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SHORT COMMUNICATIONS

Activity of *Pseudomonas rathonis* T Cells as the Receptor Component of Membrane Biosensors for Detecting Surfactants

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Surface-active compounds (surfactants) are widely used in household and various industries as components of detergent powders, emulsifiers, corrosion inhibitors, foaming, waterproofing, antistatic, and flotation agents, etc. [1]. The ever-growing production of surfactants has led to increasing accumulation of these compounds in the environment. Indeed, sewage from some industries may contain up to 10 g/l of anionic and nonionic detergents [2], which, when reaching bodies of water, kill aquatic microflora and spoil water. Although surfactants themselves are relatively nontoxic to warm-blooded animals, they promote the accumulation of toxic compounds in living organisms, thereby enhancing the toxic effect of these compounds [2].

Surfactants occurring in the environment can be detected by chemical methods and by methods that employ biosensors, including those of microbial origin [3–5]. Microbial sensors are composed of a receptor, which represents intact cells responding to changes in the environment, and a detector, which transforms the cell response to a physicochemical signal. Microbial sensors offer the advantage of simple construction and operation, low cost of production and analysis, high sensitivity, and easy use.

The aim of the present work was to evaluate the surfactant-induced response of *Pseudomonas rathonis* cells working as the receptor element of a membrane biosensor and to determine the stability of such response during the long-term storage of cells or cell-containing receptors. The response of cells to surfactants was evaluated in terms of induced changes in the cell respiration.

The *Pseudomonas rathonis* strain T was obtained from the collection of the Dumanskii Institute of Colloidal Chemistry and Chemistry of Water. The strain was maintained on peptone–tryptone agar slants at 4°C with the transfer onto fresh slants at 6-month intervals.

Cells for experiments were grown on the slants at 37°C for 18 h and suspended in 50 mM K–Na phosphate buffer (pH 7.4) to a concentration of 100 mg wet cells per milliliter. The suspension was stored in a refrigerator at 4°C in sealed Eppendorf tubes.

To manufacture a receptor element, the cell suspension (after either 1 day or 7 months of storage) was applied onto Whatman GF/A chromatographic paper. Impregnated paper was partially dried in the air for 30 min and then either immediately used for the manufacturing of a biosensor or placed in a glass-stopped flask and stored at 4°C to be used at an appropriate time.

To manufacture a biosensor, the receptor element (Whatman paper with adsorbed cells) was fixed on the measuring surface of a Clark-type oxygen electrode, which was coupled to an Ingold 5313/10 amplifier (Switzerland–United States). All measurement were carried out at 20–22°C in a 50 mM K–Na phosphate buffer (pH 7.4). The biosensor signal was recorded, using an XY Recorder-4103 (Czech Republic), as the first derivative of the change in the electrode current in response to the addition of a surfactant into the measuring chamber.

In the manufacture of biosensors employing microbial cells, the preservation of the cells in a physiologically active state to provide for the sensitivity and stability of the biosensors for sufficiently long periods of time is of crucial importance. The preferred immobilization methods are the adsorption of cells on various surfaces and their incorporation into gels [6]. Our previous investigations showed that *P. rathonis* T cells immobilized in a gel can be used as the receptor element of an amperometric biosensor for detecting anionic surfactants [4]. It was also found that the stability of such a biosensor considerably depends on the type of gel used for immobilization [5].

In spite of the apparent advantages, immobilization in gels shows some disadvantages, the main of which is the decrease in the biochemical activity of immobilized cells. This prompted us to investigate the possibility of creating a biosensor based on *P. rathonis* T cells immobilized by means of adsorption on paper.

The results presented below show that biosensors whose receptor elements represent *P. rathonis* T cells immobilized by means of physical adsorption on paper

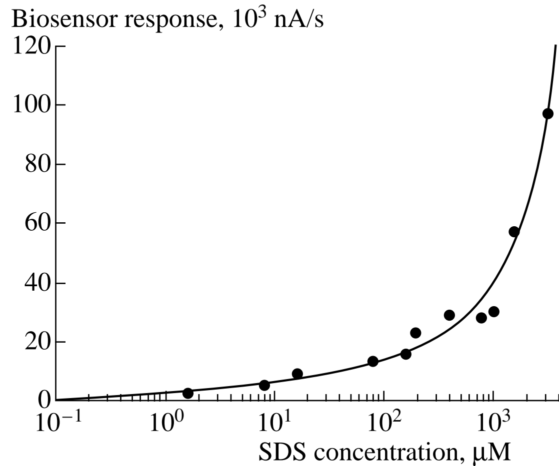

Fig. 1. Calibration curve with respect to SDS for a membrane biosensor based on *P. rathonis* T cells and a Clark-type oxygen electrode.

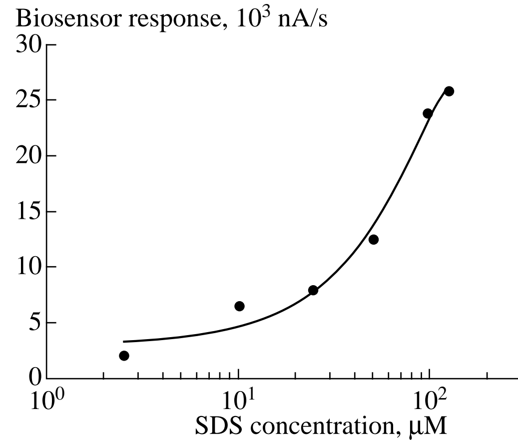

Fig. 2. Calibration curve for the membrane biosensor with respect to DEA.

