БІОСЕНСОРИ

BIOSENSORS

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INTACT RECOMBINANT CELLS OF THE YEAST *HANSENULA POLYMORPHA*, OVER-PRODUCING FORMALDEHYDE DEHYDROGENASE, AS THE SENSITIVE BIOELEMENTS FOR AMPEROMETRIC ASSAY OF FORMALDEHYDE

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Abstract

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Intact cells of the gene-engineered thermotolerant methylotrophic yeast *Hansenula polymorpha* with a high content of NAD^+- and glutathione-dependent formaldehyde dehydrogenase (FdDH, EC 1.1.1.284) were used as the biorecognition elements for amperometric assay of formaldehyde (FA). The yeast cells were immobilized on the graphite working electrode by physical fixation of the cell suspension by means of dialysis membrane (phenazine methosulphate was used as a free-diffusing redox mediator). It was supposed that the mediator reacts in cytosol with FdDH-produced NADH after entering the cells in the presence of FA. The biosensor based on recombinant yeast cells exhibited expanded linear range toward FA as compared to similar sensors based on the parental cells of *H. polymorpha (leu 1-1* and *leu 2-2)* and detection limit for it was found to be 0.1 mM. The developed biosensors are selective, inexpensive and stable over several days, as well as simple to manufacture and operate. The constructed microbial biosensors were successfully applied for FA determination in real samples of commercial chemical product (formalin), pharmaceutical (Formidron), disinfectant (Descoton forte) and rabbit vaccine against viral haemorrhage. A good correlation was observed between the biosensors' approaches and chemical methods.

Keywords: Formaldehyde, recombinant yeast cells, amperometric biosensor, analysis of real samples

Анотація

ІНТАКТНІ РЕКОМБІНАНТНІ КЛІТИНИ ДРІЖДЖІВ *HANSENULA POLYMORPHA*, НАД-ПРОДУЦЕНТИ ФОРМАЛЬДЕГІДДЕГІДРОГЕНАЗИ, ЯК ЧУТЛИВІ БІОЕЛЕМЕНТИ ДЛЯ АМПЕРОМЕТРИЧНОГО ВИЗНАЧЕННЯ ФОРМАЛЬДЕГІДУ

С. Я. Парижак, О. М. Демків, В. Шуман, М. В. Гончар

Інтактні клітини рекомбінантних термотолерантних метилотрофних дріжджів *Hansenula polymorpha* з високим вмістом NAD^+- і глутатіон-залежної формальдегіддегідрогенази (ФдДГ, КФ 1.1.1.284) використано як біоселективні елементи для амперометричного визначення формальдегіду (ФА). Дріжджові клітини іммобілізували на графітовому робочому електроді фізичною фіксацією клітинної суспензії за допомогою діалізної мембрани (феназинметосульфат служив у ролі вільнодифундуючого редокс медіатора). Передбачалось, що медіатор після проникнення в клітину взаємодіє в цитозолі з NADH, який утворюється в реакції з ФдДГ у присутності ФА. Біосенсори з рекомбінантними дріжджовими клітинами мали ширший лінійний діапазон вимірювання ФА у порівнянні із сенсорами, в складі яких містилися клітини вихідних штамів *H. polymorpha (leu 1-1* і *leu 2-2)*, і поріг визначення ФА для них складав 0,1 мМ. Розроблені біосенсори є достатньо селективні, недорогі і стабільні при зберіганні протягом кількох днів, а також прості у приготуванні і експлуатації. Сконструйовані мікробні біосенсори були успішно використані для визначення ФА в реальних зразках: дезінфікуючих та фармацевтичних засобах, вакцині проти вірусної геморагічної хвороби кролів. Показано добру кореляцію результатів, отриманих біосенсорним підходом, та хімічними методами.

Ключові слова: формальдегід, рекомбінантні дріжджові клітини, амперометричний біосенсор, аналіз реальних зразків

Аннотация

ИНТАКТНЫЕ РЕКОМБИНАНТНЫЕ КЛЕТКИ ДРОЖЖЕЙ *HANSENULA POLYMORPHA*, СВЕРХ-ПРОДУЦЕНТЫ ФОРМАЛЬДЕГИДДЕГИДРОГЕНАЗЫ, КАК БИОЭЛЕМЕНТЫ ДЛЯ ЧУВСТВИТЕЛЬНОГО ОПРЕДЕЛЕНИЯ ФОРМАЛЬДЕГИДА

