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Short communication

# Electrochemical assay of melanoma biomarker in human blood

Xiaoying Wang [a,b], Hao Li [b], Xiaoxi Li [b], Yangyang Chen [c], Yongmei Yin [a,*], Genxi Li [b,c,**]

[a] Department of Oncology, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, PR China
[b] Department of Biochemistry and State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing 210093, PR China
[c] Laboratory of Biosensing Technology, School of Life Sciences, Shanghai University, Shanghai 200444, PR China

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ABSTRACT

We report here an electrochemical assay to detect S100B, a serum biomarker of melanoma. This assay takes advantage of the 1:2 binding between S100B and its specific binding peptide. The first peptide immobilized on a gold electrode can capture S100B, while a second peptide modified with Gly–His–Lys copper ion chelating motif can generate signal. The chelated copper ion can also amplify the signal by catalyzing the oxidation of o-phenylenediamine (OPD) to electroactive 3-diaminophenazine (DAP). By using square wave voltammetry, a limit of detection as low as 0.1 nM can be achieved. This method has also demonstrated favorable recovery in the complex serum sample, indicating potential clinical application in the future.



## 1. Introduction

Melanoma is one of the most aggressive and life-threatening forms of cancer, which is characterized by not only highly unpredictable patterns of dissemination but also high risk of recurrence and mortality [1,2]. In order to provide crucial clinical information about the tumor burden, progression, prognostics and treatment response, timely monitoring the highly efficient biomarker of melanoma cancer is greatly and urgently required [3–5]. Currently, lactate dehydrogenase is the most prominent melanoma serum marker used in clinical routine [4]. Recently, it was reported that S100B, a 21 kDa dimer protein, was even superior to lactate dehydrogenase [5]. So in this work we aimed to propose a method to assay S100B in patient serum with electrochemical technique.

The applications of peptide and other artificial targeting ligands for electrochemical detection of proteins have received increasing interest [6–11]. So, different from the traditional methods for S100B assay such as enzyme-linked immunosorbnent assay (ELISA) and immunohistochemistry by using antibody, here we propose to use peptide as a simplified equivalent to antibody, while electrochemistry, instead of spectroscopy, is employed for the measurement. On the one hand, electrochemical technique is known to be simple, rapid, and cost-effective [11]. On the other hand, several merits of peptides such as synthetic accessibility and amenability to modification make them a compelling choice as targeting ligand for protein assay [12,13]. Moreover, it has been reported that a specific binding peptide can bind with S100B in a 2:1 ratio [14]. So, taking advantage of these facts, we have proposed a strategy to assay the melanoma biomarker in this work. As is illustrated in Scheme 1, the capture peptide is firstly immobilized on the electrode surface to provide the intrinsic recognition of S100B. After it has captured S100B from the test solution, the signal peptide is then employed to bind with the captured S100B. For signal generation, the signal peptide has been modified with a Gly–His–Lys motif (GHK), a strong chelator of copper ion [15,16]. So, the chelated copper ions may produce electrochemical signal in proportion to S100B abundance. Moreover, since the chelated copper ions can catalyze the oxidation of o-phenylenediamine (OPD) to electroactive 3-diaminophenazine (DAP) [17], amplified signal response can be achieved, thus the sensitivity of our strategy can be greatly improved. Our approach has also been applied to analyze spiked serum samples with different concentrations of S100B. The obtained results demonstrate not only favorable feasibility but also satisfactory recovery, so this electrochemical method may have promising clinical applications in the future.

