Biosensors and Bioelectronics 77 (2016) 284–291

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Leverage principle of retardation signal in titration of double protein via chip moving reaction boundary electrophoresis

Liu-Xia Zhang [a,1], Yi-Ren Cao [a,1], Hua Xiao [a], Xiao-Ping Liu [a], Shao-Rong Liu [b], Qing-Hua Meng [c], Liu-Yin Fan [a,b,**], Cheng-Xi Cao [a,*]

[a] Laboratory of Analytical Biochemistry and Bioseparation, State Key Laboratory of Microbial Metabolism, School of Life Science and Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, China
[b] Department of Chemistry and Biochemistry, Oklahoma University, Norman, OK 73019, USA
[c] School of Chemistry and Chemical Engineering, Shanghai Jiao Tong University, Shanghai 200240, China

ARTICLE INFO

Article history:
Received 12 July 2015
Received in revised form
28 August 2015
Accepted 1 September 2015
Available online 16 September 2015

Keywords:
Chip electrophoresis
Leverage principle
Moving reaction boundary
Protein titration
Retardation signal

ABSTRACT

In the present work we address a simple, rapid and quantitative analytical method for detection of different proteins present in biological samples. For this, we proposed the model of titration of double protein (TDP) and its relevant leverage theory relied on the retardation signal of chip moving reaction boundary electrophoresis (MRBE). The leverage principle showed that the product of the first protein content and its absolute retardation signal is equal to that of the second protein content and its absolute one. To manifest the model, we achieved theoretical self-evidence for the demonstration of the leverage principle at first. Then relevant experiments were conducted on the TDP-MRBE chip. The results revealed that (i) there was a leverage principle of retardation signal within the TDP of two pure proteins, and (ii) a lever also existed within these two complex protein samples, evidently demonstrating the validity of TDP model and leverage theory in MRBE chip. It was also showed that the proposed technique could provide a rapid and simple quantitative analysis of two protein samples in a mixture. Finally, we successfully applied the developed technique for the quantification of soymilk in adulterated infant formula. The TDP-MRBE opens up a new window for the detection of adulteration ratio of the poor food (milk) in blended high quality one.

1. Introduction

Protein content analysis of biosample is a fundamental demand of research for medical diagnosis (Liu et al., 2011; Yoo et al., 2014), proteomics (van de Waterbeemd et al., 2013; Gianazza et al., 2014), bioengineering (Scott and Knight, 2009) and food safety (Setford et al., 2002). Many analytical methods have been proposed for protein content determination in biological samples, including Kjeldahl method (Kjeldahl, 1883; Moore et al., 2010), Dumas method (Thudichum and Wanklyn, 1869; Colenbrander and Martin, 1971), biosensor method (Chen et al., 2014; Wustoni et al., 2015) as well as dye binding method (Park and King, 1974; Sherbon, 1974). Among these methods, Kjeldahl method has been utilized as the most popular and routine tool for protein content detection in biosamples since the 20th century (Scales,1916). Whereas, this method may be interfered by non-protein nitrogen (NPN) components in the samples (Ai et al., 2009).

In 2008, there was a melamine adulteration in infant formula which led to kidney failure of thousands of children (Ingelfinger, 2008; Fodey et al., 2011). After this incidence, many efforts were dedicated to detect the melamine in the milk products (Ai et al., 2009; Zhu et al., 2010; Fodey et al., 2011; Cao, et al., 2013; Dai et al., 2014; Huy et al., 2014; Ni et al., 2014; Niu et al., 2015). But these all above written methods have a drawback that they create NPN disturbance during protein content analysis.

Besides NPN reagents mentioned above, numerous poor nutritional value milk products were also illegally blended into the good ones due to economical motivation (Pizzano and Salimei, 2014; Scholl et al., 2014; Mafra et al., 2004). To solve this issue, many analytical methods have been used for the characteristic of adulterated milk or spoiled food, including the polyacrylamide gel electrophoresis (PAGE) (Chrambach and Rodbard, 1971), SDS-PAGE

* Corresponding author.
** Corresponding author at: Laboratory of Analytical Biochemistry and Bioseparation, State Key Laboratory of Microbial Metabolism, School of Life Science and Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, China.
E-mail addresses: lyfan@sjtu.edu.cn (L.-Y. Fan), cxcao@sjtu.edu.cn (C.-X. Cao).
[1] The first two authors have equal contribution to the work, the first author designed the experiment, the second author developed the theory, both of them conducted the experiments.

http://dx.doi.org/10.1016/j.bios.2015.09.001
0956-5663/

(Schägger and von Jagow, 1987), polymerase chain reaction (Mafra et al., 2004) and ELISA (Pizzano and Salimei, 2014) etc. The former two were based on the fingerprint difference of protein in normal sample and forgedone, and the latter two were relied on the monitoring of specific DNA or protein antigen existing in poor milk product. Those methods could qualitatively identify whether good milk was adulterated or not. However, there is still rare report on the rapid, simple and quantitative detection of ratio of poor milk in illegally-adulterated good one, to the authors' knowledge.

To address these issues, the concept of electrophoresis titration was developed for rapid determination of protein content without the interference of NPN reagent (Wang et al., 2013, 2014; Guo et al., 2013). This technique is based on the concept of moving reaction boundary (MRB), including the moving reactive front (Deman, 1970; Deman and Rigole, 1970; Cao, 1998; Cao et al., 2008; Dong et al., 2013). In a MRB system, the reactive cation (H^+) and anion (OH^-) moves in opposite directions under an electric field, and react together and form a reaction boundary at the meeting point (the online Supplementary information). An equal equivalent reaction between the reactive cation and anion is always present in the MRB system. Thus, this boundary moves towards cathode if the flux of reactive cation is higher than reactive anion, and vise versa. We can directly observed the boundary movement with naked eyes if a chemical or fluorescent indicator exists in the boundary system. Based on this principal, MRBE titration was developed for protein content analysis of biological samples and milk products without the NPN disturbance (Wang et al., 2013; Guo et al., 2013). Further, the concept of retardation signal was introduced for the simple, sensitive and high speed monitoring of protein based MRB titration (Wang et al., 2014). Whereas, there is still no report on monitoring adulteration ratio of the poor food (milk) in blended high quality one.

