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Peptide Biosensors for the Electrochemical Measurement of Protein Kinase Activity

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The kinase activities are elucidated using the novel redox-active cosubstrate adenosine 5′-[γ-ferrocene] triphosphate (Fc-ATP), which enables the kinase-catalyzed transfer of a redox active γ-phosphate-Fc to a hydroxyamino acid. In this report, a versatile electrochemical biosensor is developed for monitoring the activity and inhibition of a serine/threonine kinase, casein kinase 2 (CK2), and protein tyrosine kinases, Abl1-T315I and HER2, in buffered solutions and in cell lysates. The method is based on the labeling of a specific phosphorylation event with Fc, followed by electrochemical detection. The electrochemical response obtained from the "ferrocenylated" peptides enables monitoring the activity of the kinase and its substrate, as well as the inhibition of small molecule inhibitors on protein phosphorylation. Kinetic information was extracted from the electrochemical measurements for the determination of K_m and V_m values, which were in agreement with those previously reported. Kinase reactions were also performed in the presence of well-defined inhibitors of CK2, 4,5,6,7-tetrabromo-2-azabenzimidazole, 2-dimethylamino-4,5,6,7-tetrabromo-1*H*-benzimidazole, and *E*-3-(2,3,4,5-tetrabromophenyl)acrylic acid as well as the nonspecific kinase inhibitors, staurosporine and *N*-benzoylstaurosporine. On the basis of the dependency of the Fc signal on inhibitor concentration, K_i of the inhibitors was estimated, which were also in agreement with the literature values. The performance of the biosensor was optimized including the kinase reaction, incubation with Fc-ATP, and the small molecule inhibitors. Peptide modified electrochemical biosensors are promising candidates for cost-effective in vitro kinase activity and inhibitor screening assays.

Protein phosphorylation is a universal regulatory mechanism that plays a critical role in the transmission of signals controlling a diverse array of cellular functions including cell growth, survival differentiation, and metabolism.[1−3] Emphasizing the importance of protein phosphorylation is the existence of more than 500 protein kinases encoded within the human genome with more than 200 of these enzymes implicated in human diseases. Accordingly, in addition to elucidation of signal transduction mechanisms that control fundamental cellular processes, accurate detection of protein kinase activity is expected to provide insights regarding the underlying basis of disease. The emergence of protein kinases as therapeutic targets that have been highlighted by the success of the protein kinase inhibitor, Imatinib (Gleevec), for molecular-targeted therapy further reinforces the need for robust platforms to monitor protein kinase activities.

Existing methods for the measurement of protein kinase activities include fluorescence, radioactive, and surface-plasmon resonance-based systems that employ enzymatic reactions or phospho-specific antibodies to detect kinase activities.[4,5] Simple and cost-effective methods that can be readily adapted for multiplexed kinase detection are desirable to enable kinase activity profiling for diagnostic applications and to accelerate the in vitro elucidation of cellular signal transduction pathways. Electrochemical biosensors have recently been reported using biotin- and thio-conjugated ATP for the detection of phosphorylation reactions. However, additional labels were necessary for the electrochemical detection.[6,7] Electrochemical tyrosine oxidation was found as an effective label-free way to detect tyrosine phosphorylation reactions on surface-immobilized peptides.[8,9] Recently, Willner reported the development of a field-effect transistor device for the label-free electrical detection of CK2 activity[10] and demonstrated regeneration of the sensing surface after the treatment of the phosphorylated peptides with alkaline phosphatase (ALP). In order to simplify the detection process in a single-step, we previously demonstrated using Fc-ATP and purified components that a serine/threonine kinase, protein kinase C (PKC), activity could be detected using an electrochemical biosensing system.[11a] Schematic illustration of our electrochemical principal for the detection of kinase-catalyzed phosphorylation using Fc-ATP as the

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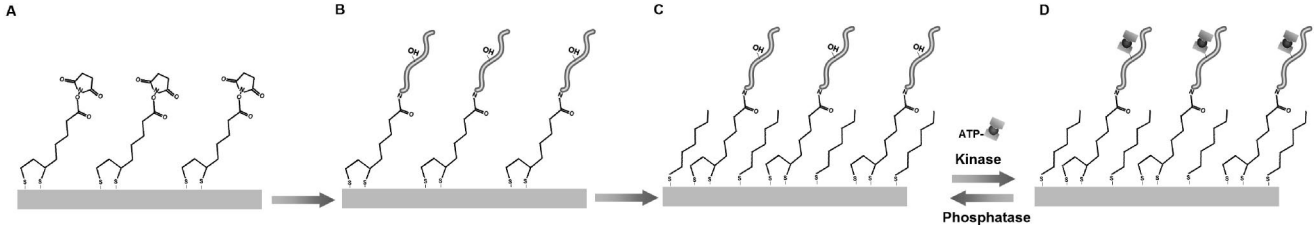
Scheme 1. Schematic Representation of the Covalent Immobilization of Substrate Peptides on the Gold Microelectrode Surface[a]



[a] Using the active-esters of lipoic acid and the electrochemical method for monitoring of the kinase-catalyzed phosphorylation and dephosphorylations of the surface-bound peptides using ferrocene-conjugated ATP (Fc-ATP) as the redox-active cosubstrate.