## 2. Experimental

### 2.1. Materials and chemicals

Capture peptide (K(11-mercaptoundecanoic acid, MUA)-KRLRRSAHARKETEFLRLKRTRLGLE, Lyophilized powder, purity > 95%) and signal peptide (D(GHK)-KRLRRSAHARKETEFLRLKRTRLGLE, Lyophilized powder, purity > 95%) were custom-synthesized from Shanghai Science Peptide Biological Technology Co., Ltd. Recombinant human S100B (Lyophilized powder, purity > 95%) was purchased from Sino Biological Inc. Tris (2-carboxyethyl) phosphine hydrochloride (TCEP), 9-mercapto-1-nonanol (MN) and bovine serum albumin (BSA) were obtained from Sigma Aldrich Chemical Co. Spectra/Pro 7 Standard

* Corresponding author. Tel.: +86 25 68136043.
** Correspondence to: G. Li, Nanjing University. Tel.: +86 25 83593596.
*E-mail addresses:* ym.yin@hotmail.com (Y. Yin), genxili@nju.edu.cn (G. Li).

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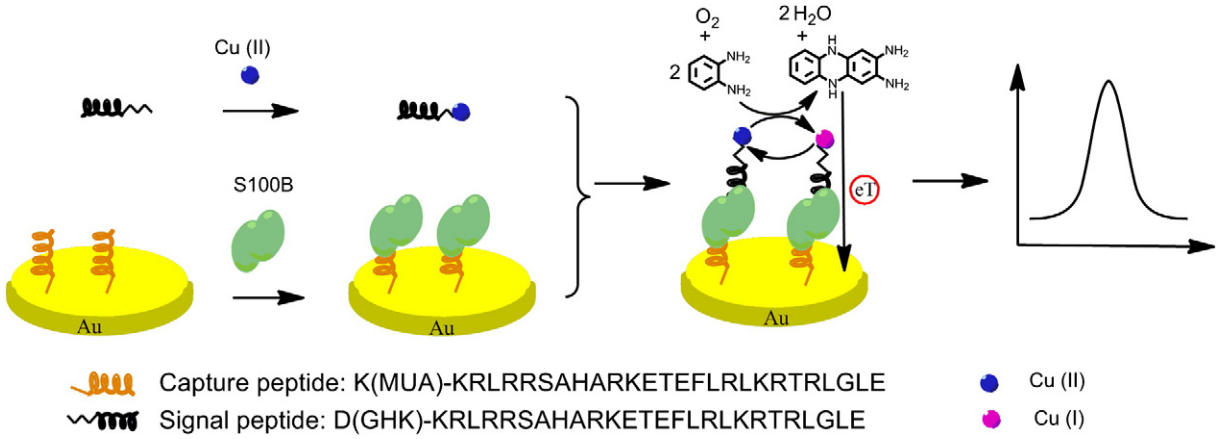


**Scheme 1.** Schematic illustration of the mechanism for peptide-based S100B assay.

Grade Regenerated Cellulose dialysis membranes were purchased from Spectrum Laboratories Inc. Other chemicals were of analytical grade and used as received. The stock solution of capture peptide and signal peptide were prepared by dissolving the powder with 20 mM Tris–HCl (pH 7.5). Desired concentrations of standard sample S100B was prepared by dissolving the powder with buffer of 20 mM Tris–HCl containing 1 mM TCEP (pH 7.5). All aqueous solutions were prepared by using double-distilled water, which was purified with a Milli-Q purification system to a specific resistance of >18 MΩ cm.

Venous blood sample from a healthy volunteer was collected. This investigation was approved by the local ethics committee. Pretreatment of fresh blood sample was conducted as follows. Venous blood sample was separated by centrifugation at 2500 rpm for 10 min, followed by collecting and dialyzing the supernatant. Then the prepared serum from the healthy volunteer was spiked with various concentrations of S100B (0.2 nM, 2 nM, 20 nM).

### 2.2. Preparation and treatment of gold electrode

Gold electrode (3 mm diameter) was first polished by P3000 and P5000 silicon carbide paper and then 1 μm, 0.3 μm alumina slurry respectively. The electrode was then sequentially ultrasonicated in ethanol and doubly distilled water for 5 min to remove adhesive particles. After that, it was soaked in piranha solution (concentrated $H_2SO_4$: 30% $H_2O_2$ = 3:1) for 5 min and further in nitric acid (50%) for 30 min. Finally, the electrode was electrochemically activated by 0.5 M $H_2SO_4$.

