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Cyclic voltammetry study of glucose and insulin interactions with supported lipid membrane

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Abstract

The effect of D-glucose and insulin on conducting properties of supported bilayer lipid membranes (s-BLM) modified by anthraquinone-2-sulphonic acid (AQS) at the presence of potassium ferricyanide was studied by means of cyclic voltammetry (CV). Both the oxidation and the reduction current peaks were found to decrease at the presence of glucose in concentration range varying from 10 to 320 mM. The influence of insulin on membrane properties is ambiguous. While the pretreatment of membrane with 20 mU l^{-1} of insulin evoked slight increase of the current with unchanged course of the dependence of peak current on glucose, the decrease of conductance was observed above 10^5 mU l^{-1} of insulin.

Keywords: s-BLM; Glucose; Insulin; Cyclic voltammetry; Biosensor

1. Introduction

High stability of supported lipid bilayer membranes (s-BLM) and incorporation of electrochemically active molecules into the lipid matrix opens up the possibility for the construction of electrochemical biosensors based on s-BLM [1,2].

Glucose carriers mediate the transport of glucose across the biological membrane. Though the successful attempts of immobilization of glucose transporters into the planar lipid bilayer membranes [3] and the supported lipid bilayer membranes [4] for glucose biosensing have been made, glucose or its derivatives by itself could alter biophysical properties of the lipid matrix [5,6]. Glucose transport by carriers becomes activated by the binding of insulin to the specific receptors in the plasma membrane, but there is likewise an evidence of nonreceptor mediated mechanism of insulin–membrane interaction [7,8].

This study deals with the investigation of the effect of glucose and insulin on the conducting properties of s-BLM modified with anthraquinone-2-sulphonic acid (AQS) by cyclic voltammetry.

2. Experimental

S-BLMs were made of 5% egg phosphatidylcholine (Sigma) in ethanol and *n*-dodecane (Sigma) with 1 mM AQS (Sigma). Potassium ferricyanide, D-glucose and the rest of the chemical compounds were of the analytical grade. Neutral solution of biosynthetic human insulin was purchased from NovoNordisk. Hanks solution with 10 mM phosphate buffer prepared in deionised water was adjusted to pH=7.4. S-BLMs were formed as described previously [9] on a tip of a Teflon-coated platinum wire (diameter 0.5 mm, cross-section area 1.96×10^{-3} cm^2). Membrane self-assembly was monitored at 50 mV and 10 kHz by the capacitance plot. The electrode with lipid layer was kept in bathing electrolyte for several hours till the membrane became stabilized and suitable for the cyclic voltammetry measurements. AC measurement as well as recording of cyclic voltammograms (CV) was performed with an electrochemical analyzer IMbe (Zahner Elektrik). An Ag/AgCl electrode was used as reference. The concentrations of $K_3[Fe(CN)_6]$, glucose and insulin in the bathing electrolyte were changed during the cyclic voltammetry experiments by addition of the appropriate amount of the concentrated solution. The response was monitored after 30 min when the system was stabilized, i.e. CV did not changed in time. The electrical capacitance of s-BLM was also measured. The parameters of the s-BLM were measured at room temperature (21±1 °C).

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3. Results and discussion

Membrane parameters reached the steady state values several hours after immersing a platinum wire with a lipid drop into the bathing electrolyte. In our experiments, the specific capacitance of s-BLM per unit area for the stabilized membranes reached 0.06 μF cm^{-2}. This value is considerably lower than the specific capacitance of the free standing bilayer lipid membranes (BLM). Analysis of the electrical parameters of lipid layers on a rough metal support (a tip of metal wire) lead the authors of Ref. [10] to the essential revision of s-BLM model as a simple lipid bilayer on a solid support. According to the work cited above, rather than a bilayer, a monolayer and even a three-layer structure appeared on the metal surface, interrupted by "islands" of uncovered metal support. During our experiments, s-BLMs were stabilized at a higher thickness and seems to have multilayer structure. Therefore, the occurrence of the uncovered areas of metal support and consequently direct contact of electrolyte with platinum seems to be less probable due to the rather low specific capacitance of s-BLMs obtained in our work.

CVs of s-BLMs modified with AQS were recorded in the presence of $K_3[Fe(CN)_6]$ (Fig. 1). Concentrations of potassium ferricyanide at which the oxidation and reduction peaks have occurred strongly depended on the parameters of the s-BLM in the steady state. Generally, they were observed at 200 mM ferricyanide concentration. From CV measured between 0 and 800 mV at voltage scan rate 50 mV s^{-1}, well-pronounced oxidation and reduction peaks can be noticed at 660 and 525 mV, respectively. Peak-to-peak separation as well as the ratio of peak currents indicates possible irreversibility of electron transport. The dependence of relative change of peak current on the glucose concentration clearly shows pronounced membrane response to glucose (Fig. 2). The peak current of s-BLMs treated with glucose in the concentrations exceeding physiological ones (10–320 mM) was found to decrease; at the concentration exceeding 300 mM, the oxidation and the reduction peaks became even completely extinct (not shown). The results obtained evidence that glucose can effectively influence the properties of AQS modified s-BLM and block all "active sites" in the membrane.