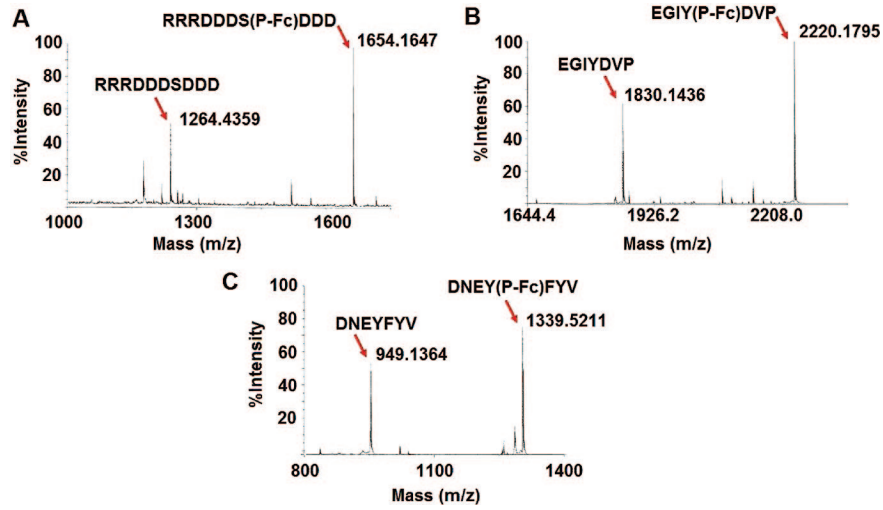


Figure 1. MS plot for the kinase-catalyzed phosphorylation of the substrate peptides for (A) CK2, (B) Abl1-T315I, and (C) HER2/ErbB2 using Applied Biosystems 4700 Proteomics Analyzer with a 2,5-dihydrobenzoic acid (DHB) matrix (10 mg/mL) and a 1:1 mixture with the sample. The sample containing the phosphorylated peptides were enriched and purified using the standard protocol of the Phosphopeptide Isolation kit (ThermoScientific Pierce).

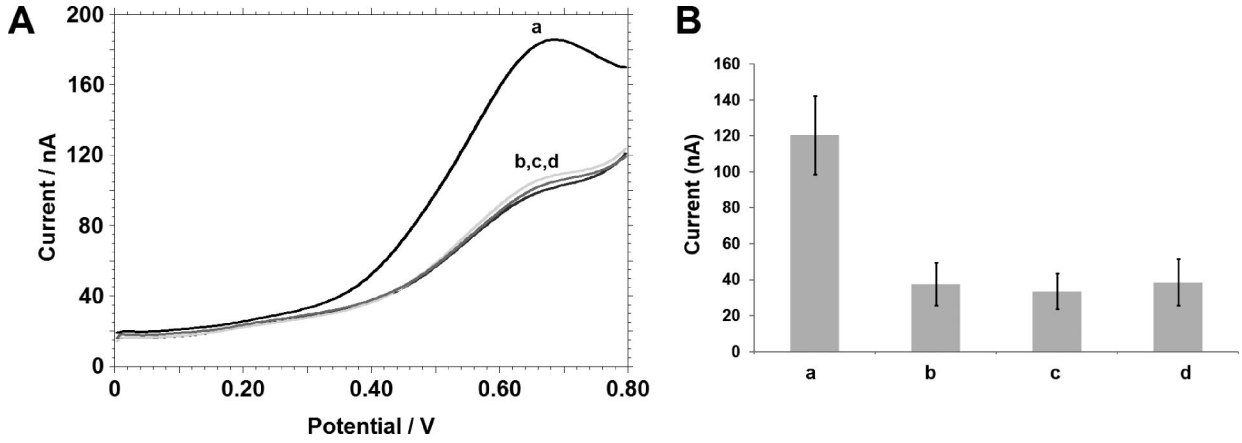


Figure 2. (A) Square wave voltammetry (SWV) of the CK2-catalyzed phosphorylation reactions performed in the cell lysates and (B) plot for the SWV-based detection of the CK2α expression state in various cell lysates. Error bars indicate the standard deviation of three consecutive measurements.

cosubstrate is shown in Scheme 1. The substrate peptide is immobilized covalently on the Au electrode surface via the sulfur bond of a lipoic acid ester. Kinase-catalyzed reaction transfers γ-phosphate-Fc group to the serine/threonine or tyrosine residues of the peptide. The Fc group attached to the peptide is electrochemically observed using cyclic voltammetry (CV). To extend this work, we sought to validate the utility of this method for the detection of another well-described protein serine/threonine kinase, casein kinase-2 (CK2), and two clinically important protein tyrosine kinases, Abl1-T315I and HER2/ErbB2, and to evaluate this method for measuring protein kinase inhibitor potency.