С. Я. Парижак, О. М. Демкив, В. Шуман, М. В. Гончар

Интактные клетки рекомбинантных термотолерантных метилотрофных дрожжей *Hansenula polymorpha* с высоким содержанием NAD^+- и глутатион-зависимой формальдегиддегидрогеназы (ФдДГ, КФ 1.1.1.284) использованы как биоселективные элементы для амперометрического измерения формальдегида (ФА). Дрожжевые клетки иммобилизовали на графитовом робочем электроде путем физической фиксации клеточной суспензии с помощью диализной мембраны (феназинметосульфат был использован в роли свободно-диффундирующего редокс медиатора). Предполагалось, что медиатор после проникновения в клетку взаимодействует в цитозоле с NADH, который образуется в результате реакции NAD^+ с ФдДГ в присутствии ФА. Для биосенсоров с рекомбинантными дрожжевыми клетками наблюдали более широкий линейный диапазон измерения ФА по сравнению с сенсорами, в составе которых были клетки родительских штаммов *H. polymorpha (leu 1-1* і *leu 2-2)*, и порог определения ФА для них составлял 0,1 мМ. Разработанные биосенсоры достаточно селективны, недорогие и стабильны при хранении на протяжении нескольких дней, а также просты в приготовлении и работе. Сконструированные микробные биосенсоры были успешно использованы для измерения ФА в реальных образцах: дезинфицирующих и фармацевтических препаратах, вакцине против вирусной геморрагической болезни кроликов. Показано хорошую корреляцию результатов, полученных биосенсорным подходом, и химическими методами.

Ключевые слова: формальдегид, рекомбинантные дрожжевые клетки, амперометрический биосенсор, анализ реальных образцов

1. Introduction

Formaldehyde (FA) is a ubiquitous environmental contaminant of our planet. It is found in paints, clothes, medicinal and industrial products, and is a component of diesel and gasoline exhaust, as well as being endogenously produced in all living organisms as a result of metabolism (methionine, histamine, methanol, and methylamine), spontaneous dissociation of 5,10-methylene tetrahydrofolate, or oxidative demethylation of DNA and RNA [1,2, 3-8]. FA is one of the chemical mediators of apoptosis and induces gene mutations in bacteria, fungi, yeast, *Drosophila* larvae, and causes chromosomal aberrations and sister chromatid exchange in the cultured rodent and human cells [9-11]. As a cross-linking agent, it readily reacts with thiol and amine groups, causing polymerization of proteins [12]. In semicarbazide-sensitive amine oxidase (SSAO)-related pathogenesis of Alzheimer's disease, FA interacts with β-amyloid and produces irreversibly cross-linked neurotoxic amyloid-like complexes. The potential effect of FA on protein misfolding may be significant, even if FA remains in the human body for only a short time [13-16]. All these facts have been the topic of considerable interest and convincingly demonstrate a need for development of simple, cheap, sensitive, and selective methods for FA analysis.

The existing enzymatic methods of FA assay are laborious, not enough selective and specific, and are still unavailable at the world market. To solve this problem, a number of attempts to develop biosensors for the detection of FA were reported [17-22] including amperometric sensors [23-25], potentiometric detection schemes [19, 26 — 29] and optical sensors [21, 30]. Biosensors for FA detection are usually based on either highly-purified alcohol oxidase (AOX) or bacterial FA dehydrogenase. However, due to a non-sufficient stability of FA dehydrogenase and to a broad specificity of AOX, they did not succeed in the practical applications [23].

Previously, it was shown that cells of the methylotrophic yeast *H. polymorpha*, capable of metabolizing FA, can be employed as FA-selective biorecognition elements in the pH-FET-based potentiometric biosensors [26, 28, 29]. Microbial sensors for FA assay based on genetically modified intact and permeabilised cells of the yeast *H. polymorpha* with a high level of AOX activity were also constructed [31].

This paper reports the development of microbial sensors for FA determination, based on the use of genetically modified intact cells of methylotrophic yeast *H. polymorpha*, over-producing FdDH, as FA-sensitive bioelements. The recombinant strains are able to produce a high quantity of FdDH (4-6-fold when compared to the parental strain). The use of cell-based amperometric biosensors looks very promising due to several advantages over conventional enzyme electrodes: simplicity, cheapness, stability and versatility.

2. Materials and methods

2.1. Chemicals

Ethylenediaminetetraacetic acid (EDTA) was from Serva (Heidelberg, Germany); $(NH_4)_2SO_4$, Na_2HPO_4, KH_2PO_4, $MgSO_4$, $CaCl_2$ were from Merck (Darmstadt, Germany), ferrocene, formalin and methanol were obtained from Merck-Schuchardt (Hohenbrunn, Germany). Methylene blue was obtained from Riedel-de Haën (Seelze, Germany); paraformaldehyde, phenazine methosulphate, phenylmethylsulfonyl fluoride (PMSF), potassium hexacyanoferrate (III), potassium hexacyanoferrate (II), methylglyoxal, 2,6-dichlorophenolindophenol (DCIP), phenazine methosulfate (PMS) were from Sigma (Deisenhofen, Germany). Glutathione (reduced) was from Fluka (Buchs, Switzerland), NAD^+ and NADH were from Gerbu Biotechnik (Gailberg, Germany). Nafion, butyraldehyde, propionaldehyde and acetaldehyde were from Aldrich (Deisenhofen, Germany). Dialysis membranes (cut-off 10 kDa) were from Biomol (Hamburg, Germany). The cathodic electrodeposition polymer 2*CPOs* was synthesized following previously published procedures [32, 33].