The specific capacitance of s-BLM after the treatment with glucose was considerably higher than initial values and reached approximately 0.34 μF cm^{-2}.

Though an electron transfer between ferricyanide and Pt electrode through AQS modified s-BLM is supposed to be the main cause of redox reaction, a direct electron transfer of electroactive species through the structural defects in the lipid layer induced by the modification of membrane with AQS is equally possible.

The increase of membrane capacitance and the decrease of the elasticity modulus $E_{\perp}$ of s-BLM treated with high glucose concentration (0.3 M) have been demonstrated in Ref. [5]. The changes of membrane parameters due to the solvent redistribution from the thicker regions of s-BLM could also influence the distribution of mediator molecules through s-BLM or their orientation in the membrane, resulting in the reduction of the current of either oxidation and reduction peak after the application of glucose.

Taking into consideration a possible extension of the measurements to probing for glucose in human blood, physiological concentration of insulin (20 mU l^{-1}=0.134 nM) was examined. The treatment of the s-BLM with insulin evoked the slight increase of redox current. Following addition of glucose produced the similar reduction of peak current as for the previous experiments with glucose. In contrast to the effect of 20 mU l^{-1} of insulin, the treatment with insulin from 1 to 30 μM brought about peak reduction in the whole observed interval (Fig. 3). After CV experiments examining the influence of insulin in the concentration range from 1 to 30 μM on the s-BLM properties, the moderate decrease of the specific membrane capacitance was observed—it changed from the initial average value 0.09 μF cm^{-2} to 0.07 μF cm^{-2} for the set of seven membranes.

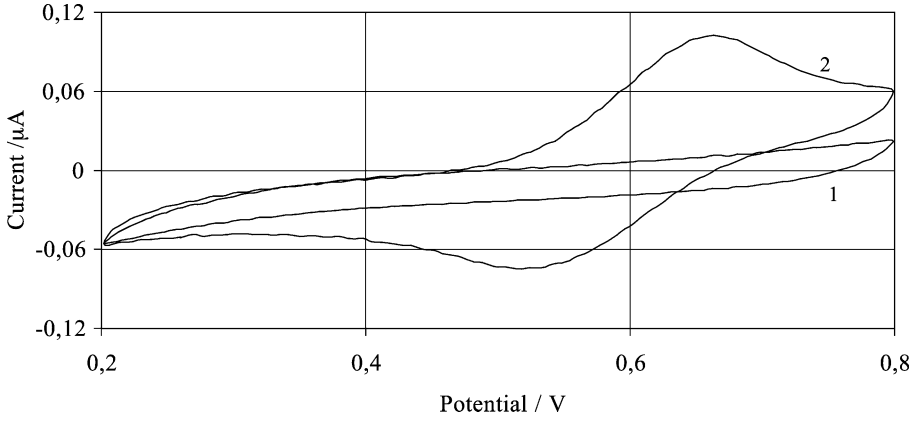

Fig. 1. Cyclic voltammogram of s-BLM modified with 1 mM AQS (1) and s-BLM modified with 1 mM AQS in the presence of 120 mM $K_3[Fe(CN)_6]$ (2). Scan rate is 50 mV s^{-1}.

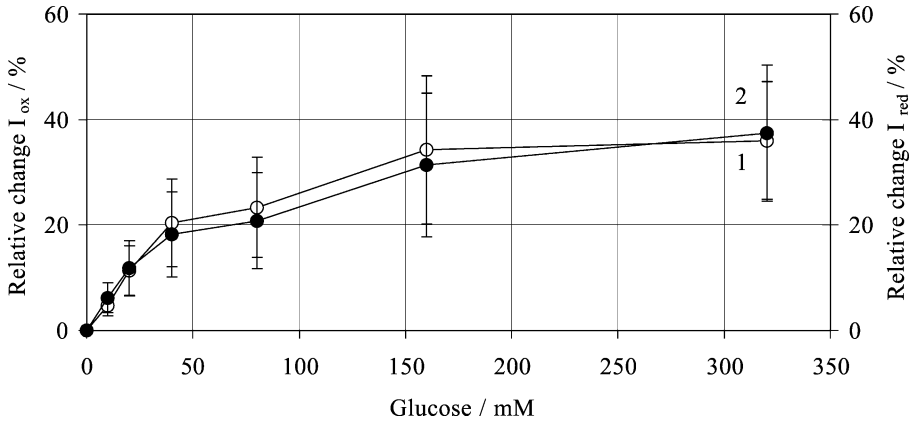

Fig. 2. Dependence of the relative change of oxidation (○) and reduction (●) peak current of the self-assembled phosphatidylcholine membranes modified with 1 mM AQS on the glucose concentration. Results are means of five experiments±S.E.