EXPERIMENTAL SECTION

Enzymes and Substrate Peptides. The CK2α and CK2α′ forms of CK2 and the peptide substrate (RRRDDDSDDD) were prepared in D. W. Litchfield's laboratory. Human osteosarcoma U2-OS cells with tetracycline-regulated expression of CK2 were prepared as described earlier.[11b]

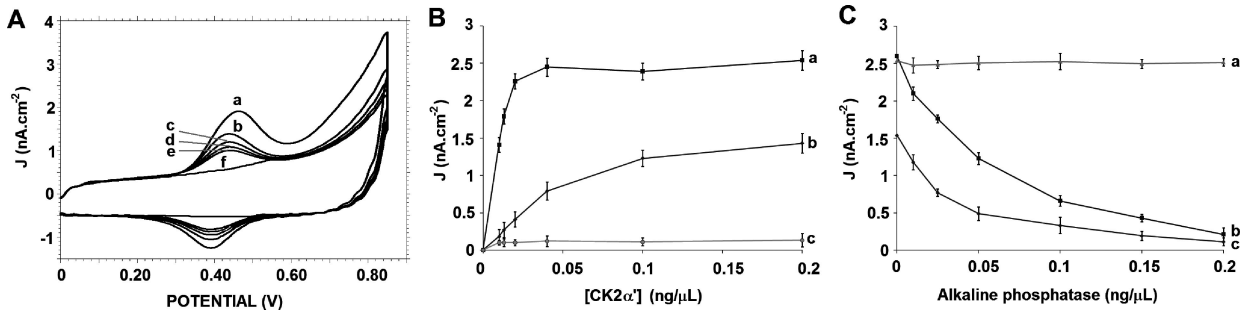


Figure 3. (A) Cyclic voltammetry (CV) for CK2α′-catalyzed phosphorylation of substrate peptide (RRRDDD**S**DDD) in the presence of CK2α′ at a concentration of (a) 0.04, (b) 0.02, (c) 0.008, (d) 0.005, (e) 0.0025 ng/μL, and (f) the control experiment in the absence of the enzyme. (B) Effect of the CK2α′ concentration on the current responses using substrate peptide modified electrodes (a) in the assay buffer, (b) in the presence of HeLa cell lysate at a 1:10 (v/v) ratio with the assay buffer, and (c) the control experiment was performed using the substrate peptide for Abl1-T315I (EGI**Y**DVP) in the presence of cell lysate at a 1:10 (v/v) ratio with the assay buffer for Abl1-T315I. (C) Plot for the dependence of the anodic current responses on the concentration of the alkaline phosphatase (ALP) with the substrate peptide immobilized on the surface (a) control experiment was performed by titrating the phosphorylated peptide with the electrolyte, (b) in the absence, and (c) in the presence of the HeLa cell lysate at a 1:10 (v/v) ratio with the CK2 assay buffer.