All chemicals were of analytical-reagent grade and all the solutions were prepared using HPLC-grade water. Formaldehyde solution (1 M) was prepared by hydrolysis of the corresponding amount of para-formaldehyde in water (300 mg; 10 ml water) by heating the suspension in a sealed ampoule at 105 °C for 6 h.

2.2. Strains and cultivation conditions

The following yeast strains were used in the present study: *H. polymorpha* NCYC 495 (*leu*1-1) and *H. polymorpha* CBS 4732 (*leu*2-2*)* — parental strains from collection of the Institute of Cell Biology (Lviv, Ukraine); Tf 11-6 and Tf 22-126 — recombinant FdDH-overproducing strains [34, 35].

Yeast cells (recombinant and parental) were cul-

tivated in flasks on a shaker (200 rpm) at 30 °C until the middle of the exponential growth phase (~24 h) in a medium containing (g/L): $(NH_4)_2SO_4$ — 3.5; KH_2PO_4 -1.0; $MgSO_4x7H_2O$ — 0.5; $CaCl_2$ — 0.1; yeast extract — 0.5 with the supplement of standard microelements (Demkiv *et al.*, 2005). As a carbon source, 1% methanol was used. For cultivation of the strains *leu 1-1* and *leu 2-2*, leucine was added up to 40 mg/L.

After washing, the cells were re-suspended in 0.05 M K, Na-phosphate buffer, pH 8.0 (PB), containing 1 mM PMSF and 1mM EDTA with the following lyophilization and kept at 0 °C. Before each experiment, lyophilised yeast cells were re-suspended in the corresponding volume of initial buffer to final cell concentration 33 mg/ml and allowed to swell during 30 min at room temperature. Just after the swelling, the suspension of the cells was used in the electrochemical experiments.

2.3. Construction of cells-based amperometric biosensors

2.3.1. Electrodes

Graphite rods (type RW001, 3.05 mm diameter, Ringsdorff Werke, Bonn, Germany) were used as working electrodes. First, they were sealed in glass tubes by means of epoxy glue forming disk electrodes, then polished with emery paper of decreasing size and cleaned in ultrasonic bath. The properties of amperometric biosensors were evaluated by means of constant-potential amperometry in a three-electrode configuration with a Ag/AgCl/KCl (3 M) reference electrode and a Pt-wire counter electrode.

2.3.2. Immobilization of recombinant FdDH-producing yeast cells

Physical adsorption of the cells and fixation by means of dialysis membrane: 5 µl of fresh suspension of intact cells (10 mg/ml) was put on the surface of the carbon electrode and dried for 5 min at room temperature. On the top of a cells-modified electrode, 3 µl of a 50 mM neutralized solution of reduced glutathione and 2 µl of 25 mM NAD^+ were dropped. After drying (2-4 min), 5 µl of a 1% neutral Nafion solution were dropped on the sensor surface. The Nafion membrane was allowed to dry for 20 — 25 min at +4 °C. Then, the electrode was covered with a piece of standard dialysis membrane (1x1 cm). An "O"-ring with a diameter of about 5 mm was used for membrane holding. Before use, the electrode was washed with 50 mM PB, pH 8.0. Phenazine methosulfate was used as a free-diffusing redox mediator (10 ml of a 1 mM solution of the mediator in 50 mM PB, pH 8.0 was added to the electrolyte solution). In these experiments, the glass cell was wrapped with aluminium foil as phenazine methosulfate is light sensitive.

Immobilization of recombinant cells by entrapment within the polymer layer of a cathodic electrodeposition paint 2CPOs: 5 µl of fresh suspension of intact cells (10 mg/ml), 2 µl of cathodic paint (*2CPOs*), 3 µl of a 50 mM neutralized solution of reduced glutathione and 2 µl of 25 mM NAD^+ were mixed and dropped onto the surface of a graphite electrode. In a miniaturized electrochemical cell, the cathodic paint was precipitated using a potentiostatic pulse sequence with pulses to a potential of -1200 mV for 0.2 s and a resting phase at a potential of 0 mV for 5 s [32]. At the applied cathodic potential, water is reduced at the electrode surface leading to an increase of the pH-value in a diffusion zone in front of the working electrode surface. Subsequently, the cathodic paint is deprotonated imposing a significant change in its solubility which leads to the precipitation of the polymer on the electrode surface simultaneously entrapping the cells. Then, the electrode was covered with Nafion membrane. Microbial electrodes were stored in 50 mM PB, pH 8.0, at 4 °C.

