight at 4 $^{\circ}\text{C}$.

Response curves to be reported here were obtained by placing the sensing surface of the optical fiber biosensor into a set volume of buffer, recording the final steady-state intensity, adding a known volume of the appropriate standard to the buffer, and again recording the steady-state intensity. Standard additions were continued and intensity levels recorded until the entire concentration range had been covered. Absorbance at each concentration was calculated as the logarithm of the ratio of the initial intensity (i.e., the incident value) and the intensity of interest ($\log(I_0/I)$).

The buffer consisted of 1.0 M 2-amino-2-methyl-1-propanol (AMP), pH 10.4, with 1.0 mM magnesium chloride. This buffer was employed owing to the previous studies regarding optimal conditions for the action of alkaline phosphatase on *p*-nitrophenyl phosphate (5).

RESULTS AND DISCUSSION

The basic design of the optical fiber biosensor involves an enzyme and some type of light scattering material held at the common end of a bifurcated fiber bundle. As shown in Figure 2 the light scattering material in this sensor is a nylon membrane. Incident radiation is transported to the enzyme/scattering layer interface through one fiber bundle. This light is scattered by the nylon mesh and a fraction of the scattered light is carried to the detector system through the other fiber bundle where the light intensity is monitored.

Figure 3 shows schematically the processes which occur at the optical fiber surface. As the enzymatic substrate diffuses from the bulk solution into the enzyme layer, the enzymatic reaction is catalyzed and the spectrophotometrically detectable

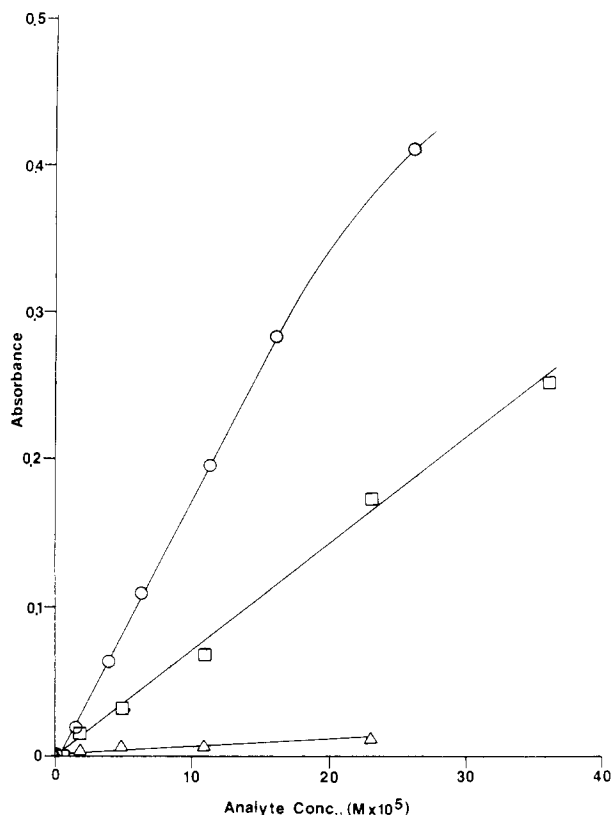
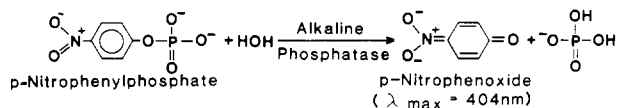


Figure 4. Response of optical fiber biosensor without enzyme to (O) *p*-nitrophenoxide and (Δ) *p*-nitrophenyl phosphate, and with enzyme to (□) *p*-nitrophenyl phosphate.

product is generated. This product will absorb a particular fraction of the back scattered radiation; hence, a decrease in light intensity is observed as product builds up. When the rate of product generation is exactly counterbalanced by the rate of product diffusion away from the optical fiber surface, a steady-state product concentration will be established and a corresponding steady-state absorbance value will be attained. A linear relationship is anticipated between the bulk substrate concentration and the steady-state absorbance value as long as the kinetics of the catalyzed reaction are first order with respect to the substrate. This condition is maintained when the substrate concentration is sufficiently below the effective K_M value for the immobilized enzyme (6).