The prepared clean gold electrode was incubated with 50 μl assembly solution (2.5 μM capture peptide and 5 mM TCEP, pH 7.5) at 4 °C for 16 h. Afterward, the electrode was dipped in 100 μM MN solution at room temperature for 3 h to block the nonspecific binding on the electrode surface.

### 2.3. Detection of S100B

The peptide-modified gold electrode was incubated with the test solution containing S100B or the prepared supernatant of venous blood samples from malignant melanoma patient for 2.5 h at 30 °C (calcium chloride was added to the samples to achieve a final concentration of 5 mM to facilitate S100B–peptide interaction). At the same time, 2.5 μM signal peptide solution was mixed with copper chloride in a 1:1 concentration ratio at 30 °C to form copper-signal peptide complex [16]. After that, the target-bound electrode was thoroughly rinsed with double-distilled water and incubated with the solution containing the copper-signal peptide complex for 2.5 h at 30 °C. Then signal response of the chelated copper ion was recorded. To amplify signal, the copper-ion labeled electrode was inserted in 1 ml Tris–HCl (20 mM, pH 7.5) buffer solution containing 1 μl HCl and excessive OPD (2.5 mg) for 30 min 50 °C water bath. Electrochemical response of DAP was subsequently recorded.

### 2.4. Electrochemical measurements

All the electrochemical measurements were performed at room temperature on CHI660D Electrochemical Analyzers (CH Instruments, USA) with a conventional three-electrode system, which consists of a gold electrode, a saturated calomel electrode (SCE) and a platinum wire. To avoid the interference from the reduction of oxygen, solutions applied below were thoroughly purged with nitrogen gas for 10 min before measurements. Experimental parameters of square wave voltammetry are as follows. For the coordinated copper ion, scan range, 0.4–−0.1 V, step potential, 4 mV, frequency, 15 Hz, amplitude, 25 mV. For the generated DAP, scan range, −0.2 to −0.6 V, while the others are not changed.

## 3. Results and discussion

As is shown in Fig. 1A, no wave on the square wave voltammograms (SWVs) can be observed in the absence of target S100B, while an apparent peak can be observed if the test solution contains S100B. Moreover, with the increase of S100B concentration, the response of chelated copper ion gradually increases, so this obtained peak of the chelated copper ion can be used to quantify the occurrence of the target protein. Certainly, since the chelated copper ion can catalyze the oxidation of OPD, a much higher peak can be obtained to more sensitively show the occurrence of the target. Before the generated DAP is employed for the measurement, we have also optimized the several important experimental conditions of the assay. Firstly, the interaction time between the target protein S100B and the capture peptide is investigated and 2.5 h is found to be sufficient for the binding to complete. Secondly, to ensure that the catalytic ability of copper ion can be fully exploited, the amount of OPD is examined. Experimental results reveal that 1.5 mg/ml OPD is enough to give the highest peak current, so we have chosen an excessive amount (2.5 mg/ml) for the following experiments. By making use of the catalytic signal, various concentrations of S100B are introduced to evaluate the possibility to sensitively detect S100B. As shown in Fig. 1B, the peak currents show a gradual increase along with the concentration of S100B, and a positive correlation can be obtained in the concentration range from 0.1 to 25.6 nM with a detection limit of 0.1 nM. The average coefficient of variation is below 5%, showing an acceptable and desirable precision and reproducibility.

To evaluate the specificity of our method, control experiments have been further performed (Fig. 1C). Compared with the peak current of S100B, insignificant response is observed, although the used amount of BSA (300 nM) is much higher than that of S100B (3 nM).