2.3.3. Redox mediators

Potassium hexacyanoferrate(III), methylene blue, phenazine methosulfate and 2,6-dichlorophenolindophenol (DCIP) were used as free-diffusing redox mediators. 10 ml of a 1 mM solution (0.5 mM for methylene blue) of the selected redox mediator in 50 mM phosphate buffer, pH 8.0, was added to the electrolyte solutions.

In contrast, ferrocene was initially dissolved in acetone. For the preparation of the mediator-containing sensing layer, 4 µl of a 10 mM ferrocene solution was dropped on the surface of the electrode at room temperature before the electrodeposition of the cathodic paint (see section 2.3.2). Electrodeposition of Prussian blue was performed by means of cyclic voltammetry (10 cycles from 0.4 to 1.3 V with a scan rate of 10 mV s^{-1} in 50 mM phosphate buffer, pH 8,0) in a 10 mM solution of $K_4[Fe(CN)_6]$. After the electrodeposition, Prussian blue-modified electrodes were rinsed in 50 mM phosphate buffer, pH 8.0.

2.3.4. Amperometric measurements

Amperometric experiments were carried out using an Autolab PGstat12 potentiostat (Eco Chemie, Utrecht, Holland) controlled by the GPES4.9 software. Measurements started after the steady-state current became established. The electrode with immobilized yeast cells was placed into the electrochemical cell containing 20 ml buffer solution at 25 °C under continuous stirring. After 20 min of the background current stabilizing, the experiments were started by addition of FA up to 30 mM. In the course of the experiments, the modified electrodes were stored in 50 mM PB, pH 8.0 at 4 °C. All measurements were repeated at least 3 times. Operational stability of the obtained microbial sensors was evaluated using a previously described automatic sequential-injection analyzer [36].

2.4. Chemical methods of FA analysis

Chemical assay of FA was performed by two methods with a usage of chromotropic acid [37] and 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) [38].

2.5. Statistics

Statistic treatment of the data and the level of correlation between experimental values have been calculated using computer program *Origin 6.0* and *Microsoft Excel*.

3. Results and discussion

3.1. Design of cell-based biosensor

Cells of recombinant strains Tf 11-6 and Tf 22-126, overproducing thermostable FdDH, were used for preparation of microbial FA-selective amperometric biosensors. These "sensing" cells, grown in methanol medium, possess 4-6-fold higher activity (4~6 U/mg) of FdDH in cell-free extracts when compared to the wild type parental strains. A high content of rFDH in the recombinant cells Tf 11-6 and Tf 22-126 correlates with a higher *FLD1* gene copy [34, 35]. It makes these cells ideal as the bioactive elements in construction of FA cell sensors. For evaluation of a FA-biosensor prototype based on intact cells, different red-ox mediators were tried to establish the electron transfer between FdDH located in the cellular cytoplasm and the electrode surface. For the developed biosensors, phenazine methosulfate (free-diffusing redox mediator) exhibited the best electron transfer characteristics (Fig. 1). The amperometric responses of intact recombinant yeast cells of *H. polymorpha* Tf 11-6 immobilised on the bare carbon electrodes in the presence of different mediators are presented in Fig. 1. It was assumed that PMS penetrates through cell membrane, collect electrons from a reduced form NADH and returns back to the electrode (3.05 mm graphite rod electrodes and of 0.0 mV potential were chosen for PMS oxidation). Architecture of cells-based biosensor and principal scheme of FA monitoring using PMS as a mediator, as well as a supposed mechanism of PMS reaction cycle in yeast cells are shown in Fig. 2. Taking into account that lyophilised cells are heterogeneous: a part of the cells would have the damaged membranes, whereas another part is still native, we co-entrapped NAD^+ and glutathione in the bioactive layer and covered it by a negatively charged Nafion membrane. This membrane prevents leakage of the cofactors due to electrostatic interactions.

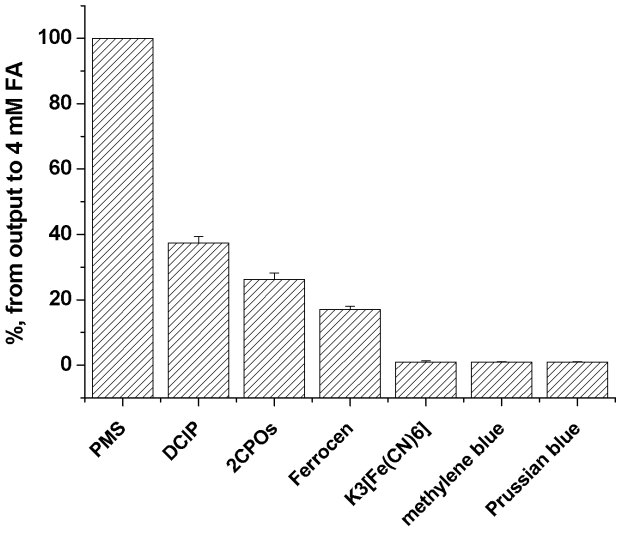

Fig. 1. Evaluation of the suitability of different red-ox mediators for recombinant (Tf 11-6) cells-based amperometric biosensor. Free-diffusing redox mediators: PMS (0 mV vs. Ag/AgCl), DCIP, $K_3[Fe(CN)_6]$, methylene blue; electrodeposited electrocatalysts: ferrocene, Prussian blue and the Os-complex modified cathodic electrodeposition paint *2CPOs* (+200 mV vs Ag/AgCl)