For these feasibility studies alkaline phosphatase has been selected as the immobilized enzyme because this biocatalyst is well characterized biochemically, relatively stable, and readily available. *p*-Nitrophenyl phosphate has been employed as the model substrate because it is an excellent substrate for this enzyme and because the reaction product, *p*-nitrophenoxide, has a large molar extinction coefficient (5). The catalyzed reaction involved is shown below. The



wavelength of maximum absorbance for *p*-nitrophenoxide is 404 nm; hence, a 404.7-nm narrow-band-pass filter has been positioned immediately before the photomultiplier so that the light intensity at this wavelength is selectively monitored. The narrow-band-pass filter has a 10-nm band-pass at half intensity.

Figure 4 shows the results of these feasibility studies with the *p*-nitrophenyl phosphate biosensor. As predicted, linear relationships are observed between the measured absorbance

($\log(I_0/I)$) and the concentration of the species of interest. The circles show the response observed for the enzymatic product, *p*-nitrophenoxide, when two 5 μm pore size nylon membranes are employed without the enzyme. The triangles show the response of this non-enzyme-containing biosensor to the enzymatic substrate, *p*-nitrophenyl phosphate. It can be seen that little response is observed in the absence of immobilized alkaline phosphatase. Finally, the squares show the response to *p*-nitrophenyl phosphate by the biosensor with alkaline phosphatase immobilized on the inner nylon membrane. The excellent linearity of this latter curve is quite encouraging and clearly demonstrates the feasibility of the proposed approach to biosensors. Moreover, the range of linearity of this response curve extends well over 1 order of magnitude which is better than that of previously reported fiber optic sensors (1, 2). Previous systems have depended on monitoring the change in light intensity owing to a change in an indicator concentration. This biosensor system, however, relies on directly measuring the build up of a colorimetric species which allows for the extended linear range.

The slope measured for the response to *p*-nitrophenoxide is considerably lower than that anticipated when a 0.4 cm path length (see Figure 2) and a molar extinction coefficient of 19050 $\text{L mol}^{-1} \text{cm}^{-1}$ (5) are used. Calculations give an expected slope of 7680 L mol^{-1} ; however, a least-squares estimate of the slope for the curve shown in Figure 4 reveals a value of only $1713 \pm 168 \text{ L mol}^{-1}$. In addition, the slope of the *p*-nitrophenyl phosphate curve has been estimated to be $732 \pm 14 \text{ L mol}^{-1}$ which is lower than that for *p*-nitrophenoxide. One factor which helps to explain the difference in slopes between the substrate and product is the variation in the inner nylon mesh. After alkaline phosphatase immobilization, the inner nylon mesh has a different appearance and most likely different scattering properties. Thus, the effective path lengths of the two sensors are most likely different which should account for some of the variation. Other factors which might affect the response slope include immobilized enzyme concentration, relative diffusion coefficients for the substrate and product, and distance between the enzyme layer and the optical fiber bundle. Detailed studies concerning the effect of each of these parameters on the response slope are currently being performed.

The results which are presented here suggest that enzyme-based optical fiber sensors are feasible. With improvements in biosensor design, better response can be expected and with the immobilization of other enzymes, biosensors for new analytes should be possible. The development of enzyme-based fiber optic sensors is continuing.

LITERATURE CITED

- (1) Peterson, J. I.; Vurek, G. G. *Science* **1984**, *224*, 123-127.
- (2) Seitz, W. R. *Anal. Chem.* **1984**, *56*, 16A-34A.
- (3) Hornby, W. E.; Morris, D. L. In "Immobilized Enzymes, Antigens, Antibodies and Peptides"; Westall, H. H., Ed.; Marcel Dekker: New York, 1975.
- (4) Mascini, M.; Iannello, M.; Paleschi, G. *Anal. Chim. Acta* **1983**, *146*, 135-148.
- (5) "The Measurement of the Catalytic (Activity) Concentration of Seven Enzymes in NBS Human Serum SRM 909" *NBS Spec. Publ. (U.S.)* **1983**, No. 260-88.
- (6) Carr, P. W.; Bowers, L. D. "Immobilized Enzymes in Analytical and Clinical Chemistry"; Wiley: New York, 1980.

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