МЕТОДИ БІОФІЗИЧНИХ ДОСЛІДЖЕНЬ

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INDICATOR DYE BEHAVIOR IN THE SYSTEM GLUCOSE OXIDASE + GLUCOSE

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Using the absorption spectroscopy technique the group of indicator dyes including bromocresol purple, phenol red, neutral red, auramine O and acridine orange has been examined from the viewpoint of the dye applicability to the design of optical glucose biosensors. The absorbance changes of bromocresol purple, phenol red and neutral red observed in the glucose oxidase + glucose system were interpreted in terms of the dye sensitivity to the pH reduction occurring while glucose is catalytically oxidized. The arguments in favor of neutral red interaction with glucose oxidase are obtained.

KEY WORDS: glucose biosensor, glucose oxidase, indicator dyes

During the past decade biosensing has become one of the most rapidly developing area of fundamental and applied research [1]. Biosensors are analytical devices whose operation is governed by the biological recognition element combined with the signal transducer [2]. Molecular recognition and specific association of the biological element with the analyte in question is followed by the formation of measurable signal proportional to the analyte concentration. One important line of biosensor development involves the glucose sensing [3]. Glucose biosensors have found numerical applications in clinical diagnostics, process control, food analysis, *etc.* The vast majority of glucose biosensors were fabricated using the electrochemical transducers [4,5]. However, considerable attention was also paid to the design of optical glucose sensors [6]. The optical biosensors offer advantages of miniaturization, electrical passivity, combination of biochemical and spectroscopic selectivity, disposability, high information density and low cost [7,8]. Most of the optical biosensors are indirect, *i.e.* they contain a reagent whose optical properties (absorbance, fluorescence intensity, lifetime or polarization, *etc.*) change on the analyte binding to the biological sensor component. The absorption-based sensors seem to be the simplest among the optical sensors of the other types from the viewpoint of detection principle and instrumental configuration [8]. The operation of these sensors is based on measuring the changes in the absorbance of a dye sensitive to a certain biochemical reaction, particularly, to the reaction of glucose oxidation. This reaction is catalyzed by glucose oxidase (GO) according to the scheme:

$$\text{D - glucose} + O_2 \xrightarrow{\text{Glucose Oxidase}} \text{D - gluconolactone} + H_2O_2 \quad (1)$$

$$\text{D - gluconolactone} + H_2O \longrightarrow \text{D - gluconic acid} \quad (2)$$

As seen from this scheme, glucose oxidation is followed by the formation of hydrogen peroxide, consumption of oxygen and pH decrease in the reaction medium. Therefore, there exist several possibilities for selection of the dyes applicable for the sensor elaboration. These dyes can be tentatively divided into four groups: i) the dyes sensitive to hydrogen peroxide; ii) pH indicators; iii) oxygen-sensitive dyes; iv) the dyes appropriate for monitoring the changes in GO conformation during the catalytic reaction. The main steps of the dye screening can be outlined as follows: i) evaluating the dye sensitivity to the enzymatic reaction of glucose oxidation in solution; ii) examining the behavior of the dye co-immobilized with the enzyme on a certain support; iii) choice of the immobilization conditions ensuring a distinct relationship between the measured optical parameter and analyte concentration. Despite a considerable progress in the glucose optical biosensing there still exists a need for the dyes sensitive to the glucose oxidation reaction. The present study was undertaken to evaluate the applicability of a series of indicator dyes including bromocresol purple, phenol red, acridine orange, auramine O and neutral red to designing the absorbance-based glucose biosensors. In accordance with the above steps of the dye screening our studies were focused on detecting the changes in dye absorption spectra in the course of enzymatic glucose oxidation in solution.

MATERIALS AND METHODS

Glucose oxidase from *Aspergillus niger* (25 units/mg, catalase activity $\leq$ 50 % (GO1), and 130 units/mg, catalase activity $\leq$ 4 % (GO2)) was purchased from Fluka (Germany). Bromocresol purple (BCP), phenol red (PR), acridine orange (AO), auramine O (AU) and neutral red (NR) were obtained from Reakhim (Russia). Glucose was purchased from Sigma (Germany).