3.2. Properties of the cell-based biosensor

Bioanalytical characteristics of the constructed biosensors were studied in detail: kinetics, dynamic and linear range, selectivity, dependence of sensors output on temperature. Biosensors demonstrated a good sensitivity, high selectivity to FA, and a good

stability. Linear range for developed *cells Tf11-6*-NAD^+-GSH-Nafion-modified electrode towards FA is shown in Fig. 3. For recombinant cells-based biosensor, a linear detection range for FA was 0.25 — 8 mM. The optimal pH-value for the developed biosensors was in the range of 7.6 to 8.3 with an optimal temperature between 45-50 °C (data not shown), due to a higher thermostability of the used enzyme and thermotolerance of recombinant yeast cells.

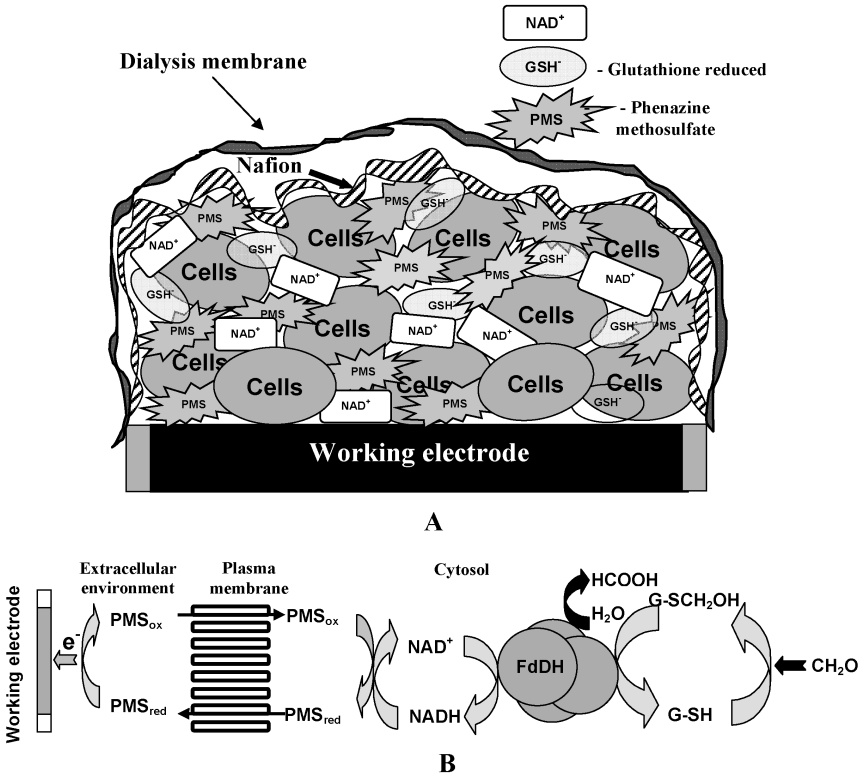

Fig. 2. **A**. Architecture of cells-based biosensor; **B**. The scheme of intracellular red-ox reactions coupled with electrochemical oxidation of the free-diffusing mediator PMS; 0.0 V vs Ag/AgCl electrode for cells-based sensor

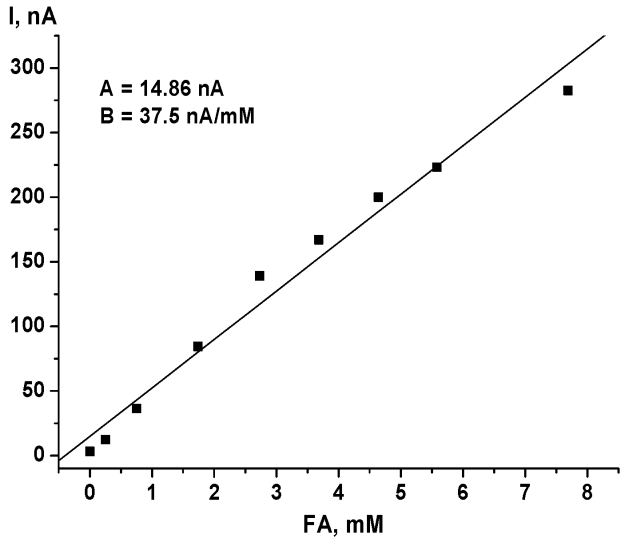

Fig. 3. Linear concentration range for biosensor with an architecture *cells Tf11-6*-NAD^+-GSH-Nafion (3.05 mm diameter graphite disk electrode; PMS — free-diffusing redox mediator; 0 mV vs. Ag/AgCl)

