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Portable and sensitive detection of protein kinase activity by using commercial personal glucose meter

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ABSTRACT

Protein kinases (PKs), which play critical regulatory roles in the cellular signal transduction pathways, have been regarded as important molecular targets for clinical diagnosis as well as pharmaceutical development. Most of the existing PK assays rely heavily on the use of expensive and sophisticated instruments, which are only available in specialized labs. Herein we wish to report a new sensing strategy for portable and sensitive detection of PK activity by use of a commercial personal glucose meter (PGM) for signal readout with the assistance of Zr^{4+}-functionalized magnetic beads (ZrMBs). In this design, ZrMBs can selectively capture PK-induced biotin-phosphopeptide substrate against the unphosphorylated ones. Streptavidin (STV) acts as a bridge to further bring biotin-invertase onto the surface of ZrMBs in correlation with the amount of immobilized biotin-phosphopeptides. Finally, the anchored invertase on the ZrMBs can catalyze the hydrolysis of sucrose to glucose with millions of turnover, which is monitored by the PGM with amplified signal. Although only common PGM is used for signal readout, as low as 0.0001 U/μL protein kinase A (PKA) can be clearly detectable in this study and the sensitivity is well comparable to those assays by using large-scale equipment. Therefore, due to the distinct features of PGM such as low cost, simple operation, wide accessibility and portability, this proposed assay shows great potential for the point-of-care testing of PKs activities in the future.

1. Introduction

Phosphorylation of cellular proteins by protein kinases (PKs) is the most important post-translational modification mechanism in the signal transduction pathways, which plays vital roles for the regulation of various cellular biological processes [1,2]. Aberration of PK activities may lead to abnormal levels of protein phosphorylation states, which is profoundly associated with the etiology of many human diseases [3,4]. As such, the levels of PK activities may be regarded as an indicator for clinical diagnosis and more importantly, PKs are also important molecular targets for the pharmaceutical intervention of several diseases [5,6]. In this regard, sensitive, low-cost and simple assay for quantitative detection of PK activities is of great significance.

Traditionally, radioactive methods used to be considered as the standard protocols for assaying PK activities by use of ^{32}P-labeled adenosine triphosphate (γ-^{32}P-ATP) [7,8]. Since the radioactive labels are harmful to the environment and human health, significant efforts have been devoted to the development of non-radioactive PK assays. Up to now, a variety of PK assays have been established by using various analytical techniques, such as fluorescence [9,10], flow cytometry [11], quartz crystal-microbalance [12], electrochemistry [13–15], mass spectrometry [16], colorimetric methods [17,18], magnetic resonance imaging [19] as well as electrogenerated chemiluminescence [20]. All of these methods have their own advantages and they have made great advances for evaluating PK activities. However, unfortunately, a majority of these methods require laboratory-based expensive and

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sophisticated instruments that are only available in specialized labs. Therefore, it still remains a great challenge to develop simple, low cost and practical PK assays that can be used in the field in order to keep face with expectations in future for the point-of-care testing (POCT) of PK activities.

As for POCT, the personal glucose meter (PGM) may be the most successful device that enables in-home medical diagnosis. Due to its compact small size, low cost, simple operation and reliable quantitative results, PGM has been widely accepted by millions of diabetic patients for daily monitoring of blood glucose levels. Notably, very recently, based on the elegant design of enzyme-catalyzed transformation of PGM-inert sucrose (or amylon) to PGM-detectable glucose, Lu's group and some other groups have successfully applied the commercial PGMs to the detection of a broad range of non-glucose targets including antibodies, DNA, metal ions and small molecules [21–25].

Inspired by these pioneering works, we wish to report herein a new strategy for portable, low cost and sensitive detection of PK activities by use of a PGM as the signal transducer. The key design of our strategy is to use invertase as a "bridge" to interpret the PK-induced phosphopeptide recognition events into measurable readout by the PGM. In this work, Zr^{4+}-functionalized magnetic beads (ZrMBs) are used to specifically capture the kinase reaction-produced biotin-phosphopeptides over the unphosphorylated peptides. Afterward, streptavidin (STV), which has multiple binding sites for biotin, acts as the cross-connection to bring the biotin-invertase onto the ZrMBs in correlation with the amount of captured biotin-phosphopeptides. So the amount of anchored invertase on ZrMBs will be proportional to the initial activity of PK. Finally, the invertase can catalyze the hydrolysis of sucrose into glucose with millions of turnover and thus transform the PK activity into the levels of glucose detectable by PGM.