The buffer used in the experiments was 5 mM sodium-phosphate, pH 6.8. The dye absorption spectra were recorded at 25 °C with CM 2203 spectrometer (SOLAR, Belarus).

RESULTS AND DISCUSSION

The experiments were designed to answer three main questions: i) whether the dye absorption spectrum in GO solution differs from that in buffer; ii) how the glucose addition affects the dye absorbance and iii) to what extent the glucose-dependent absorbance changes are determined by the pH shift in a medium. As seen in Figs. 1, A and 2, A the absorption spectra of BCP and PR did not undergo any marked changes in the presence of glucose oxidase suggesting that the dye binding to the protein, if any, is very weak. This is not surprising since both forms (protonated and deprotonated) of sulfophthalein dyes BCP and PR possess negative charge [9], which prevents their association with GO, being negatively charged at neutral pH (the protein isoelectric point is 4.2).

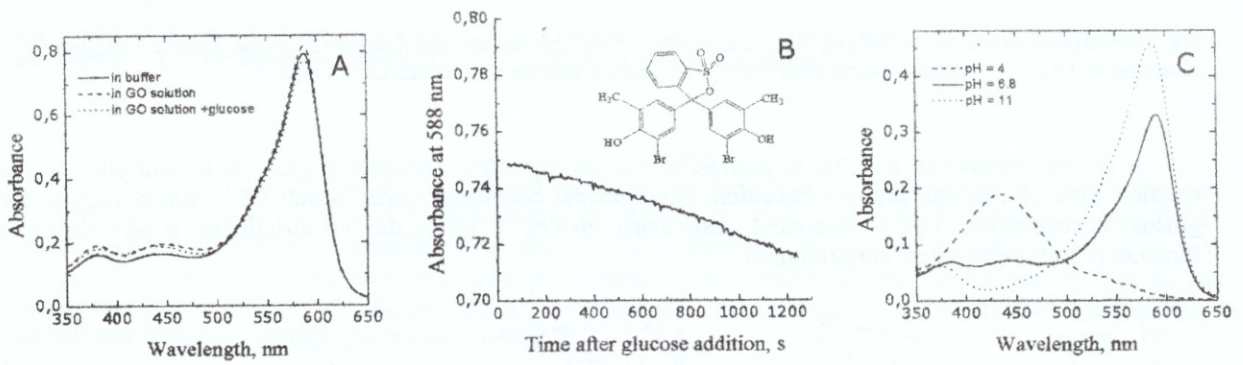

Fig. 1 Absorption spectra of bromocresol purple (A,C) and kinetics of the dye absorbance changes (B) on the glucose oxidation catalyzed by GO. The protein concentration (GO1) – 1.9 mg/ml, glucose concentration – 22 mM.

After glucose addition to the dye-GO mixture the BCP absorbance at the wavelength 588 nm (corresponding to absorption maximum (λ_{max}) of deprotonated dye form, Fig. 1, C) exhibits slow decrease consistent with the pH reduction. Likewise, increased absorbance of the protonated PR form (λ_{max} = 434 nm) can be attributed to the lowered pH.