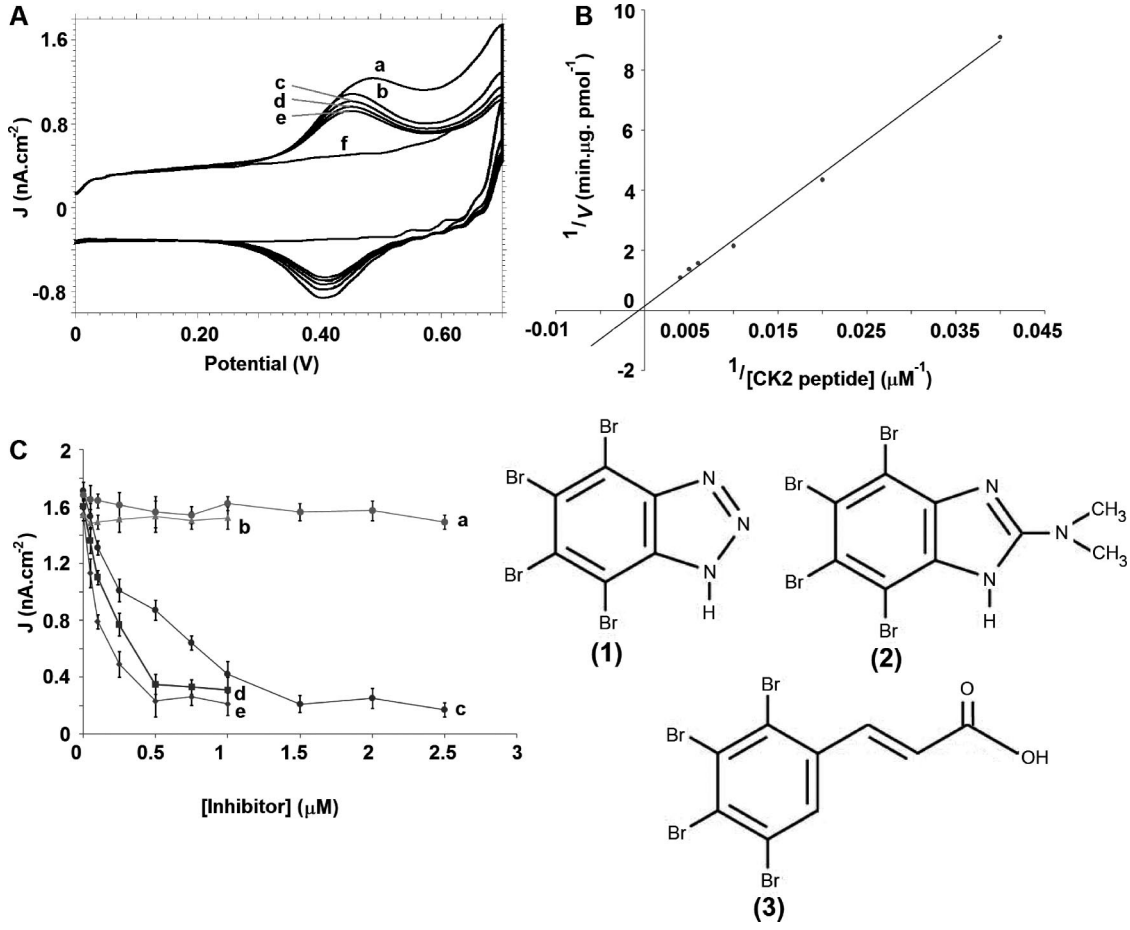


Figure 4. (A) Cyclic voltammograms for the inhibition of CK2α-catalyzed phosphorylation with the substrate peptide in the presence of the inhibitor, **1**, TBB (4,5,6,7-tetrabromo-2-azabenzimidazole) (a) 250, (b) 500, (c) 750, (d) 800, and (e) 900 nM and (f) control experiment in the absence of CK2α. (B) Lineweaver−Burk plot for the determination of kinetics of the CK2α′-catalyzed phosphorylation of the immobilized substrate peptide at varying surface density conditions on the Au microelectrode surface as described in the text. (C) Control experiments were performed by (a) titrating the phosphorylated substrate peptide on the surface with the assay buffer, which showed the stability of the electrochemical responses, and (b) EGI**Y**DVP was not inhibited upon exposure to **1** and demonstrated the specificity of the inhibition reactions, (c) the current reponses decreased rapidly in the presence of **2** (2-dimethylamino-4,5,6,7-tetrabromo-1*H*-benzimidazole), and (d) **3** (*E*-3-(2,3,4,5-tetrabromophenyl)acrylic acid) in the HeLa cell lysate.

The purified recombinant human HER2/ErbB2 kinase and Abl1-T315I mutant kinase and their substrate peptides, FLT3 (DNE**Y**FYV) and Signal Transduction Protein (STP, EGI**Y**DVP) peptides were purchased from Cell Signaling Technology (MA). Alkaline phosphatase (10 000 U/mL) was purchased from New England Biolabs (ON, Canada).

Peptide Immobilization on the Surface. Homemade gold microelectrodes (12.5 μm i.d.) were incubated with 5 mM NHS-