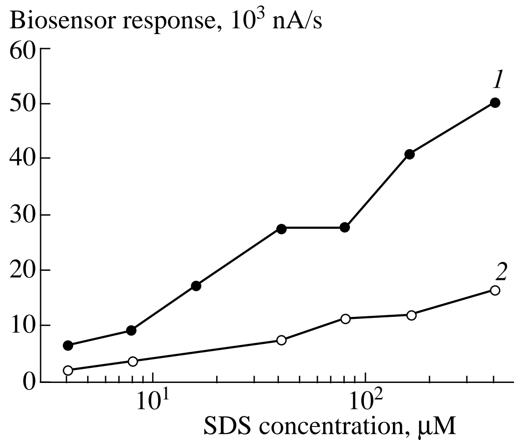

Fig. 3. The effect of storage of the suspension of *P. rathonis* T cells for (*1*) 1 day and (*2*) 7 months on the sensitivity of the membrane biosensor to SDS.

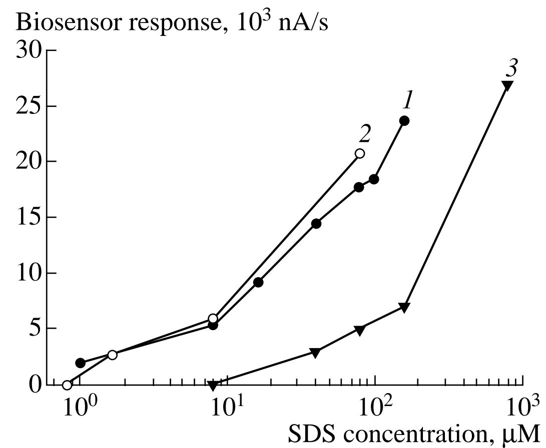

Fig. 4. The effect of storage of immobilized *P. rathonis* T cells (in the form of the receptor element) for (*1*) 1 day, (*2*) 7 days, and (*3*) 1 year on the sensitivity of the biosensor to SDS.

(hereafter called membrane biosensors) can be used for detecting the anionic surfactant sodium dodecyl sulfate (SDS). Figure 1 presents a calibration curve constructed for such a biosensor within the range of SDS concentrations between 1.6 μM and 3.12 mM. The lower limit of SDS detection (1.6 μM or 0.46 mg/l) was close to that of the biosensor whose receptor element represented cells immobilized in an agar film (hereafter called film biosensor) [3, 4]. The sensitivity of the membrane biosensor was slightly higher than that of the film biosensor. The reason for this is twofold. First, the diffusion of SDS molecules to the cells immobilized in the agar film is hindered. Second, the value of the response of cells to SDS probably depends on the cell wall permeability, which increases under the action of the surfactant [7]. If so, the response of the gel-immobilized cells to SDS could decrease owing to the protective effect of the gel.

Within the range of SDS concentrations from 0.2 to 1 mM (Fig. 1), the calibration curve showed a plateau, whose formation can be accounted for by drastic changes in the physicochemical properties of aqueous solutions of surfactants at their concentrations exceeding critical micelle concentration (CMC) [1]. It can be suggested that the CMC of SDS in 50 mM K–Na phosphate buffer is close to 0.2 mM.

Among the other anionic, cationic, and nonionic surface-active components of detergent powders, such as sodium alkyl sulfate, sodium alkylbenzene sulfonate (C_{12}), imidostat, diethanolamide (DEA), and sulfoethoxylate, only the nonionic surfactant DEA could

induce a sufficiently high response of immobilized cells at concentrations ranging from 2.5 to 125 mg/l (Fig. 2).

Figure 3 shows the effect of storage of *P. rathonis* T cells, used for the manufacture of the receptor element of the membrane biosensor, on its sensitivity to SDS, and Fig. 4 shows the effect of storage of the receptor element on the sensitivity of the biosensor. It can be seen that the sensitivity of the membrane biosensor with the receptor element manufactured from cells stored for 7 months decreased by an order of magnitude as compared with the cells stored for 1 day. In particular, the response of the first variant of the biosensor to 80 μM SDS was almost the same as the response of the second variant to 8 μM SDS (Fig. 3). As for the storage of cells in the immobilized state (i.e., in the form of the receptor element), it is evident that 7-day storage impaired neither the lower limit of sensor sensitivity nor the value of its response to SDS (Fig. 4). On the other hand, as with the 7-month storage of the cell suspension, the 1-year storage of the receptor element led to an order-of-magnitude fall in the biosensor sensitivity to SDS. Nevertheless, in both variants of long-term storage, the sensitivity of the membrane biosensor remained sufficiently high to detect SDS at concentrations of 100 μM and higher (Figs. 3 and 4).

To conclude, the membrane biosensor based on *P. rathonis* T cells and a Clark-type oxygen electrode is sensitive to the anionic surfactant SDS and the nonionic surfactant DEA with the lower limits of detection equal to 0.46 and 2.5 mg/l, respectively. The insensitivity of this biosensor to the other surface-active components of detergent powders (sodium alkyl sulfate, sodium alkylbenzene sulfonate, imidostat, and sulfoethoxylate) suggests that it can be used for the selective detection of SDS and DEA in detergent-containing solutions and wastewaters. The sensitivity of the membrane biosensor remains at a relatively high level after a 7-month storage of the cell suspension or a 1-year storage of the receptor element in a refrigerator at 4°C.

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