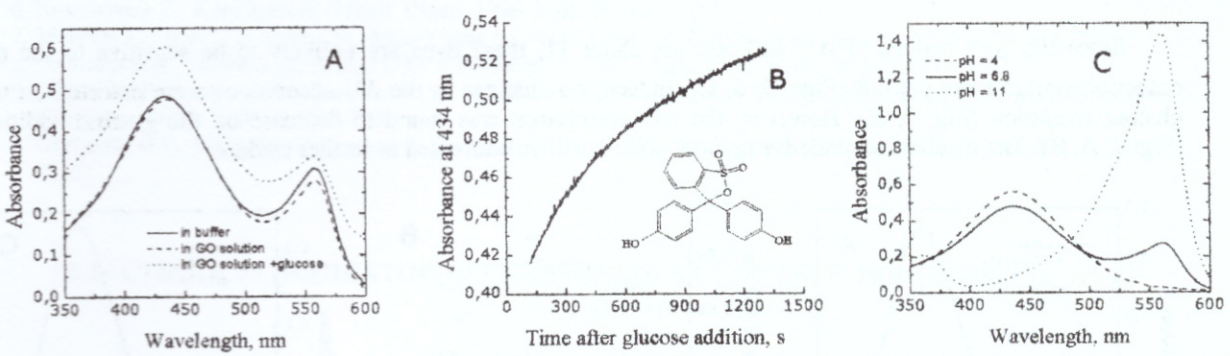

Fig. 2 Absorption spectra of phenol red (A,C) and kinetics of the dye absorbance changes (B) on the glucose oxidation catalyzed by GO. The protein concentration (GO1) – 1.9 mg/ml, glucose concentration – 22 mM.

The NR absorbance changes observed on glucose addition (increase in the concentration of the protonated dye form, λ_{max} =525 nm) can also be explained in terms of the pH decrease (Fig. 3, B). It is evident that all the above pH-dependent effects can be significantly enhanced by increasing the enzyme concentration in the reaction medium or by employing the GO preparation with higher catalytic activity. In principle, these effects might be expected since the ionization constants of the aforesaid dyes in water (pK_a^w) are about 6.4 (BCP), 8.0 (PR) [9] and 6.6 (NR), *i.e.* pK_a^w values are close to pH of the reaction medium (6.8). [10]. However, the dye binding to GO can affect its protolytic behavior, yielding the apparent ionization constant different from pK_a^w. This may be the case, particularly, for NR whose association with GO is displayed in the dye absorption spectrum (Fig. 3, A). The NR–GO complex is likely to be stabilized mainly by electrostatic interactions, because the protonated NR form is positively charged [10].

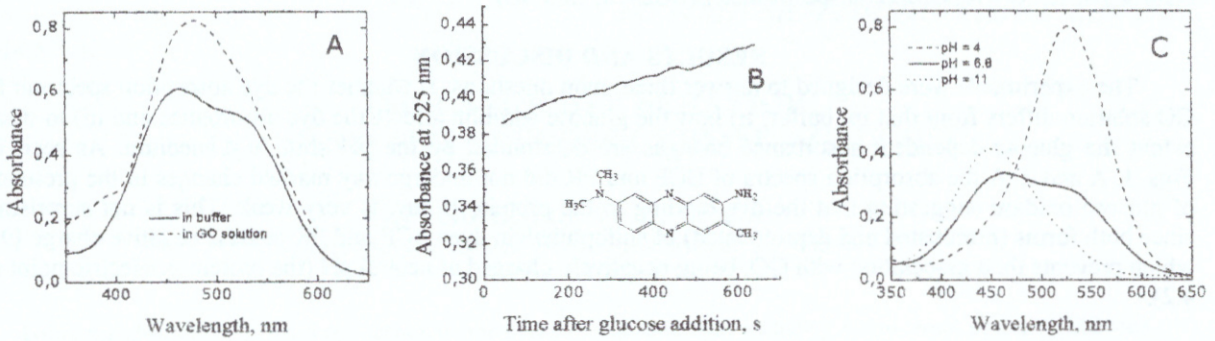

Fig. 3 Absorption spectra of neutral red (A,C) and kinetics of the dye absorbance changes (B) on the glucose oxidation catalyzed by GO. The protein concentration (GO2)– 0.9 mg/ml, glucose concentration – 23 mM.

It is also noteworthy that the magnitude of the pH-dependent absorbance changes is controlled by the reaction time of catalytic glucose oxidation, experimental conditions under which this reaction occurs, and glucose concentration. This is illustrated, particularly, in Fig. 4, where the NR absorbance is presented as a function of time and glucose concentration.