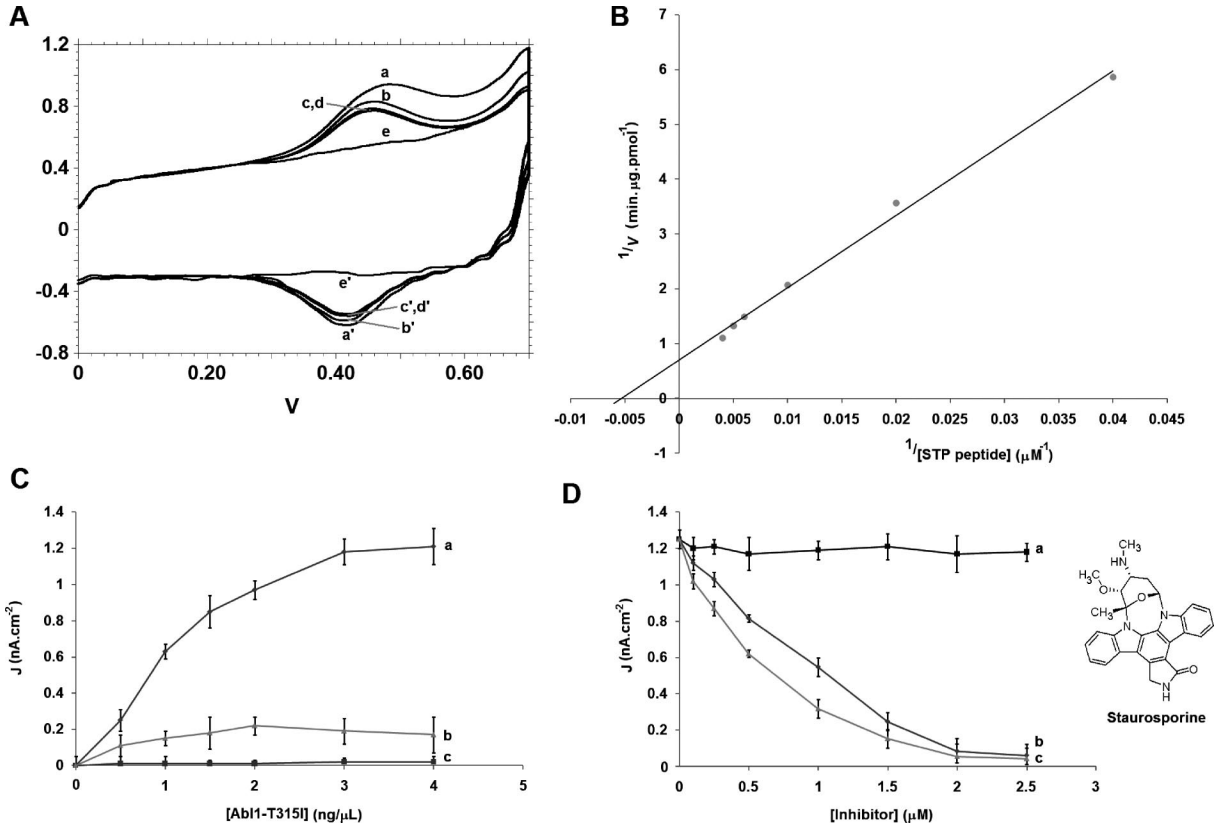


Figure 5. (A) CV for the inhibition of tyrosine kinase-catalyzed phosphorylation with the signal transduction protein (STP) peptide (EGI**Y**DVP) in the presence of Abl1-T315I at a concentration of (a) 3, (b) 1.5, (c) 1, (d) 0.75 ng/μL, and (e) in the absence of the enzyme. CV measurements were taken at 500 mV/s in blank 2 M $NaClO_4$. (B) Lineweaver−Burk plot for the determination of the kinetics of the Abl1-T315I-catalyzed phosphorylation of the immobilized STP peptide at varying concentrations on the surface as described in the text. (C) Plot for the dependence of the anodic current responses on the amount of the Abl1-T315I kinase in the presence of the HeLa cell lysate at a 1:10 (v/v) ratio with the kinase buffer with (a) the STP peptide immobilized on the surface, (b) a control experiment using the FLT3 peptide (DNE**Y**FYV), which is the substrate for HER2/ErbB2, and (c) a second control experiment involved the CK2 substrate peptide (RRRDDD**S**DDD). (D) Plot for the dependence of current responses on the concentration of the general protein kinase inhibitors, (b) staurosporine and (c) *N*-benzoylstaurosporine; the control experiments involved the titration of the phosphorylated STP peptide with the buffer titration in the presence of HeLa cell lysates (a).

lipoic acid ester in ethanol for 15 h (Scheme 1A). Then, the electrodes were incubated with the substrate peptides in the presence of 2 mM *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDC) in 0.1 M 2-(*N*-morpholino) ethanesulfonic acid (MES, pH 6) for 2 h (Scheme 1B). The microelectrodes were incubated with mercaptopolyethylene glycol 5′000 monomethyl ether (PEG-thiol 5′000) solution (1:100 v/v) in ethanol for 10 min for the blocking of the remaining Au surfaces (Scheme 1C).

CK2-Catalyzed Phosphorylation. The activity of CK2α and CK2α′ kinases were measured using the following conditions: 50 mM Tris HCl (pH 7.5), 10 mM $MgCl_2$, 150 mM NaCl, and 100 μM ATP-Fc in a total reaction volume of 25 μL. The substrate peptide modified electrodes were incubated at 37 °C for 2 h. After the incubation period, the electrodes were washed multiple times using 2 M $NaClO_4$. After the washing process, the electrodes were immersed into 2 M $NaClO_4$ for the electrochemical measurement using a Ag/AgCl reference electrode, which was connected with the electrolyte via a salt bridge, and a Pt wire was used as the counter electrode. Cyclic voltammetry (CV) was recorded at a scan rate of 500 mV/s using CHInstruments potentiostat (TX). With the use of the same three-electrode system, square-wave voltammetry (SWV) was performed with an increment of 4 mV at an amplitude of 25 mV and frequency of 20 Hz.

HER2/ErbB2 Catalyzed Phosphorylation. The activity of HER2/ErbB2 kinase was measured using the following conditions: 5 mM MOPS (pH 7.2), 2.5 mM β-glycerophosphate, 5 mM $MnCl_2$, and 100 μM Fc-ATP in the presence of FLT3 peptide substrate (and 10 ng/μL kinase in a total reaction volume of 25 μL). The substrate peptide modified electrodes were incubated at 37 °C for 2 h. After following the same washing procedures as described above, the CV measurements were recorded using the same parameters.

Abl1-T315I-Catalyzed Phosphorylation. The activity of Abl1-T315I kinase was measured using the following conditions: 60 mM HEPES (pH 7.5), 5 mM $MgCl_2$, 5 mM $MnCl_2$, 3 μM Na_3VO_4, 400 μM ATP, peptide substrate (STP), and the kinase in a total reaction volume of 25 μL. The substrate peptide modified electrodes were incubated at 37 °C for 2 h. After following the same washing procedures, the CV measurements were recorded using the same parameters as described above.

ALP-Catalyzed Dephosphorylation. The reaction conditions for the ALP included 100 mM NaCl, 50 mM Tris-HCl, and 10 mM $MgCl_2$ (pH 7.9). The reaction was performed for 2 h at 37 °C. One unit is defined as the amount of enzyme that hydrolyzes 1 μmol of *p*-nitrophenylphosphate to *p*-nitrophenol in a total reaction volume of 1 mL in 1 min at 37 °C. After following the same

