Following a protein kinase activity using a field-effect transistor device

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Received (in Cambridge, UK) 22nd May 2007, Accepted 9th July 2007
First published as an Advance Article on the web 30th July 2007
DOI: 10.1039/b707677k

The specific phosphorylation of a peptide-functionalized ion-sensitive field-effect transistor device by casein kinase II in the presence of ATP enables the electronic readout of the protein kinase activity; treatment of the phosphorylated surface with alkaline phosphatase results in the regeneration of the active sensing surface.

Field-effect transistor (FET) devices are attracting growing interest as electronic transducers for the label-less detection of biorecognition events or biocatalytic transformations.[1] Two general strategies are used to apply FETs for biosensing. In one method, the degree of charging of the gate surface by the biorecognition events, or the biocatalytic processes, is used to control the gate potential, thus enabling the readout of the biosensing events.[2] For example, the transduction of DNA hybridization events,[3] the analysis of aptamer–substrate complexes[4] or the analysis of tyrosinase activity by the association of dopamine to a boronic acid ligand associated with the gate surface[5] represent biosensors based on the charging (or discharging) of the gate surface. In a second approach, the gate is modified by a redox-active unit, and the biocatalytic transformations alter the equilibrium of the reduced/oxidized states of the modifier, thus controlling the gate potential. For example, FET devices modified with the redox-active units of pyrroloquinoline quinone[6] or dopamine[5] were used to sense the activities of NAD^+-dependent enzymes (and their substrates) and the activity of tyrosinase, respectively.

The protein kinases are a large family of enzymes that modulate the activity of proteins by phosphorylation. They regulate the majority of cellular pathways, especially those involved in signal transduction. One of the most versatile of the protein kinases is casein kinase II (CK2), a serine/threonine-selective protein kinase, which can phosphorylate more than 160 proteins. It has been shown that CK2 is involved in signal transduction, transcriptional control, apoptosis, cell cycle and more.[7]

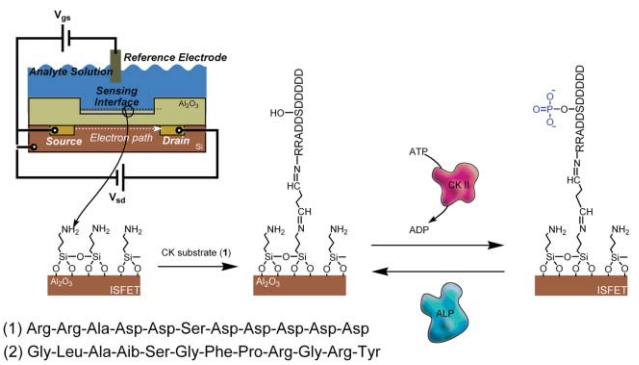
Aberrant activity of CK2 has been implicated in a number of diseases. For example, both reduced amount and reduced activity of CK2 were found in neurons of patients with Alzheimer's disease. On the other hand, elevated amounts of CK2 were found in various types of cancer.[8] It also plays a major role in the life cycle of HIV-1, and it has been found that CK2 is a selective target of HIV-1 transcriptional inhibitors.[9]

Therefore, quantifying CK2 activity from different tissue samples, and having a method for screening for CK2 inhibitors are of great interest to the medical community. Traditionally, kinase activity is quantified using radiolabeled [γ^{32}P]-ATP. More recent studies used an antibody against a phosphorylated amino acid residue,[10] or a biotin coupled to the g-phosphate of ATP,[11] as reporters of the phosphorylation step. In both cases an additional label (such as a fluorescent dye) is needed for the detection.

In the present study we present the use of a FET device for the label-less electrical detection of CK2 activity. We demonstrate the specificity and reuse of the sensor device.

For the detection of kinase activity, the gate surface was functionalized with the peptide (**1**), as depicted in Scheme 1. This peptide includes the serine residue adjacent to aspartic acid, and it represents the sequence-specific peptide that is recognized by CK2. The primary modification of the Al_2O_3 gate of an ISFET device was achieved by the treatment of the ISFET with 3-aminopropyltriethoxysilane (0.2 μl, 10% (v/v) solution in toluene) at room temperature for 12 h. Peptides (**1**) or (**2**) were covalently linked to the aminosiloxane-functionalized gate interface by treatment of the gate with glutaric dialdehyde (0.2 μl, 10% (v/v) solution in water) at room temperature for 20 minutes. The chips were rinsed with water and with a HEPES buffer solution (10 mM, pH 6.3), and then treated with (**1**) or (**2**) (0.2 μl, 1 mM in HEPES buffer solution) for 20 minutes.