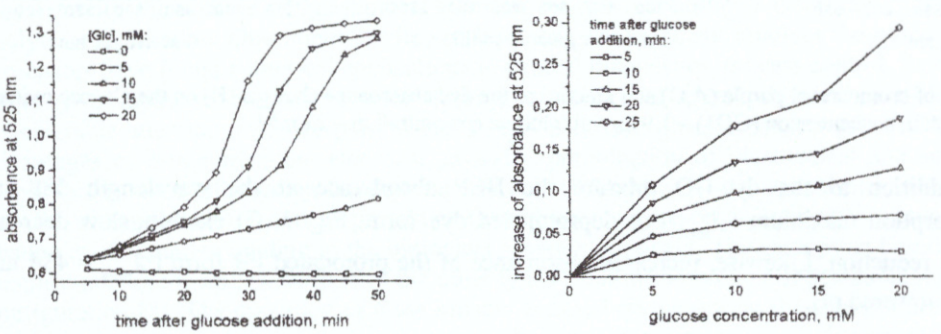

Fig. 4 Absorbance changes of neutral red detected at varying glucose level and protein concentration (GO2) – 1.1 mg/ml.

Since the pK_a^* values of AU and AO are about 11, these dyes are unlikely to be sensitive to the pH reduction started from pH 6.8 (Figs. 5, 6, C). Indeed, no changes in the AU absorbance were detected on the glucose oxidation (Fig. 5, B). However, the AO absorbance was found to decrease on the glucose addition (Fig. 6, A, B). The mechanism underlying these effects will be addressed in further studies.

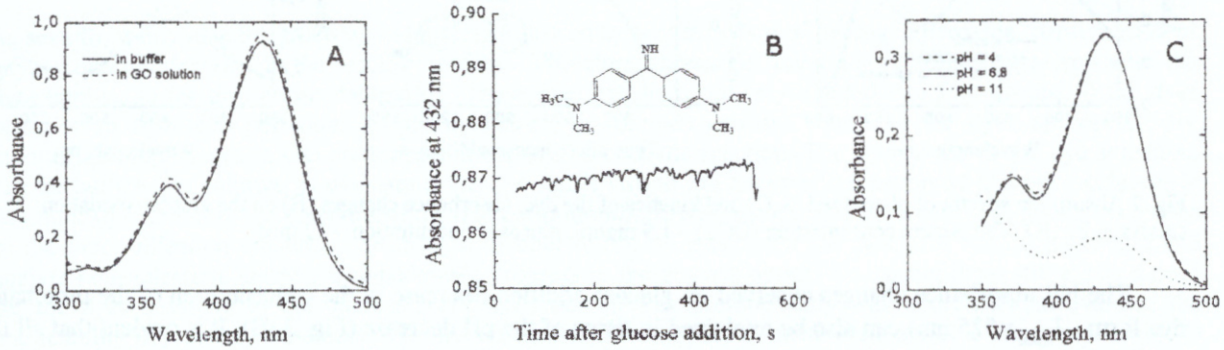

Fig. 5 Absorption spectra of auramine O (A,C) and kinetics of the dye absorbance changes (B) on the glucose oxidation catalyzed by GO. The protein concentration (GO1) – 1.9 mg/ml, glucose concentration – 22 mM.

The control experiments showed that hydrogen peroxide does not influence the AO absorbance. Since the AO protonated form is positively charged [10], the dye interaction with GO is highly probable, although this process does not manifest itself in the AO absorption spectrum (Fig. 5, A).

