Biochimica et Biophysica Acta 1854 (2015) 460–468

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Multidimensional electrostatic repulsion–hydrophilic interaction chromatography (ERLIC) for quantitative analysis of the proteome and phosphoproteome in clinical and biomedical research

Stefan Loroch [a], Tim Schommartz [b], Wolfram Brune [b], René Peiman Zahedi [a], Albert Sickmann [a,c,d,*]

[a] Leibniz-Institut für Analytische Wissenschaften—ISAS—e.V., Otto-Hahn-Straße 6b, 44227 Dortmund, Germany
[b] Heinrich-Pette-Institut—Leibniz-Institut für Experimentelle Virologie, Martinistraße 52, 20251 Hamburg, Germany
[c] University of Aberdeen, School of Natural & Computing Sciences, Meston Building, Meston Walk, Old Aberdeen, AB24 3UE, Germany
[d] Medizinische Fakultät, Ruhr-Universität Bochum, Universitätsstraße 150, 44801 Bochum, Germany

ARTICLE INFO

Article history:
Received 20 October 2014
Received in revised form 10 January 2015
Accepted 15 January 2015
Available online 22 January 2015

Keywords:
ERLIC
Proteomics
Phosphoproteomics
Multidimensional separation
Clinical research

ABSTRACT

Quantitative proteomics and phosphoproteomics have become key disciplines in understanding cellular processes. Fundamental research can be done using cell culture providing researchers with virtually infinite sample amounts. In contrast, clinical, pre-clinical and biomedical research is often restricted to minute sample amounts and requires an efficient analysis with only micrograms of protein. To address this issue, we generated a highly sensitive workflow for combined LC-MS-based quantitative proteomics and phosphoproteomics by refining an ERLIC-based 2D phosphoproteomics workflow into an ERLIC-based 3D workflow covering the global proteome as well. The resulting 3D strategy was successfully used for an in-depth quantitative analysis of both, the proteome and the phosphoproteome of murine cytomegalovirus-infected mouse fibroblasts, a model system for host cell manipulation by a virus. In a 2-plex SILAC experiment with 150 μg of a tryptic digest per condition, the 3D strategy enabled the quantification of ~75% more proteins and even ~134% more peptides compared to the 2D strategy. Additionally, we could quantify ~50% more phosphoproteins by non-phosphorylated peptides, concurrently yielding insights into changes on the levels of protein expression and phosphorylation. Beside its sensitivity, our novel three-dimensional ERLIC-strategy has the potential for semi-automated sample processing rendering it a suitable future perspective for clinical, pre-clinical and biomedical research.

1. Introduction

The identification of quantitative changes in protein abundance and post-translational modifications provides novel insights into pathogenesis, progression and effects of human diseases. Aberrant protein expression and protein phosphorylation can be linked to a variety of diseases [1–5]. Indeed, many drug treatments induce changes on the level of protein expression and/or phosphorylation [6–9]. For understanding the complex underlying cause and effect relations, quantitative (phospho-) proteomics is becoming a key technique in clinical and biomedical research [10–12].

In the past years, several quantitative proteomics and phosphoproteomics strategies have been developed [13–15], many employ multidimensional separation of enzymatically digested proteins and subsequent analysis by LC-MS [14]. Notably, current strategies often have two major disadvantages. First, proteome and phosphoproteome analysis are conducted separately in two different approaches, requiring more sample material then actually necessary. Second, phosphoproteome analysis often requires a huge amount of starting material, up to milligrammes of protein (recently summarized by Ficarro *et al.* [16]). Consequently, such strategies are inadequate for analysing most clinical or biomedical samples [10,15]. Current efforts for building up bio bank facilities to provide researchers with exactly the required samples, but obviously not with infinitive amounts, even underscore the necessity of low sample amount strategies [17]. Hence, proteomics and phosphoproteomics workflows have to be successively refined to enable comprehensive and reliable quantitative analysis consuming a minimum of sample material. To achieve the required reduction of sample material, sensitive proteomics and phosphoproteomics have to be combined to a single workflow, rather than in two separate ones, to provide quantitative information on both (I) the level of protein expression and (II) the level of protein phosphorylation.