Fig. 1(A), curve (a) shows the changes in the gate-to-source potential, ΔVgs, upon the treatment of the peptide-functionalized gate with CK2, 0.012 U, and adenosine triphosphate, for different time intervals. As the time interval for interaction with CK/ATP is prolonged the changes in ΔVgs increase, and they level off after 20 minutes to a value of ΔVgs ~ 40 mV. Control experiments revealed that no changes in the gate-to-source potential are observed upon treatment of the (**1**)-functionalized device with CK or ATP alone, implying that both the enzyme and ATP are essential to induce the chemical reaction on the gate surface. Furthermore, the attachment of the foreign peptide, (**2**), to the gate surface, and the interaction of the (**2**)-modified peptide with



Scheme 1 Modification of an ISFET device for the specific analysis of casein kinase II activity and for the recycling of the sensing interface by alkaline phosphatase.

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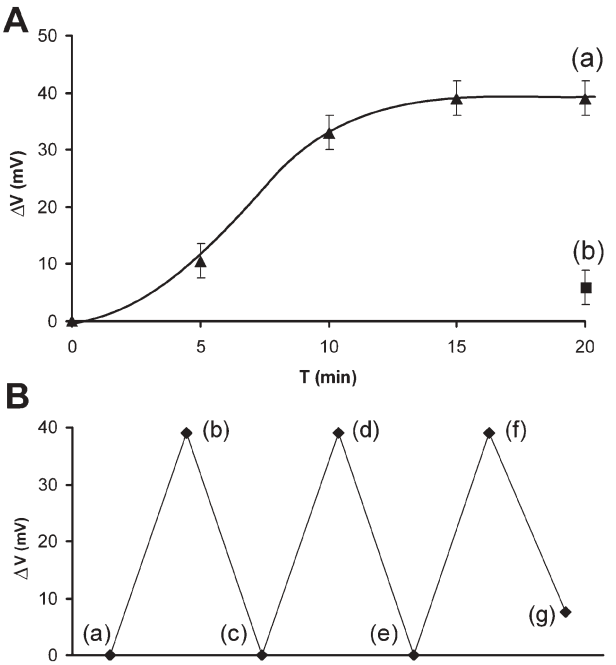

Fig. 1 (A) (a) Time-dependent changes in the source-to-gate potential upon the treatment of the (**1**)-functionalized device with CK2, 0.012 U, and ATP, 100 μM. (b) Potential change of the (**2**)-modified device upon treatment with CK2, 0.012 U, and ATP, 100 μM, for 20 minutes. (B) Cyclic source-to-gate potential changes corresponding to (a) (**1**)-modified ISFET device before treatment with CK2/ATP. (b) After treatment with CK2/ATP. (c) After treatment of the phosphorylated gate with alkaline phosphatase. (d) and (e) After a second cycle of phosphorylation with CK2/ATP and alkaline phosphatase, respectively. (f) and (g) After a third cycle of phosphorylation with CK2/ATP and alkaline phosphatase, respectively. Phosphorylation was performed in the presence of CK2, 0.012 U, and ATP, 100 μM, for 20 minutes. Hydrolysis of the phosphorylated surface was performed in the presence of alkaline phosphatase, 0.004 U for 20 minutes. All measurements were performed in 20 mM Tris-HCl buffer solution, pH = 7.5, that included 50 mM KCl and 10 mM $MgCl_2$.

CK/ATP did not yield any noticeable potential changes, ΔVgs = 6 mV after 20 minutes of reaction, Fig. 1(A), point (b). These results are consistent with the fact that CK and ATP specifically phosphorylate the sequence-specific peptide. The resulting negatively charged phosphate units alter the gate potential and lead to the observed potential changes. As the reaction with the enzyme/ATP is prolonged the content of the phosphate units increases, leading to higher ΔVgs values. The saturation of the surface groups by the phosphate units leads to the saturation value of ΔVgs = 40 mV.