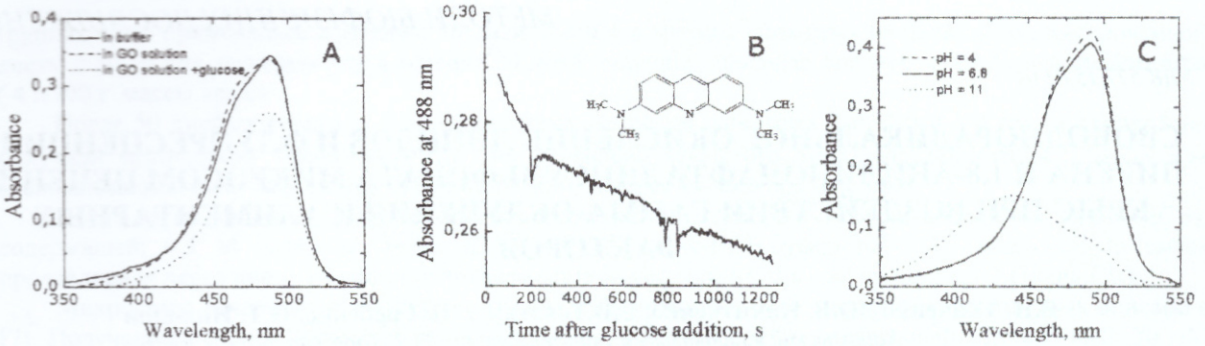

Fig. 6 Absorption spectra of acridine orange (A, C) and kinetics of the dye absorbance changes (B) on the glucose oxidation catalyzed by GO. The protein concentration (GO1) – 1.9 mg/ml, glucose concentration – 22 mM.

CONCLUSIONS

In summary, the present study indicate that indicator dyes bromocresol purple, phenol red and neutral red are suitable for probing the glucose oxidation reaction catalyzed by glucose oxidase. These dyes are sensitive to the pH reduction in the reaction medium. The two of the examined dyes, neutral red and acridine orange seem to be capable of forming complexes with glucose oxidase. The kinetics of glucose-induced changes in AO absorbance invites further investigations. All the examined dyes, except auramine O, are applicable to the design of low cost absorbance-based glucose biosensors.

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REFERENCES

1. Rich R.L. and Myszka D.G. // J. Mol. Recognit. 2002. V.15. P. 352-376.
2. Vo-Dinh T., Cullum B. // Fresenius J. Anal. Chem. 2000. V.366. P. 540-551.
3. Fraser D.M. // Medical Device Technology. 1994. V.5. P. 44-47.
4. Ravi Shankaran D., Uehara N., Kato T. // Biosensors and Bioelectronics. 2003. V.18. P. 721-728.
5. Bianco P. // Reviews in Molecular Biotechnology. 2002. V.82. P. 393-409.
6. Rosenzweig Z., Kopelman R. // Anal. Chem. 1996. V.68. P. 1408-1413.
7. Mehrvar M., Bis C., Scharer J.M., Moo-Young M., and Luong J.H. // Analytical Sciences. 2000. V.16. P. 677-692.
8. Potyrailo R.A., Hobbs S.E., Hieftje G.M. // Fresenius J. Anal. Chem. 1998. V.362. P. 349-373.
9. Mchedlov-Petrossyan N.O., Isaenko Yu.V., Salamanova N.V., Alekseeva V.I., and Savvina L.P. // Journal of Analytical Chemistry. 2003. V.58. P. 1018-1030.
10. Gorbenko G.P., Mchedlov-Petrossyan N.O., and Chernaya T.A. // J. Chem. Soc., Faraday Trans. 1998 V.94. P. 2117-2125.

ВЛАСТИВОСТІ ІНДИКАТОРНИХ БАРВНИКІВ В СИСТЕМІ ГЛЮКОЗООКСИДАЗА + ГЛЮКОЗА

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З використанням методу абсорбційної спектроскопії групу індикаторних барвників, що включала бромкрезоловий пурпуровий, феноловий червоний, нейтральний червоний, аурамін О та акридиновий оранжевий, було досліджено з точки зору можливого застосування цих барвників для розробки оптичних біосенсорів на глюкозу. Зміни поглинання бромкрезолового пурпурового, фенолового червоного та нейтрального червоного, що спостерігались в системі глюкозооксидаза + глюкоза свідчать про чутливість цих барвників до зниження pH реакційного середовища при каталітичному окисленні глюкози. Отримані докази на користь зв'язування нейтрального червоного з глюкозооксидозою.

КЛЮЧОВІ СЛОВА: біосенсор на глюкозу, глюкозооксидаза, індикаторні барвники