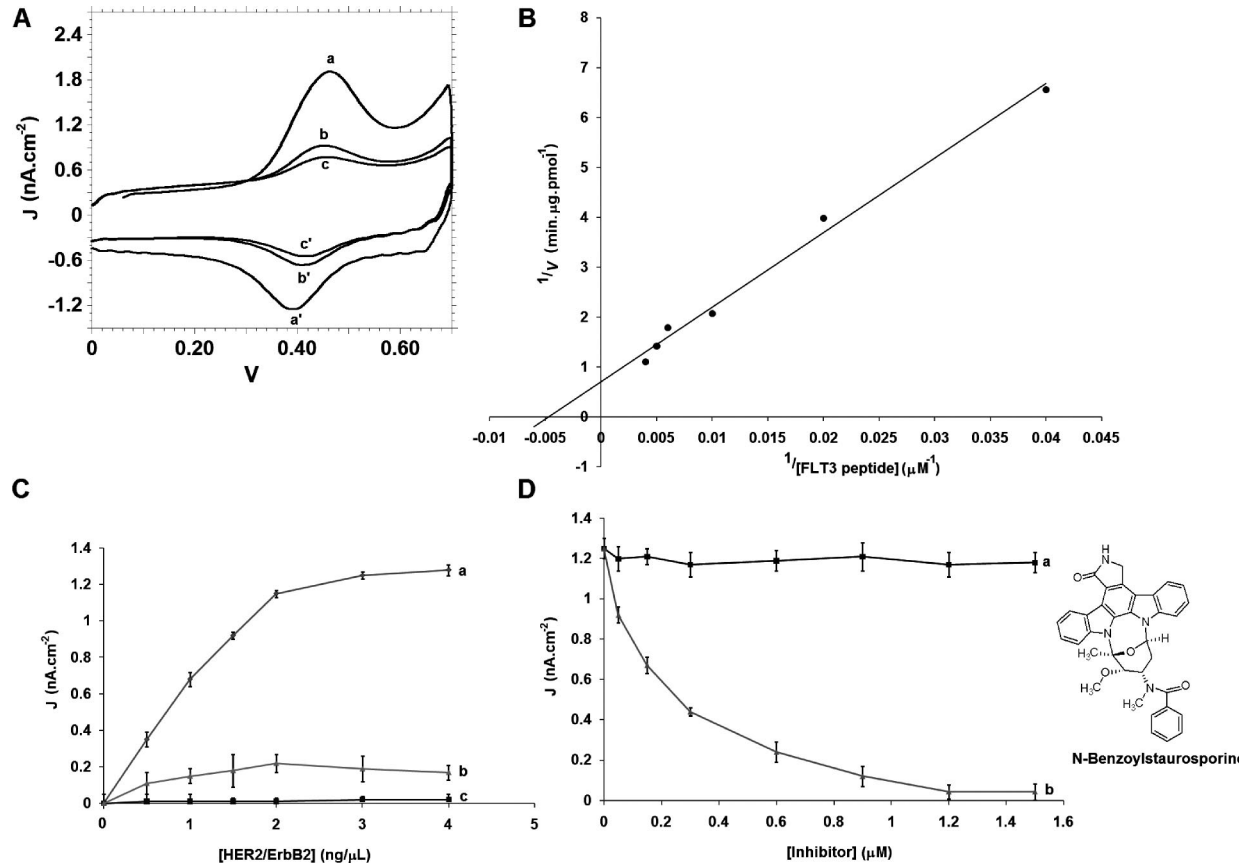


Figure 6. (A) Cyclic voltammograms for the inhibition of tyrosine kinase-catalyzed phosphorylation with the FLT3 peptide (DNE**Y**FYV) in the presence of HER2/ErbB2 at a concentration of (a) 4, (b) 0.5, (c) 0.25 ng/μL. CV measurements were taken at 500 mV/s in blank 2 M $NaClO_4$. (B) Lineweaver−Burk plot for the determination of kinetics of the HER2/ErbB2-catalyzed phosphorylation of the immobilized FLT3 peptide at varying concentrations. (C) Plot for the dependence of the anodic current responses on the amount of the HER2/ErbB2 kinase with (a) the FLT3 peptide immobilized on the surface in the presence of HeLa cell lysate at a 1:10 (v/v) ratio with the kinase buffer, (b) control experiment with STP peptide (EGI**Y**DVP) resulted in a slight increase in the current responses, (c) the CK2 substrate peptide (RRRDDD**S**DDD) in the presence of HeLa cell lysate at a 1:10 (v/v) ratio with the kinase buffer. (D) Plot for the dependence of *J* responses on the concentration of (b) *N*-benzoylstaurosporine, whereas (a) the phosphorylation of the immobilized FLT3 peptide was not affected with blank buffer titration in the presence of HeLa cell lysates.

washing procedures as described above, the CV measurements were recorded using the same parameters.

Cell Lysate Pretreatment. For the reactions containing cell lysates, 20×10^6 HeLa cells, which were obtained from the D. W. Litchfield's laboratory, were lysed in 1 mL of lysis buffer (50 mM Tris (pH 8), 150 mM NaCl, 10% glycerol, 0.5% Triton X-100) containing 1 mM phenylmethanesulphonylfluoride (PMSF, Pierce) by rotation for 10 min. During the phosphorylation reactions, Halt phosphatase inhibitor cocktail (Pierce) in a 1:1 ratio (v/v) with the cell lysate was used for suppressing the serine, threonine, and tyrosine phosphatase activites. The cell debris was collected at 12 000 rpm, and the supernatant was stored at 4 °C until use in subsequent reactions. For the reactions containing HeLa lysates, the lysate solution was mixed with the kinase reaction buffer at a ratio of 1:10 (v/v) and applied to the phoshorylation or dephosphorylation reactions as described above.

