



Review

A review on preparative and semi-preparative offgel electrophoresis for multidimensional protein/peptide assessment



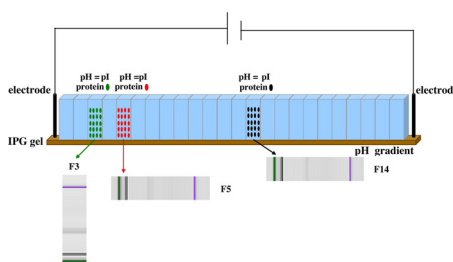
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HIGHLIGHTS

- A review on preparative and semi-preparative offgel electrophoresis.
- Offgel electrophoresis trends.
- Protein pellet treatments for offgel electrophoresis.
- Offgel electrophoresis coupling with electrophoretic/chromatographic techniques.
- Offgel electrophoresis for metal-protein complexes assessment.

GRAPHICAL ABSTRACT



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ABSTRACT

Mass spectrometry (MS) techniques are commonly used for protein identification and further analysis of selected protein spots after high resolution 2-D electrophoresis. Complementary gel-free approaches have been developed during the last few years and have shown to be useful tools in modern proteomics. The development and application of various gel-free electrophoresis devices for performing protein fractionation according to the pI differences is therefore a topic of interest. This review describes the current state of isoelectric focusing (IEF) gel-free electrophoresis based on the Agilent offgel 3100 fractionator. The review includes, therefore, (i) an overview on IEF as well as other previous IEF gel-free electrophoresis developments; (ii) offgel fundamentals and future trends; (iii) advantages and disadvantages of current offgel procedures; (iv) requirements of isolated protein pellets for further offgel fractionation; (v) offgel fraction requirements to perform the second dimensional analysis by advance electrophoresis and chromatographic techniques; and (vi) effect of the offgel operating conditions on the stability of metal-protein complexes.

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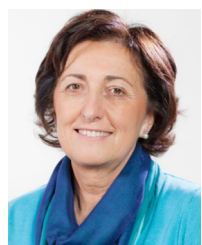
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1. Introduction

Proteomics is the study of quantitative changes of protein expression levels and their application to drug discovery, diagnosis and therapy [1]. The difficulties due to protein similarities in complex samples, as well as the assessment of low abundance proteins, have conventionally been overcome by applying two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) for isolation/separation purposes. First dimension separation (protein separation on the basis of differences in their isoelectric points (pI)) is commonly performed by isoelectric focusing (IEF) techniques; whereas, further protein separation according to MWs is achieved by sodium dodecylsulfate (SDS)-PAGE. The potential of well-established high resolution 2D-PAGE (IEF and SDS-PAGE) for protein separation before identification and analysis of protein spots with MS techniques has been shown in different reviews [2–4]. Other strategies based on non-gel (gel-free) approaches, such as multidimensional protein identification technology (MuDPIT) or shotgun proteomics (proteins are first digested by proteolysis and then analysed by several chromatographic techniques with MS detection), have been developed during the last few years [5]. The development and application of

gel-free separation techniques have therefore gained importance when separating peptides by size exclusion (SE) and reverse phase (RP) high performance liquid chromatography (HPLC) techniques with tandem mass spectrometry (MS/MS) detection [6,7]. The findings from MuDPIT approaches have shown the detection of proteins which do not appear in a 2-D gel, although proteins included in a 2-D gel partially do not show up in this chromatographic-based technique [5]. In addition, other gel-free approaches, such as preparative immobilised pH gradient (IPG) IEF where peptides can be recovered from the liquid phase (offgel electrophoresis), can lead to efficient fractionation and improved protein identification [8]. Gel-free techniques and high resolution 2-D electrophoresis are therefore complementary tools in modern proteomics studies.

The aim of the current review is the critical discussion of the possibilities of IEF based gel-free electrophoresis methods (formally known as offgel electrophoresis) as a preparative stage, mostly for peptide separation according to their pIs (first dimension) before further separations, mainly by RP chromatography (second dimension). General IEF fundamentals as well as an offgel overview including trends have been introduced. Advantages and drawbacks associated with offgel electrophoresis have

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