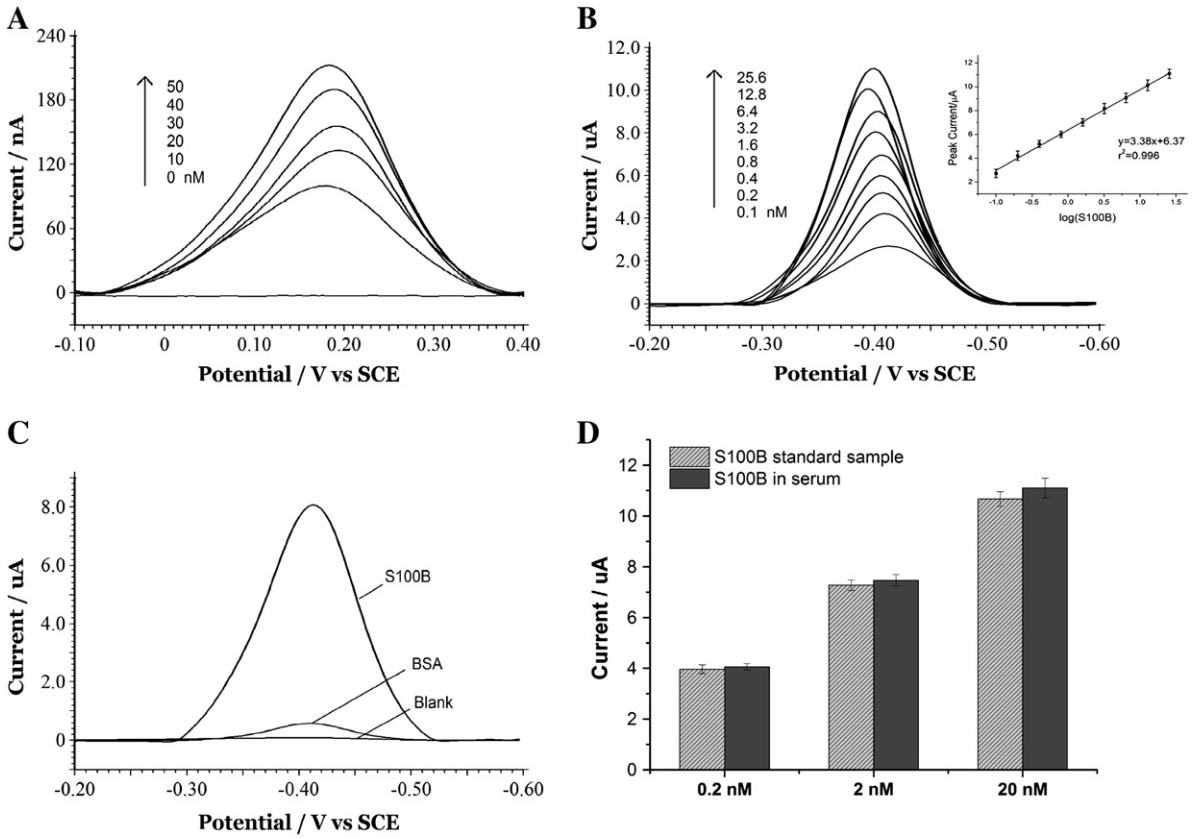


**Fig. 1.** (A) SWVs obtained at the capture peptide-modified electrode after sequential incubation with S100B at different concentrations shown on the graph and 2.5 μM signal peptide chelated by copper ion. (B) SWVs of DAP for the assay of S100B with different concentrations. DAP is generated by OPD oxidation catalyzed by chelated copper ion from electrode treated as described in panel (A). Inset shows the calibration curve, and the error bars indicate standard deviation (n = 3). (C) SWVs of generated DAP to show the selectivity of the proposed method. Concentrations of S100B and BSA are 3 nM and 300 nM respectively. (D) Comparison of the detected signal in spiked serums and in standard S100B samples. Error bars indicate standard deviation (n = 3).

