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A tunable isoelectric focusing via moving reaction boundary for two-dimensional gel electrophoresis and proteomics

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ABSTRACT

Routine native immobilized pH gradient isoelectric focusing (IPG-IEF) and two-dimensional gel electrophoresis (2DE) are still suffering from unfortunate reproducibility, poor resolution (caused by protein precipitation) and instability in characterization of intact protein isoforms and posttranslational modifications. Based on the concept of moving reaction boundary (MRB), we firstly proposed a tunable non-IPG-IEF system to address these issues. By choosing proper pairs of catholyte and anolyte, we could achieve desired cathodic and anodic migrating pH gradients in non-IPG-IEF system, effectively eliminating protein precipitation and uncertainty of quantitation existing in routine IEF and 2DE, and enhancing the resolution and sensitivity of IEF. Then, an adjustable 2DE system was developed by combining non-IPG-IEF with polyacrylamide gel electrophoresis (PAGE). The improved 2DE was evaluated by testing model proteins and colon cancer cell lysates. The experiments revealed that (i) a tunable pH gradient could be designed via MRB; (ii) up to 1.65 fold improvement of resolution was achieved via non-IPG-IEF; (iii) the sensitivity of developed techniques was increased up to 2.7 folds; and (iv) up to about 16.4% more protein spots could be observed via the adjustable 2DE as compared with routine one. The developed techniques might contribute to complex proteome research, especially for screening of biological marker and analysis of extreme acidic/alkaline proteins.

1. Introduction

A series of robust techniques have been developed for proteomics [1,2], functional genomics [3,4] and systemic biology [5]. For example, two-dimensional gel electrophoresis (2DE) [6,7] and nano-liquid chromatography (Nano-LC) [8] are developed for separation of complex protein samples and tryptic peptides, respectively. Powerful nano-LC–MS/MS is proposed for high throughput and sensitive identification of tryptic peptides [1,8]. Meanwhile, many soft ionization techniques (e.g., matrix-assisted laser desorption/ionization [9,10] and electrospray ionization (ESI) [11,12]) are advanced for structural characterization of intact proteins. Generally, there are two classic proteomic strategies. One is the top-down strategy, and the other is the bottom-up approach. Interestingly, 2DE can be the most powerful separation tool for both approaches.

In the bottom-up strategy [1,5], protein spots in 2DE are digested into peptides, and then LC–MS/MS is used for the detection of tryptic peptides for protein identification. The bottom-up strategy has many merits, including good sensitivity, high throughput and great convenience as well as automation [12,13]. However, this strategy has its own weaknesses. First, many important data of protein structure are not available at the end because only a sequence of tryptic peptide was detected. Second, different protein isoforms with the same peptide sequences may not be distinguishable because of low sequence coverage of protein. Third, data on post-translational modifications (PTMs) of protein are usually lost. Finally, LC–MS/MS based peptide-centric analyses may complicate protein quantification due to the coexistence of protein isoforms. These defects make structural analyses of intact protein very challenging, especially for the PTMs of proteins [13].

To overcome these defects, different top-down proteomic approaches have been developed [13]. In 2004, Takáts et al.

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proposed a novel MS sampling under ambient conditions with desorption electrospray ionization [14]. Qiao et al. [15,16] developed an ambient ionization strategy and ESI for in situ ionization of proteins or peptides in the PAGE and coupled IPG-IEF to ESI-MS directly. Thus, protein samples could be firstly separated by IPG-IEF, then in situ detected by rapidly scanning gel strip via ESI-MS. This novel top-down strategy can retain the structure information of intact proteins. Furthermore, this approach avoids gel staining and spot extraction, greatly improved the throughput and reduced labor intensity, running time, contaminant and sample loss [15,16].

The IPG-based IEF has been invented as an excellent electrophoretic technique [17,19] to address the cathodic or anodic drifting of pH gradient in Svensson's IEF [20,21], and used in the routine 2DE method for 30 years. However, the IPG-based IEF and 2DE techniques have been facing with the following challenges. First, many denature reagents and solubilizers (urea, thiourea, CHAPS, dithiothreitol, sugar and sorbitol [18,22,23]) were used for improving the solubility of protein in the routine IPG-IEF, making studies of PTMs and protein isoforms very complex and inconvenient. However, a native IPG-IEF without the use of denature reagents and solubilizers resulted in serious random protein precipitate [24] (Section 3.4), greatly affecting resulting sensitivity, resolution, quantitation and reproducibility and making comparative proteomics (e.g., cancer serum vs. normal control serum) dubious. Second, tunable local-zoomed pH gradient in IEF and 2DE was required for high resolution separation of complex protein isoforms and PTMs. However, once the pH gradient of IPG-IEF was immobilized, it could not be adjusted for high resolution separation of many protein isoforms and PTMs [7,25]. Third, sensitivities of routine IPG-IEF and 2DE are still poor [12,13], making identification of low abundance proteins impossible. All of these issues obviously weakened the power of 2DE on characterization of protein isoforms and PTMs [15,16].