Calculation of Kinetic and Inhibition Constants. The initial time dependence of the kinase reactions were determined at these varying peptide concentrations. The phosphorylation reaction was stopped after 5, 15, 30, 60, 90, 120, and 150 min, and the measurement of the attached Fc was carried out. The reciprocals of the reaction rate values were plotted against the reciprocal of the peptide concentration, which gave a linear Lineweaver−Burk curve. The equation of this curve defines the kinetic data, where the *y* intercept is $1/V_{max}$. If the *y* is set to 0 and the equation is solved for *x*, the *x* intercept becomes equal to $-1/K_m$.

RESULTS AND DISCUSSION

CK2 is frequently overexpressed in tumors or leukemic cells and exhibits oncogenic activity in mice. A peptide biosensor to monitor CK2 activity was first prepared by attaching a peptide substrate RRRDDD**S**DDD for CK2 covalently to a surface-immobilized layer of lipoic acid-succinimide esters (Scheme 1A). The succinimide group on the lipoic acid reacted selectively and efficiently to immobilize the peptides (Scheme 1B), while the polyethyleneglycol (PEG) groups suppressed the nonspecific binding of proteins to the biosensor (Scheme 1C). The application of a solution containing recombinant CK2 and Fc-ATP into HeLa cell lysates resulted in robust phosphorylation of the immobilized peptide substrate as indicated by electrochemical measurements of the surface-confined Fc molecules (Scheme 1D).

Initially, we evaluated the enzymatic modification of kinase-specific peptide RRRDDD**S**DDD[12] for a well-described serine/

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threonine kinase, CK2, using mass spectroscopy with Fc-ATP as the cosubstrate. Our results clearly demonstrate that CK2 transfers the desired redox group to the target peptide. Additional reactions were carried out using the substrate peptides for Abl1-T315I and HER2/ErbB2, clearly showing the utility of our approach also for tyrosine kinases (Figure 1B,C).

Our general approach uses succinimide-ester lipoic acid for the formation of peptide films on the Au surface, as shown in Scheme 1A−C. As expected, the capacitive background current drops after the attachment of the Fc peptides to the surface to approximately 75% of that of the bare Au surface (data not shown). Little variation in capacitive current was observed after the attachment of the peptides to the surfaces, suggesting further evidence for a loosely packed monolayer.

From the background-subtracted CV data, the Coulombic charge was obtained by integration, which allowed the determination of the surface coverage of the peptide film. The measurements that were taken after the phosphorylation reactions using ATP-Fc were utilized for the calculations. The coverage values that were determined from 10 microelectrodes fell within the range of 10−15 pmol cm^{-2}. Furthermore, back-filling studies were carried out using PEF-thiol after each modification step of the Au microelectrodes using the $[Fe(CN)_6]^{3-/4-}$ redox couple in solution to assess the effectiveness of the peptides blocking the access of the solution-based redox compound to the Au surface. These measurements were taken in the absence of the redox-active cosubstrate ATP-Fc. The decrease in the $[Fe(CN)_6]^{3-/4-}$ redox probe signal was observed due to the limited penetration through the peptide film on the surface. Incomplete blockage of the surface was expected, because the redox probe still continued to show a negligible response even after the back-filling of the exposed Au remains with the PEG-thiol.

To assess the feasibility of measuring kinase activities in cellular extracts, electrochemical assays were performed by adding Fc-ATP to cell lysates prepared from human osteosarcoma U2-OS cells with tetracycline-regulated expression of CK2. We employed square-wave voltammetry (SWV) for the analysis in cellular extracts because the current intensity of the signals was significantly low that cyclic voltammetry (CV) measurements did not provide sufficient sensitivity to detect them. As shown in Figure 2A, the highest intensity of SWV current signal was observed for the lysates derived from cells with elevated CK2 levels. The substrate peptide modified Au microelectrodes were immersed into the cell lysates containing (a) the overexpressed CK2α, (b) endogenous CK2 levels, (c) the overexpressed kinase-dead CK2α, and (d) normal-expressed CK2α. The peak potential of the oxidation current signal was observed at about +0.6 V, which indicated that the redox process was interrupted with the presence of various compounds in the cellular extract. By comparison, lower current responses were obtained from the other cell lysates including uninduced cells or cells expressing kinase-inactive CK2α. The increase in the current signal indicated that the kinase was in an excess amount and could cause the attachment of a larger amount of Fc molecules on the peptides in comparison to the other cell lysates. Overall, the kinase activity measurements obtained by electrochemical detection are in complete correspondence with measurements previously obtained using conventional radioactive detection of CK2 activity. Figure 2B displays the average SWV current responses obtained from a set of five measurements performed on the same peptide in the cell lysate environment under the same conditions. CV measurements were used to detect phosphorylation reactions using CK2 under clean buffer conditions. The phosphorylation of the immobilized peptides (RRRDDD**S**DDD) was specific to CK2. Protein tyrosine kinases (Abl-T315I and HER2) did not catalyze phosphorylation of the CK2 peptide substrate (Figure 5C,c and 6C,c), and only background signals were observed from a peptide that was the preferred substrate of Abl-T315I (EGI**Y**DVP, Figure 3B,c). Furthermore, as shown in Figure 3C, alkaline phosphatase (ALP) reverses the phosphorylation reaction, liberating the anchored Fc from the peptides with a corresponding decrease in the electrochemical responses. Notably, as the concentration of ALP increased, the current density responses decreased rapidly and reached a minimum level with ALP concentrations over 0.1 ng/μL.