Therefore, we purposes herein are: (i) to develop the model of TDP and leverage theory of retardation signal based on MRB electrophoresis (MRBE), (ii) to design the simple and rapid chip experiments showing the validity of model and theory of TDP-MRBE, and (iii) to present the real application of the developed technique to quantitative analysis on ratio of soymilk in blended infant formula milk. The developed technique opens up a new window for monitoring the ratio of poor one in adulterated high-quality-milk (food).

2. Brief theory

The TDP model of Protein 1 and 2, and the relevant leverage principle of MRBE chip. Panel A and B display the schematic diagrams of acidic MRBE titration of Protein 1 and 2, respectively (Fig. 1). In the titration, the two proteins are immobilized via polyacrylamide gel and neutralized by hydrogen ion moving from the anode to the cathode under an electric field. The neutralization boundary can be visualized due to the acid–base indicator of fluorescein isothiocyanate (FITC) in the microchannel. From the runs of MRB, we can obtain the standard curves between the contents of two proteins (C) and their retardation signals (two curves of Protein 1 and 2 in Panel D) in accordance with our previous work (Wang et al., 2014). The two curves are Eqs. (1) and (2), respectively,

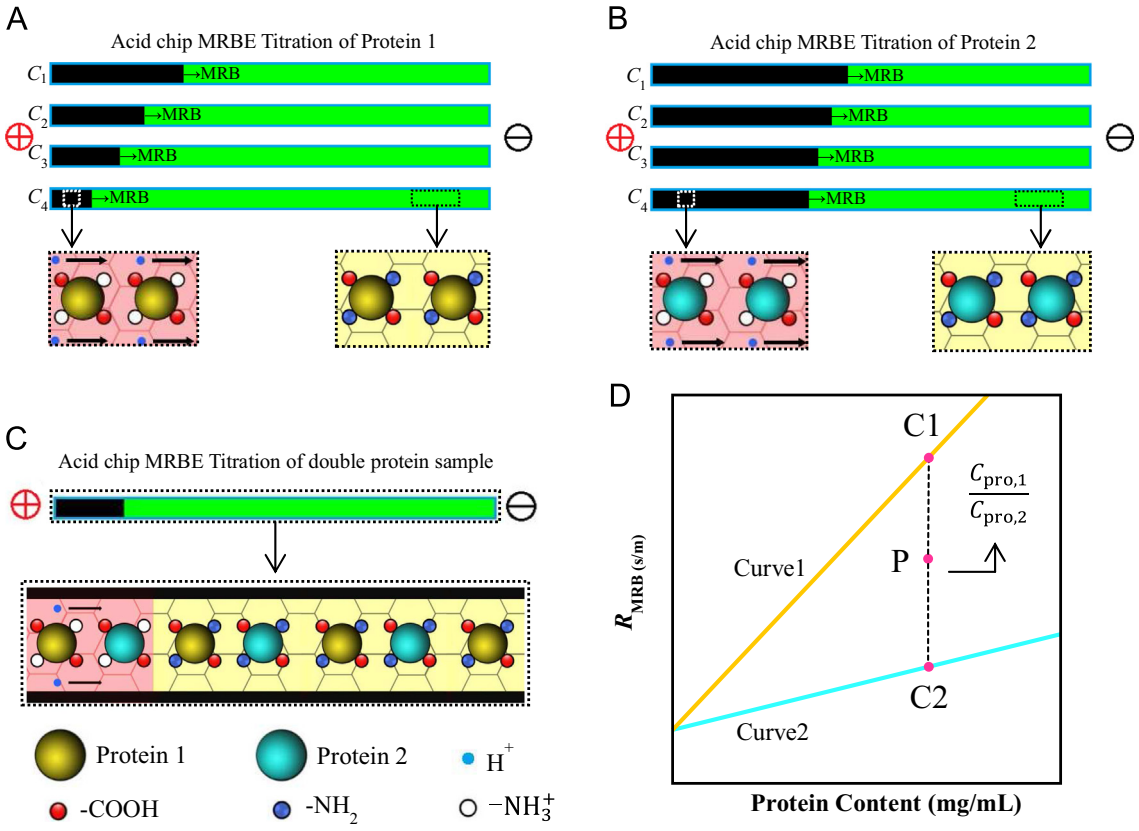

Fig. 1. TDP-MRBE chip for quantitative analysis of protein or milk sample. (A) Calibration curve 1 of Protein 1 via acid MRBE titration. (B) Calibration curve 2 of Protein 2 via acid MRBE titration. (C) Retardation signal R_{mix} measurement of the double protein sample. (D) $C_{pro,1}$ to $C_{pro,2}$ ratio calculation by leverage principle. The symbols of C_1, C_2, C_3 and C_4 denote protein contents ($C_1 < C_2 < C_3 < C_4$); the symbols '+' and '–' indicate the anode and the cathode, respectively; curve 1 and curve 2 are R_{MRB} calibration curves of protein 1 and 2, respectively; Point P indicates the value of retardation signal and total protein content of the double protein sample. The notations of $C_{pro,1}$ and $C_{pro,2}$ are the protein content of Protein 1 and Protein 2 in the sample, respectively.