For the estimation of the difference in bioanalytical characteristics of microbial sensors based on parental and genetically engineered cells, we constructed different types of sensors. It is clearly seen (Fig. 4) that recombinant cells give a higher and faster response to FA than the wild type cells. The maximal current values I_{max} for the *leu 1-1* and *leu 2-2* cells-modified sensors were 209.6 ± 5.8 nA and 180 ± 4.4 nA, respectively, while the Tf 11-6 and Tf 22-126 cells-based electrodes showed an I_{max} of 671 ± 10.3 nA and I_{max} of 574 ± 8.0 nA at the same conditions. The calculated values for K_M^{app} derived from the calibration curves were 9.56±0.59 mM and 3.64±0.23 mM for sensors based on parental (non-recombinant) *leu 1-1* and *leu 2-2* cells, while for Tf 11-6 and Tf 22-126 cells-modified electrodes these parameters were shown to be 1.84±0.09 mM and 5.95±0.18 mM.

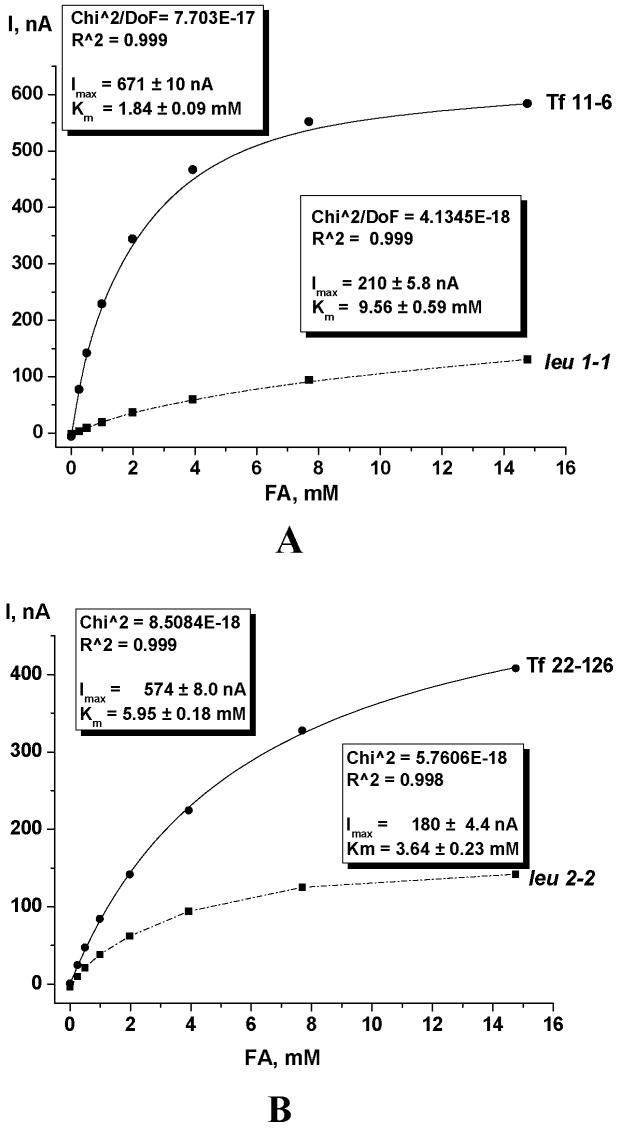

Fig. 4. FA calibration curves for microbial sensors based on wild type cells (dot lines) and recombinant cells (solid lines). 3.05 mm diameter graphite disk electrode; PMS — free-diffusing redox mediator; 0 mV vs. Ag/AgCl: **A**. *H. polymorpha leu 1-1* — parental strain and Tf 11-6 — recombinant strain; **B**. *H. polymorpha leu 2-2* — parental strain and Tf 22-126 — recombinant strain

The selectivity of the *cells Tf11-6*-NAD^+-GSH-Nafion biosensor for the determination of FA was evaluated using different structurally related to FA substances and potential interferents. Quantitatively, the ratio of the sensor output for different potentially interfering analytes compared to formaldehyde-derived response is as following: FA (100 %), methylglyoxal (10.9 %), ethanol (6 %). Output to acetaldehyde, butyraldehyde and propionaldehyde was of opposite direction (substrate reduction instead of oxidation was observed). This can be explained by functioning in the cells of the other, than FdDH, enzymes with a different specificity. Due to a highly expressed difference in sensitivity toward FA and the tested analytes, it can be assumed that the developed biosensors are suitable for the determination of FA in real samples.