The influence of insulin on the supported membrane properties is ambiguous as far as the low concentration of protein increases the conductance of s-BLM modified with AQS, the decrease of redox current is observable at the insulin concentration above 10^5 mU l^{-1}.

The membrane–insulin interaction can be effectively modified not only by major membrane structural lipid, but the additives of different origin as well [11]. AQS molecule could play a role of such an additive inducing the changes of the physical properties of the membrane and facilitating the insertion of insulin into the membrane or its absorption on membrane surface, in contrast to the lipid membrane without modifiers. It was demonstrated [12] that the increased internalization rate of insulin by synthetic liposomal membranes might result from an increase in membrane fluidity caused by chromium picolinate.

Nonspecific influence of insulin on the viscoelastic properties of the planar lipid bilayer membranes without any protein receptor or modifier demonstrated by authors of the paper [8] showed that insulin at low concentrations (10^{-11}–10^{-9} M) adsorbs on the lipid membrane with the insertion of peptidic chains into the hydrophobic region of the membrane. The change of membrane viscoelastic properties at distances exceeding the effective diameter of the peptide molecule associated with the increase of membrane surface charge at pH=7.4 caused a diminution in the Young elasticity modulus and the coefficient of dynamic viscosity that was followed by an increase in membrane conductivity. Our experiments with low insulin concentration are consistent with the results of the studies cited above. Adsorption of insulin present in physiological concentration in bathing electrolyte increases the surface potential [8] and facilitates the contact of electroactive species with the platinum electrode, resulting in an enhancement of membrane conductivity in accord with the results described in Ref. [8].

Our findings suggest that such a high concentration of insulin, as 1–30 μM, could cover the supported membrane minimizing the number of "binding sites" in s-BLM. The result is consistent with the work of Omodeo-Salè et al. [13]; according to that, high concentration of insulin may

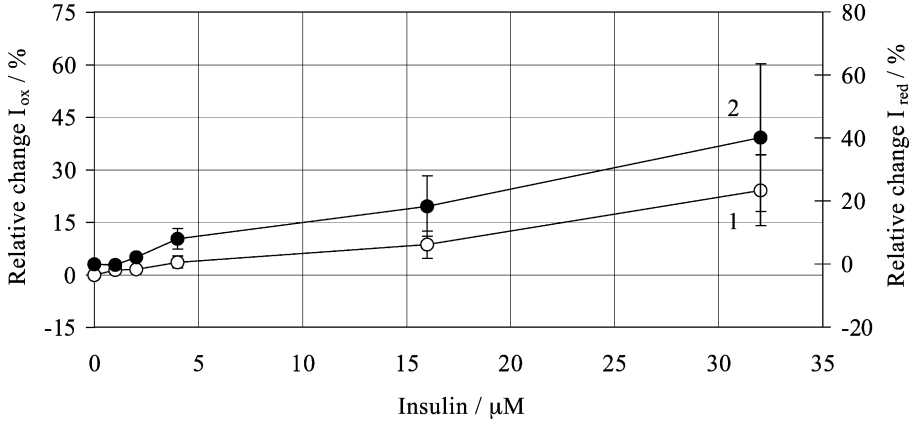

Fig. 3. Dependence of the relative change of oxidation (○) and reduction (●) peak current of the self-assembled phosphatidylcholine membranes modified with 1 mM AQS on the insulin concentration. Results are means of seven experiments±S.E.

simply "coat" the bilayer structure of the large unilamellar vesicles by hydrogen or electrostatic bonds without penetrating into the hydrophobic core of the membrane.

The exact mechanism of insulin–bilayer interaction leading to change of CV current response of supported membrane as well as the electrochemistry of the occurring redox reactions cannot be exactly deduced from our experiments. However, they might be admittedly important for the successful construction of an s-BLM-based biosensor sensitive to glucose.

4. Conclusions

The experiments revealed the influence of glucose on the electrochemical properties of s-BLM modified with AQS at the concentration above the physiological level in contrast to the unmodified membrane. At the same time, the course of the dependence on glucose was unchanged for the low concentration of insulin. We suppose that the hyperglycemic concentration of glucose change membrane fluidity. It seems possible that glucose influence the ordering of bilayer lipid membrane, which results in lower transmembrane current. The effect of concentration dependence of current in oxidation and reduction peaks could be used for constructing glucose sensor based on s-BLM.

Acknowledgements

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