One proof that phosphorylation indeed occurred on the (**1**)-modified surface was obtained by treatment of the phosphorylated gate with alkaline phosphatase that catalyzed the hydrolysis of the phosphate ester group to the neutral peptide (**1**). Upon application of the hydrolyzing enzyme, the gate potential returned to the original value after 20 minutes, Fig. 1(B), point (c). This is consistent with the cleavage and removal of the charged phosphate units. The resulting device was subsequently treated with CK/ATP and after 20 minutes the potential changes regenerated the value ΔVgs = 40 mV, Fig. 1(B), point (d). These results indicate that the serine residue of (**1**) was re-phosphorylated. By the cyclic treatment

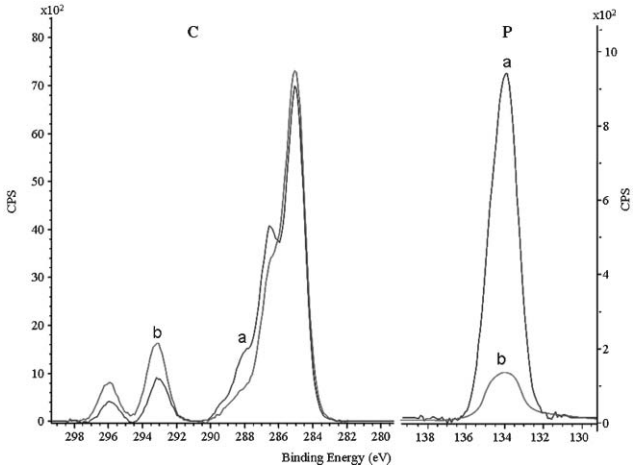

Fig. 2 XPS curves corresponding to the (**1**)-functionalized gate ISFET. (a) After treatment with CK, 0.012 U, and ATP, 100 μM, for 20 minutes. (b) After the treatment of the phosphorylated gate with alkaline phosphatase, 0.004 U, for 20 minutes. All measurements were performed in 20 mM Tris-HCl buffer solution, pH = 7.5, that included 50 mM KCl and 10 mM $MgCl_2$.

of the phosphorylated surface with alkaline phosphatase and then with CK/ATP the gate-to-source potential was switched between the base potential level and 40 mV, Fig. 1(B).

Further evidence that phosphorylation occurred on the peptide-functionalized gate was obtained by XPS measurements. Fig. 2 curves (a) and (b) show the XPS spectra of the peptide-modified gate after reaction with CK/ATP, and after application of alkaline phosphatase, respectively. After the phosphorylation process occurred, the phosphorus band at 133 eV is clearly visible. Subsequently, upon the application of the hydrolyzing enzyme, a marked decrease of *ca.* 80% in the P : C ratio can be observed.

The success in monitoring the CK activity by the (**1**)-functionalized device was then used to assay the enzyme activity, Fig. 3. In this experiment, the (**1**)-functionalized device was reacted with different concentrations of CK in the presence of ATP, 100 μM, for a fixed time interval of 20 minutes. Fig. 3 depicts the calibration curve that corresponds to the resulting changes of the

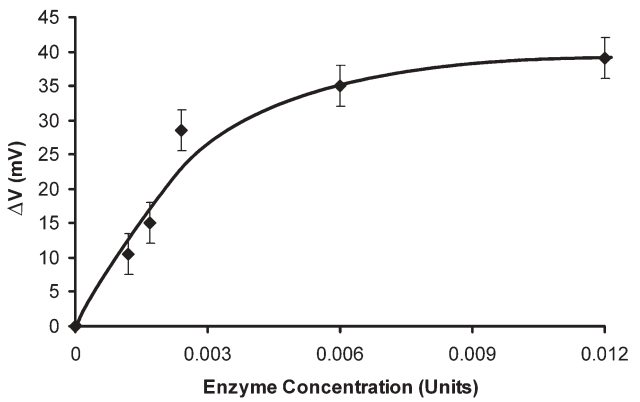

Fig. 3 Source-to-gate potential changes upon phosphorylation of the (**1**)-functionalized device with different concentrations of CK2 in the presence of ATP, 100 μM, for 20 minutes. Readouts were made in 20 mM Tris-HCl buffer solution, pH = 7.5 that included 50 mM KCl and 10 mM $MgCl_2$.

gate-to-source potential. The sensitivity limit for the detection of CK is *ca.* 1.5×10^{-3} U mL^{-1}. The shape of the curve depicted in Fig. 3 is consistent with the fact that the FET response is a logarithmic function of the phosphate surface charges (although the phosphate coverage depends linearly on the enzyme concentration).

To conclude, the present study has demonstrated the successful label-less specific analysis of casein kinase II using a field-effect transistor device. We demonstrated that the device is reusable and that the treatment of the phosphorylated sensor with alkaline phosphatase regenerated the sensing device. This approach can be extended for analyzing other phosphokinases. It seems that the major potential application of the present analytical system is to probe different inhibitors of phosphokinases for clinical evaluation.

This research is supported by Intel Co., Israel.

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