**Table 1**
The recovery of spiked S100B determined in serum samples using our method.

| Sample | Concentrations spiked (nM) | Concentrations measured (nM) | Recovery (%) |
|---|---|---|---|
| 1 | 0.2 | 0.197 | 98.5 |
| 2 | 2 | 2.074 | 103.7 |
| 3 | 20 | 20.556 | 102.78 |

Given that circulating S100B level is a valuable biomarker for melanoma patients, it is critical to assess the applicability of the proposed method in complex serum samples. To this end, serum samples spiked with different concentrations of S100B have been evaluated, and the results have been shown in Fig. 1D. So, the signal readout from the spiked samples is very comparable to that from standard samples of the same concentration. Moreover, the spiked samples also give a satisfactory recovery (Table 1). Therefore, results of the above spiking experiment may indicate favorable feasibility for clinical applications.

## 4. Conclusion

In summary, an electrochemical strategy for S100B assay is proposed. In the meantime, peptide, instead of antibody, can be used for this assay method. Our method may offer easy handling, and considerable sensitivity and selectivity. This proposed method has also been used to determine the spiked S100B in blood samples. Since S100B occupies a crucial position in improving clinical management, outcome, and survival of melanoma patients, our method might be of great potential for further clinical applications.

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## References

[1] A.J. Miller, M.C. Mihm, N. Engl. J. Med. 355 (2006) 51–65.
[2] C.M. Balch, G.C. Balch, R.R. Sharma, J. Clin. Oncol. 30 (2012) 1406–1407.
[3] S. Kruijff, E. Bastiaannet, A.H. Brouwers, W.B. Nagengast, M.J. Speijers, A.J. Suurmeijer, G.A. Hospers, H.J. Hoekstra, Ann. Surg. Oncol. 19 (2012) 620–626.
[4] R. Dummer, A. Hauschild, M. Guggenheim, U. Keilholz, G. Pentheroudakis, Ann. Oncol. 23 (2012) 86–91.
[5] F. Egberts, E.M. Kotthoff, S. Gerdes, J.H. Egberts, M. Weichenthal, A. Hauschild, Eur. J. Cancer 48 (2012) 695–702.
[6] E. Palecek, F. Scheller, J. Wang (Eds.), Electrochemistry of nucleic acids and proteins. Towards electrochemical sensors for genomics and proteomics, Elsevier, Amsterdam, 2005.
[7] O. Hammerich, J. Ulstrup (Eds.), Bioinorganic Electrochemistry, Springer, Dordrecht, 2008.
[8] E. Palecek, V. Ostatna, H. Cernocka, A.C. Joerger, A.R. Fersht, J. Am. Chem. Soc. 133 (2011) 7190–7196.
[9] V. Dorcak, V. Ostatna, E. Palecek, Electrochem. Commun. 31 (2013) 80–83.
[10] J.F. Rusling, C.V. Kumar, J.S. Gutkind, V. Patel, Analyst 135 (2010) 2496–2511.
[11] G. Li, P. Miao, Electrochemical Analysis of Proteins and Cells, Springer, Berlin, Germany, 2012.
[12] S. Pavan, F. Berti, Anal. Bioanal. Chem. 402 (2012) 3055–3070.
[13] H. Li, H. Xie, Y. Cao, X. Ding, Y. Yin, G. Li, Anal. Chem. 85 (2013) 1047–1052.
[14] S. Bhattacharya, E. Large, C.W. Heizmann, B. Hemmings, W.J. Chazin, Biochemistry 42 (2003) 14416–14426.
[15] H. Li, H. Xie, N. Yang, Y. Huang, L. Sun, G. Li, Chem. Commun. 49 (2013) 5387–5389.
[16] A. Trapaidze, C. Hureau, W. Bal, M. Winterhalter, P. Faller, J. Biol. Inorg. Chem. 17 (2012) 37–47.
[17] X. Yang, E. Wang, Anal. Chem. 83 (2011) 5005–5011.