The operational stability of the developed FA biosensors was tested using the automatic sequential injection analyser "OLGA" [36]. The sensor was integrated into specifically adapted flow-through electrochemical cells, and 12 injections of the FA standard solution per hour were performed automatically. Fig. 5 shows the peak current of the Tf 11-6 cells-modified sensor during sample injection for about 30 hours (about 360 individual measurements). All subsequent tests were performed at a constant temperature of 24 °C adding 1 mM solution of FA in 50 mM phosphate buffer, pH 8.0. The sensor demonstrated a linear drop of the current response during continuous operation over 30 hours. The current decreased to 50% of the initial sensor output after 30 hours of continuous operation. So, developed sensors exhibit satisfactory operational stability and can be used for FA measurements in real samples. The operational stability was investigated only for Tf 11-6 cells-based sensor, because this variant demonstrated the best response to FA.

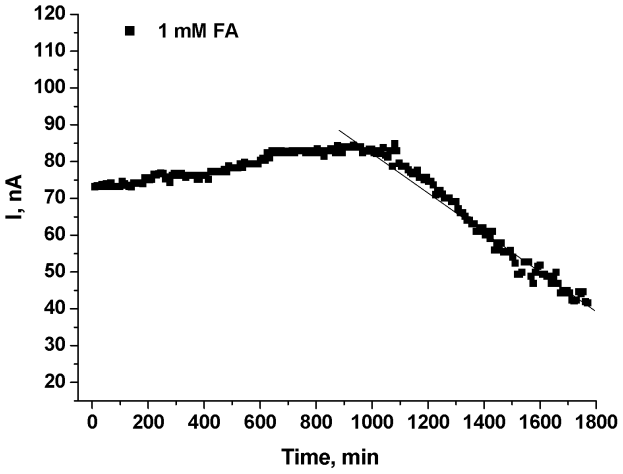

Fig. 5. Operational stability of the optimised recombinant (Tf 11-6) cells-based biosensor tested in an automatic sequential injection analyser (flow-rate 5 ml min^{-1}; sample injection every 4 min). 3.05 mm diameter graphite disk electrode; PMS — free-diffusing redox mediator; 0 mV vs. Ag/AgCl

The storage stability of the developed biosensors was found to be longer than 14 days at 4 °C (data not shown). 7.7 mM FA solution was used for the evaluation of the storage stability. After 4 days of

keeping in buffer solution in the refrigerator, the detected signal exceeded the initial one by about 20 %. This effect is often observed for amperometric biosensors and is an indicative of an equilibration of the sensor architecture, potentially leading to an improved permeability of the immobilisation matrix for the substrate.

3.3. Bioanalytical application of the cell-based biosensors

The sensors were applied for FA testing in real samples of industrial product formalin, antimicrobial agent Descoton forte, antiperspirant Formidron and rabbit vaccine against viral haemorrhage, using the multiple standard addition method, taking into consideration the possible interfering effect of real samples' components on FA assay [39]. Results of such analysis are presented in Fig. 6 and Table 1. It is clearly seen from the Fig. 6, that interfering effect of real sample components is observed for all samples, but in different extent. The maximal interfering effect is observed for Descoton forte, less — for Formidron and rabbit vaccine, and the minimal — for formalin, that follows from the slope values for calibration curves obtained on the background of real samples in different dilutions: for Descoton a difference is — 47.6 % (42 and 22 for dilutions 4000 and 4635, respectively), for Formidron — 25.7 % (0.48 and 0.35 for dilutions 250 and 81, respectively), for rabbit vaccine — 27.9 % (11.8 and 8.51 for dilutions 20 and 10) and for formalin — 0.8 % (7.15 and 7.09 for dilutions 20100 and 14300, respectively). A good correlation was observed between the data of FA testing (Table 1) by the biosenor's approaches (FdDH and cells-based), proposed enzymatic method Formatest [39] and routine chemical methods (if the standard additions method is performed).

Table 1

FA content in real samples determined by different methods: chemical and cell-based biosensor approaches

Sample/ Method	FA molar concentration (mole/L), M±m				
	Chemical methods		FdDH-based methods		
	MBTH	Chromotropic acid	Formatest	FdDH- biosensor	Cells- biosensor
Formidron	1.64± 0.61	1.48±0.26	1.53± 0.31	1.57± 0.13	1.48±0.06
Descoton forte	3.57± 0.30	3.59±0.44	3.25±0.8	3.61± 0.13	3.29±0.12
Formalin	12.6± 0.73	14.0±0.81	13.5±0.7	13.6±0.6	13.82± 0.54
Rabbit vaccine against viral haemorrhage	0.038± 0.003	0.029± 0.005	0.042± 0.004	0.041± 0.005	0.042± 0.002

4. Conclusion

Microbial amperometric FA-selective biosensors based on recombinant cells of methylotrophic yeast *H. polymorpha*, overproducing FdDH, have been constructed. Among seven tested mediators for biosensors development, PMS was chosen as the optimal one. Selectivity, operational and storage stability of the best variant of biosensor were evaluated. The developed sensors were successfully applied for FA determination in some real samples of industrial product formalin and pharmaceuticals: antimicrobial agent Descoton forte, antiperspirant Formidron, and vaccine against viral haemorrhage. A good correlation was observed between the data of FA testing by the FdDH-based biosenor's approaches and enzymatic or standard chemical methods.

