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Review

Recent applications of capillary electromigration methods to separation and analysis of proteins

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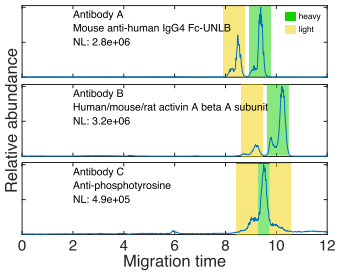
HIGHLIGHTS

- Recent developments in analysis of proteins by CE methods are reported.
- Sample preparation, preconcentration and detection methods are described.
- Review of coatings suppressing proteins adsorption to the capillary is presented.
- Separations by CZE, CITP, CIEF, ACE, EKC and CEC are discussed.
- Quality control of protein biopharmaceuticals is demonstrated.

GRAPHICAL ABSTRACT



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ABSTRACT

This review article describes the significant recent developments in analysis of proteins by capillary electromigration (CE) methods (zone electrophoresis, isotachophoresis, isoelectric focusing, affinity electrophoresis, electrokinetic chromatography and electrochromatography) during the period 2011–2015. Improvements in sample preparation, preconcentration, suppression of adsorption and control of electroosmotic flow, separations by particular CE methods, and the detection schemes used in the analysis of proteins are discussed. Innovative applications of the above CE methods for quality control of protein biopharmaceuticals, protein determination in complex biomatrices, peptide mapping of proteins, and determination of physicochemical parameters of proteins are presented.

Contents

Non-standard abbreviations: ACN, acetonitrile; BGE, background electrolyte; BSA, bovine serum albumin; CITP, capillary isotachophoresis; 2D, two-dimensional; DS, dextran sulfate; EKS, electrokinetic supercharging; EPO, erythropoietin; FASS, field-amplified sample stacking; FASI, field-amplified sample injection; FESI, field-enhanced sample injection; FETUA, fetuin A; IACE, immunoaffinity CE; LVSS, large-volume sample stacking; M-IPG-CIEF, monolithic immobilized pH gradient-based CIEF; PB, polybrene; PDA, polydopamine; PDADMAC, poly(diallyldimethylammonium chloride); POE, polyoxyethylene; PVS, poly(vinylsulfonate); rhEPO, recombinant human erythropoietin; SMIL, successive multiple ionic layer; t-ITP, transient isotachophoresis; wrFLU, wavelength-resolved fluorescence detection.

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1. Introduction

Proteins, complex biopolymers composed of amino acids, are large single- or multichain molecules that perform many essential roles in all living organisms. They function as enzymes, enzyme substrates and inhibitors, structural elements, hormones, hormone and drug receptors, antibodies or transporters that carry specific substances into or out of cells. The separation and analysis of proteins in complex biological matrices is a difficult task that requires application of sophisticated and reliable methods. Traditionally, SDS-PAGE, IEF, and 2-DE have been used for protein analysis [1–3]. At present, HPLC and UHPLC techniques with MS detection dominate among the different separation methods applied to protein analyses [4,5]. Nevertheless, capillary electromigration (CE) methods are also well suitable, and widely used for protein and proteomic analyses, offering many advantages, such as high separation efficiency, short analysis times, low sample and reagents consumption and different separation modes [6–12]. While in the field of proteomics CE methods are frequently combined with MS detection, as MS enables protein identification and quantification [13–17]; in other protein analyses, besides MS also UV-absorption detection is extensively used. Due to its relatively low concentration sensitivity, various preconcentration methods are frequently employed either before or during CE analysis of proteins. Some recent reviews were focused on enhancing the sensitivity in CE and on different preconcentration methods [18–22]. Another important issue concerning protein analysis by CE is preventing the adsorption of proteins to the inner capillary wall. For that purpose various materials and techniques have been developed [23–25].

CZE is the most frequently employed CE method for protein separations but other methods, such as CIEF, CITP, ACE, EKC, or CEC are often used as well. Main application areas concern quality control of protein biopharmaceuticals, determinations of proteins in biomatrices, their physicochemical characterization, and proteomic analyses.

The aim of this review is to present substantial developments within all above fields of protein analysis by CE methods in the last five years with the exception of proteomic analysis, which was recently reviewed elsewhere [17]. This review does not aim to be comprehensive but rather to show the significant recent works demonstrating the major progress in protein analysis by CE methods.

2. Sample preparation

Due to their miniaturized capillary format, CE methods possess generally much lower sample loading capacity than the classical chromatographic methods with much larger id columns or than slab gel electrophoretic techniques with larger gel thickness. This fact results in high mass (substance amount) sensitivity but relatively low concentration sensitivity of CE methods with the most frequently used UV-absorption detection. The concentration sensitivity of the CE analyses, however, can be increased by application of different electrophoretic or chromatographic preconcentration procedures. Electrophoretic pre-concentration methods include stacking techniques based on electric field amplification in diluted sample zone, first described by Mikkers et al. [26] and recently reviewed by Lian et al. [27]. Such techniques are: field-amplified sample stacking (FASS) [28], large-volume sample stacking (LVSS) [29], field-enhanced or field-amplified sample injection (FESI [30] or FASI introduced by Chien and Burgi [31]; and recently applied e.g. to analysis of drugs in urine [32,33]). Other concentration methods include transient isotachophoresis (t-ITP) [34], dynamic pH junction described first by Britz-McKibbin and Chen [35] and later reviewed by Kazarian et al. [36], sweeping proposed by Quirino and Terabe [37], and later applied as multistep enrichment method in MEKC [38], and electrokinetic supercharging that combines FASI and t-ITP [39,40].

FESI was employed for pre-concentration of intact proteins (cytochrome *c*, lysozyme, ribonuclease A, S-ribonuclease, α-lactalbumin, β-lactoglobulin, lactoferrin and myoglobin) before CE-MALDI-MS analysis [41]. Under optimized conditions, average signal enhancement factors for proteins were 3200 and 4800 for peak height and area, respectively, and LODs ranged from 5 to 10 nM, which were comparable with direct MALDI-MS measurement in the presence of ammonium acetate and LODs of proteins measured by state-of-art sheathless CE-ESI MS method.

Chromatographic pre-concentration methods, such as SPE [42,43] or SPME [44], are also frequently used to improve the sensitivity of CE analysis. The employment of SPE methods enables to concentrate the analytes of interest and to remove compounds