To figure out these issues mentioned above, we have developed the concept of moving reaction boundary (MRB) [26,27] from the prototype ideas of moving reactive front [28,29] and stationary neutralization boundary [30]. The concept of MRB was used to improve the sensitivity of capillary electrophoresis (CE) [31,32], the design of supermolecular boundary electrophoresis [33,34] and the advancement of total protein content titration [35,36]. Furthermore, we developed the MRB-based dynamic theory of IEF [24,26,27,37,38], which quantitatively explained the mechanism of quasi-stable pH gradient in IEF and gave the quantitative illumination on various pH gradient instabilities [39–41] and Hjertén's mobilization [42–44]. However, to the best of our knowledge, a tunable local-zoomed pH gradient in IEF has never been designed based on the concept of MRB for 2DE.

Therefore, the main purposes herein are to design a tunable non-IPG-IEF via the concept of MRB; to evaluate resolution and sensitivity of the developed system by comparing it with the routine IPG-IEF; to create an novel non-IPG-IEF based adjustable 2DE; and to validate the advantage of tunable non-IPG-IEF used as the first dimension of 2DE by using model proteins and lysates of colon cancer samples.

2. Experimental designs and methods

The details about materials, instruments and analytical software used herein, as well as procedures of staining, sample preparation and LC–MS/MS, are given in the Supporting Information.

2.1. Tunable pH gradient of non-IPG-IEF

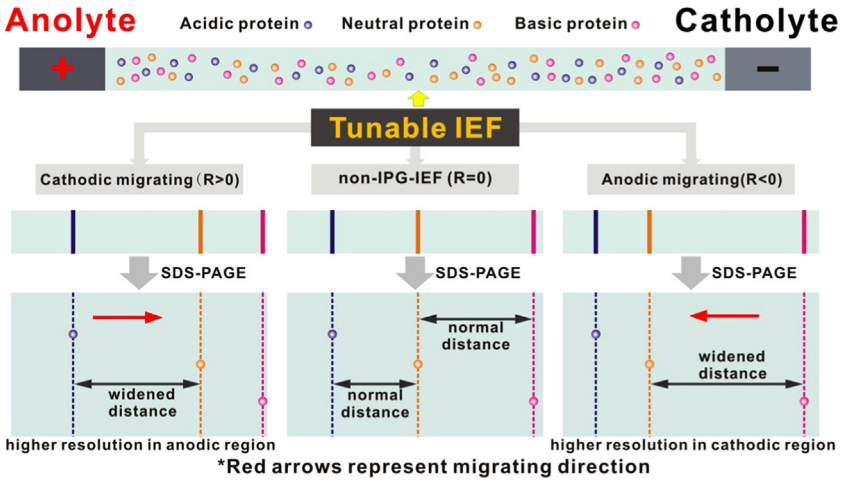
Scheme 1 shows the complete design of tunable pH gradient in non-IPG IEF via R value (viz., judgment expressions of MRB) and its combination with 2DE. In our previous works [26,27], we defined the expression for comparing fluxes of protons in an acid and hydroxyl in a base as follows:

$$R=\frac{m_+c_+\kappa_-}{m_-c_-\kappa_+}-1 \quad (\text{used for } R>0) \tag{1a}$$

$$R=1-\frac{m_-c_-\kappa_+}{m_+c_+\kappa_-} \quad (\text{used for } R<0) \tag{1b}$$

where, R is the symbol of judgment expression, κ is the conductivity (S/m); m is the ionic mobility ($m^2\,s^{-1}\,V^{-1}$); c is the equivalent concentration (equiv./l), the subscripts, "+" and "−", imply the hydrogen and hydroxyl ions, respectively. Herein, Eqs. (1a) and (1b) are used for $R>0$ and $R<0$, respectively.

As shown in Scheme 1, the significances of R value are: (i) we can design a cathodic migrating pH gradient of IEF by setting $R>0$, leading to obvious enhancement of separation of acidic proteins; (ii) conversely, we may create an anodic migrating pH gradient by using $R<0$, resulting in clear improvement of separation of alkaline proteins; and (iii) we can form a non-migrating pH gradient by choosing $R\cong 0$, increasing separation of neutral proteins. Thus, one can use tunable pH gradient of IEF to 2DE for high resolution separation of acidic, and/or neutral and/or alkaline proteins (Scheme 1). Interestingly, the non-IPG-IEF also has the merit of



Scheme 1. Diagrams of tunable non-IPG-IEF and adjustable 2DE designed via R value of MRB. The red arrows indicate the migration direction of pH gradient.