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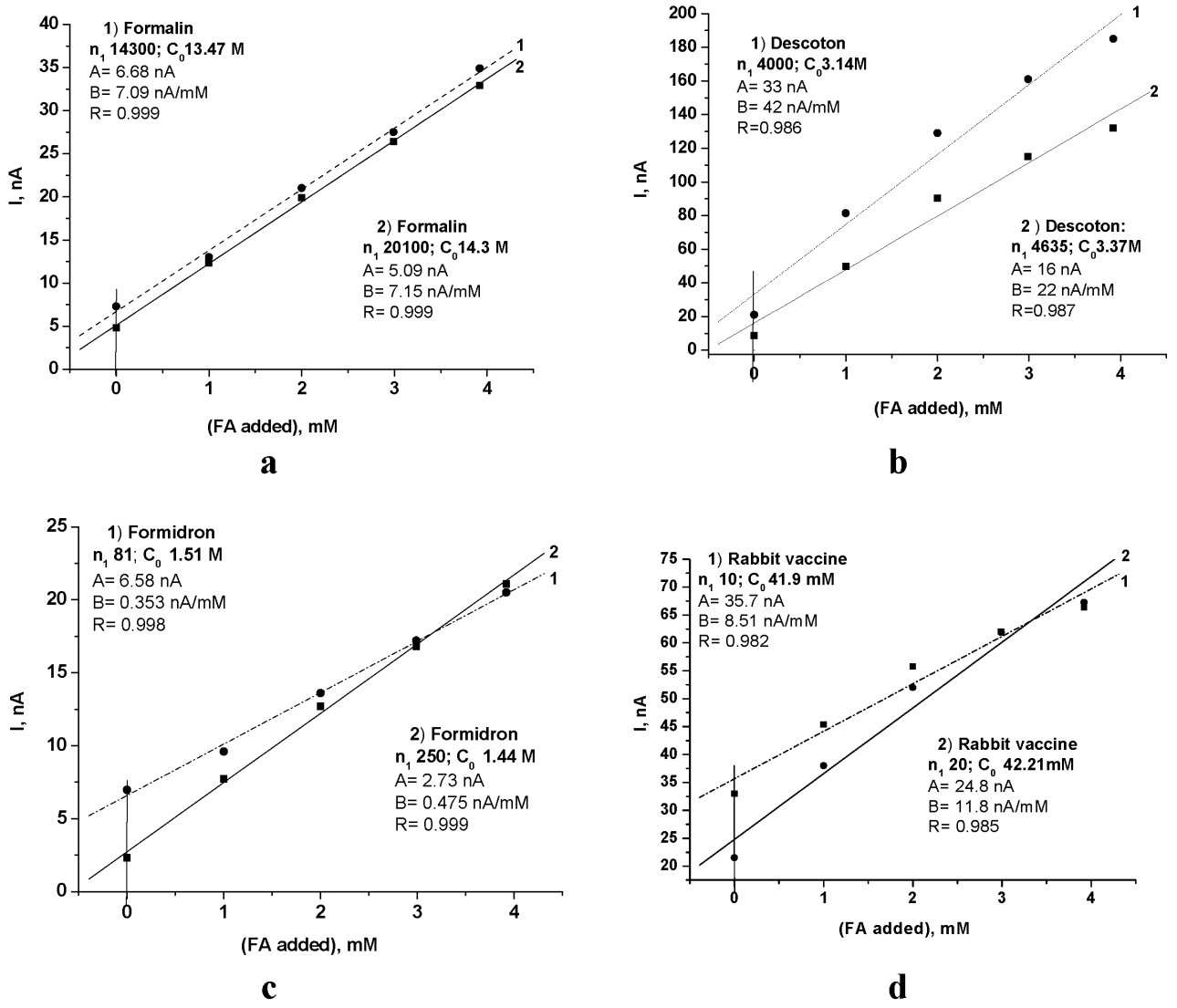

Fig. 6. Calibration curves for standard additions test for Tf 11-6 cells-based sensor with an architecture *cells Tf11-6*-NAD$^+$-GSH-Nafion, applied for FA analysis in real samples: **a** — formalin; **b** — disinfectant Deskoton; **c** — antiperspirant Formidron; **d** — rabbit vaccine against viral haemorrhage (A, B — parameters of the linear regression, n — dilution, C_0 — calculated initial concentration of FA). 3.05 mm diameter graphite disk electrode; PMS — free-diffusing redox mediator; 0 mV vs. Ag/AgCl

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