2. Experimental

2.1. Materials and reagents

cAMP-dependent protein kinase A (PKA, catalytic subunit) was purchased from New England Biolabs. Src (active) and H-89 were obtained from Millipore. PKA-specific substrate peptide (biotin-LRRASLG) and Src-specific substrate peptide (biotin-RRRRIYGEFKKK) were custom synthesized by GL Biochem (Shanghai, China). ATP and sucrose were from Sangon Biotech (Shanghai, China). Streptavidin (STV) was purchased from Promega. Invertase from baker's yeast was supplied from Sigma–Aldrich. Biotin-N-hydroxysuccinimide ester (biotin-NHS) was obtained from Alfa Aesar. The culture and drug stimulation of MCF-7 breast carcinoma cells as well as the preparation of cell lysates were performed according to previous reports [26,27]. ZrMBs (with an average diameter of 5–6 μm) were prepared according to our previous reported method [28]. The commercial personal glucose meter used in this study was an ACCU-CHEK Active glucose meter (Roche, Germany). All other reagents used in this work were of analytical grade and used as purchased without further purification.

2.2. Standard procedures for the detection of PKA activity

In a typical 100 μL PKA reaction system, biotin-LRRASLG peptides (6 μM) were treated with a certain concentration of PKA at 30 °C for 1 h in 50 mM Tris–HCl buffer (pH 7.5) containing 10 mM of $MgCl_2$ and 36 μM of ATP. After the phosphorylation reaction, the mixture was mixed with an equal volume of acetonitrile containing 1.0 mg/mL of ZrMBs and incubated for 1 h at room temperature under gentle shaking. After magnetic isolation,

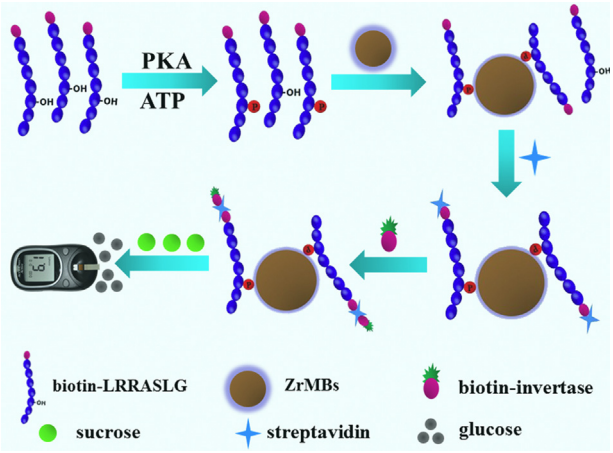

Fig. 1. Design principle of the PGM-based portable PKA assay.

the biotin-phosphopeptide-loaded ZrMBs were further dispersed in 50 μL 1% BSA and then 10 μg of STV was added to the mixture and incubated for 1 h at room temperature under shaking. The ZrMBs were then washed three times with Buffer A (0.1 M phosphate buffer with 0.2 M NaCl, pH 7.3) containing 0.1% BSA to remove unbound STV. Afterward, the isolated ZrMBs were redispersed in 100 μL Buffer A containing 0.625 mg/mL biotin-invertase and incubated for 1 h at room temperature under shaking. After removing the unbound biotin-invertase via several washing cycles, 50 μL of 0.5 M sucrose in HAc–NaAc buffer (pH 4.5) was added to the ZrMBs and incubated at 55 °C for 2 h to conduct the invertase-catalyzed hydrolysis of sucrose. Finally, 3 μL of the supernatant was tested by the PGM.

For PKA inhibition assay, the experiments were carried out via similar procedures as those for PKA assay stated above, except for the pre-incubation of a fixed PKA concentration of 0.1 U/μL and varied concentrations of H-89 (0–5 μM) in the reaction mixture.

3. Results and discussion

3.1. Design principle of the portable kinase assay

The design principle of the PGM-based protein kinase assay is illustrated in Fig. 1 by using PKA as a proof-of-concept target and biotin-LRRASLG as its specific substrate. Under the catalysis of PKA, the γ-phosphoryl of ATP will be transferred to the hydroxyl group of serine residue (S) of the biotinylated peptide substrate. We have previously reported that ZrMBs can specifically capture and enrich phosphorylated peptides through the strong interaction between the phosphate groups and Zr^{4+} with facile magnetic separation [28]. Therefore, when the biotin-peptides are phosphorylated by PKA, the phosphopeptides will specifically bind on the ZrMBs while the unphosphorylated peptides will not. After magnetic separation of unbound peptides, STV, which has four binding sites for biotin, serves as a linker to connect the biotin-phosphopeptides with biotin invertase on the surface of ZrMBs in a sandwich-type manner. The invertase-anchored ZrMBs can catalyze the hydrolysis of sucrose into glucose with millions of turnovers, which can be quantified by a PGM. According to the design mechanism, the amount of invertase anchored on the ZrMBs will be proportional to the amount of immobilized biotin-phosphopeptides as well as the initial activity of PKA. The PGM reading signal is related to the amount of glucose and further indicates the amount of the captured invertase, which eventually reflects the PKA activity in the testing sample. It should be noted that the nonspecific adsorption of STV on the ZrMBs will lead to false positive signal. So the