Table 1. Comparison of Kinetic Constants of Protein Kinases with Their Substrate Peptides Immobilized on Gold Microelectrodes[a]

protein kinase	peptide	K_m (mM)	V_{max} (μmol min^{-1} mg^{-1})	V_{max}/K_{max}
CK2α	RRRDDD**S**DDD	0.087	1.97	22.64
CK2α′	RRRDDD**S**DDD	0.098	1.78	18.16
Abl1-T315I	EGI**Y**DVP	0.182	1.67	9.18
HER2/ErbB2	DNE**Y**FYV	0.208	1.54	7.41

[a] The kinetic data were extracted from measurements using varying surface density conditions of the substrate peptides immobilized on the surface.

Based on our success with the detection of the reversible phosphorylation of kinase substrates, we expected that it would be possible to adapt this method to evaluate small molecule inhibitors. Therefore, we evaluated the ability of the peptide biosensor to assess the inhibitory activity of three recently developed CK2 inhibitors (Figure 4). Reactions were performed with CK2 and Fc-ATP, and each inhibitor (at concentrations ranging from 50 nM to 2.5 μM) was exposed onto the CK2 substrate peptide films. After incubation for 2 h at 37 °C with CK2α, the biosensors were washed and analyzed by electrochemical measurements. Table 1 shows the inhibition data for the analysis of K_i values for four kinases and five inhibitors in total. In general, the K_i values that were calculated from the electrochemical data are in agreement with literature values obtained with conventional kinase assays.[12−18]

To evaluate the utility of the electrochemical biosensor for the measurement of other kinase assays, we modified the biosensors to monitor Abl1 and HER2 protein tyrosine kinases. The first FDA-

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approved kinase inhibitor drug, Imatinib (Gleevec), has been successfully used to treat Bcr-Abl kinase associated chronic myeloid leukemia.[19] The most frequently identified mutation associated with resistance to Gleevec is T315I in the Abl1 kinase domain.[20,21] Another successful small molecule inhibitor is Trastuzumab (Herceptin), which is used as part of a treatment regimen containing doxorubicin, cyclophosphamide, and paclitaxel for the adjuvant treatment of patients with HER2-overexpressing, node-positive breast cancer.[22−24] The Abl1-T315I-based assay included the incubation of the STP peptide modified Au microelectrodes at 37 °C for 2 h in 60 mM HEPES (pH 7.5), 5 mM $MgCl_2$, 5 mM $MnCl_2$, 3 μM Na_3VO_4, and 200 μM Fc-ATP. When the substrate peptide for CK2 was immobilized on the surface, no significant current responses were observed, which evidenced the specificity of the phosphorylation reaction (Figure 5C,c). Low current responses indicated insufficient phosphorylation between the FLT3 peptide and the Abl1-T315I (Figure 5C,b). The control experiments involved the titration of the phosphorylated STP peptide with the buffer titration in the presence of HeLa cell lysates (Figure 5D,a). No significant drops were observed in the current responses indicating that the contents of the cell lysate did not affect the current signals.

The HER2-based assay included the incubation of the substrate peptide modified Au microelectrodes at 37 °C for 2 h in 5 mM MOPS (pH 7.2), 4 mM $MgCl_2$, 5 mM $MnCl_2$, 1 mM EGTA, 0.4 mM EDTA, 2.5 mM β-glycerophosphate, and 200 μM Fc-ATP. Some representative voltammograms and the data extracted from those CV measurements are shown in Figure 6. Specific activities for the two CK2 isoforms, Abl1 and HER2, on their substrate peptides are shown in Table 2. Notably, the activities determined by the electrochemical measurements compare very favorably with the literature values.[1] However, the slightly lower reaction rates seen in the electrochemical assays may arise in part through decreased accessibility of the substrate peptides anchored on the Au electrode surface and issues related to the diffusion of the enzyme to the electrode surface, followed by binding to the immobilized peptide and phosphorylation.

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Table 2. Comparison of K_i of Small Molecule Inhibitors on Protein Kinases with Their Substrate Peptides Immobilized on Gold Microelectrodes[a]

inhibitor (nM)	CK2α	CK2α′	Abl1-T315I	HER2/ErbB2
1	450	380		
2	35	20		
3	50	25		
staurosporine			550	225
N-benzoylstaurosprine			600	275

[a] The K_i values were determined with the data obtained using varying surface density conditions of the substrate peptides immobilized on the surface.

Protein tyrosine kinases were also challenged with the well-defined general inhibitors of kinases, staurosporine and its derivative, *N*-benzoylstaurosporine (Figure 5D and 6D), which are ATP-competitive inhibitors with broad-spectrum inhibitory activities. Again, the K_i values for staurosporine and its derivative that were determined by electrochemical assays were very similar to those obtained using conventional radioactive assays for Abl1[19−21] and HER2.[22−24]

CONCLUSIONS

Overall, the electrochemical assay reported here offers new prospects for monitoring protein kinase activities and promises to accelerate the identification of novel protein kinase inhibitors. These advances are particularly important in view of the involvement of many protein kinases in numerous forms of cancer and other diseases and the emergence of proteins kinases as therapeutic targets. To realize this potential, we are currently in the process of adapting the assay into a microchip format to enable high-throughput screening technologies for protein kinase profiling of patient samples and for drug discovery.

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