Owing to the usually sub-stoichiometric nature of protein phosphorylation, a comprehensive analysis requires one or more dedicated phosphopeptide enrichment steps prior LC-MS analysis. Independent of the employed enrichment techniques, even the most sensitive phosphoproteomics workflows based on affinity

* Corresponding author at: Leibniz-Institut für Analytische Wissenschaften—ISAS—e.V., Otto-Hahn-Straße 6b, 44227 Dortmund, Germany. Tel.: +49 231 1392 100; fax: +49 231 1392 200.
E-mail address: albert.sickmann@isas.de (A. Sickmann).

http://dx.doi.org/10.1016/j.bbapap.2015.01.006
1570-9639/

chromatography [18–21] or electrostatic repulsion–hydrophilic interaction chromatography (ERLIC) [22,23], require substantially more sample material than a standard LC-MS-based proteomics workflow. Since phosphopeptide enrichment actually depletes non-phosphorylated peptides, the latter ones can be recovered and used for an additional analysis of the global proteome [24,25]. This allows the combined quantitative analysis of the proteome and phosphoproteome even from small sample amounts.

We recently developed an optimized ERLIC-based strategy for highly sensitive phosphoproteomics, termed ERLIC-SCX/RP (ERLIC-1) [23]. Here we demonstrate a refined three-dimensional ERLIC-ERLIC strategy for combined quantitative proteomics and phosphoproteomics by employing two different ERLIC modes. We integrated a quantitative proteomics workflow (ERLIC-2) that allows the highly efficient analysis of non-phosphorylated peptides recovered from the phosphopeptide enrichment step (ERLIC-1). This integrated workflow represents a comprehensive proteomics/phosphoproteomics strategy, without (i) the need for employing additional sample material (Fig. 1) and (ii) loosing sensitivity in the phosphorylation analysis. We demonstrate its applicability for low sample amounts by comparing murine cytomegalovirus (MCMV)-infected mouse fibroblasts against non-infected controls, which serves as a model for host cell manipulation by a pathogenic infectious agent [26,27]. We were able to perform an in-depth analysis of both, the phosphoproteome and the proteome, in a SILAC 2-plex experiment using only 150 μg of protein per condition. The presented 3D ERLIC-ERLIC-LC-MS strategy has proven to be perfectly applicable for experiments with limited sample amounts, one of the critical demands in clinical, preclinical and biomedical research.

2. Experimental procedures

Chemicals, reagents and trypsin (T-1426) were purchased from Sigma Aldrich (Munich, Germany) in pA grade or higher, unless stated otherwise. Solvents were purchased from Biosolve (Valkenswaard, Netherlands) in ULC/MS grade. De-ionized water was generated in-house by a pure lab ultra system (ELGA, Celle, Germany). For SPE after ERLIC, Hypersep 10–200 μL SpinTips were used (Thermo Scientific, Bremen, Germany). PolyWax LP columns for ERLIC-1 and ERLIC-2 were from Poly LC, Columbia, USA)

2.1. Fibroblast cell culture and MCMV infection

Murine NIH-3T3 fibroblasts (CRL-1658) were obtained from the American Type Culture Collection (ATCC, USA). A differential 2-plex SILAC sample was generated by growing NIH-3T3 cells in SILAC-Dulbecco's Modified Eagle Medium (Thermo Scientific) containing 10% dialyzed FCS (GE Healthcare, Chalfont St Giles, United Kingdom), 100 U/mL penicillin/100 μg/mL streptomycin, 4 mM glutamine and 200 mg/L proline supplemented with 84 mg/L Arg $^{13}C_6$ and 143 mg/L Lys $^{13}C_6$ (R6K6 labelled) or 84 mg/L Arg $^{12}C_6$ and 143 mg/L Lys $^{12}C_6$ (R0K0) for five passages. For infection R6K6 labelled cells were grown in a 6-well dish and infected at a multiplicity of infection (MOI) of 5

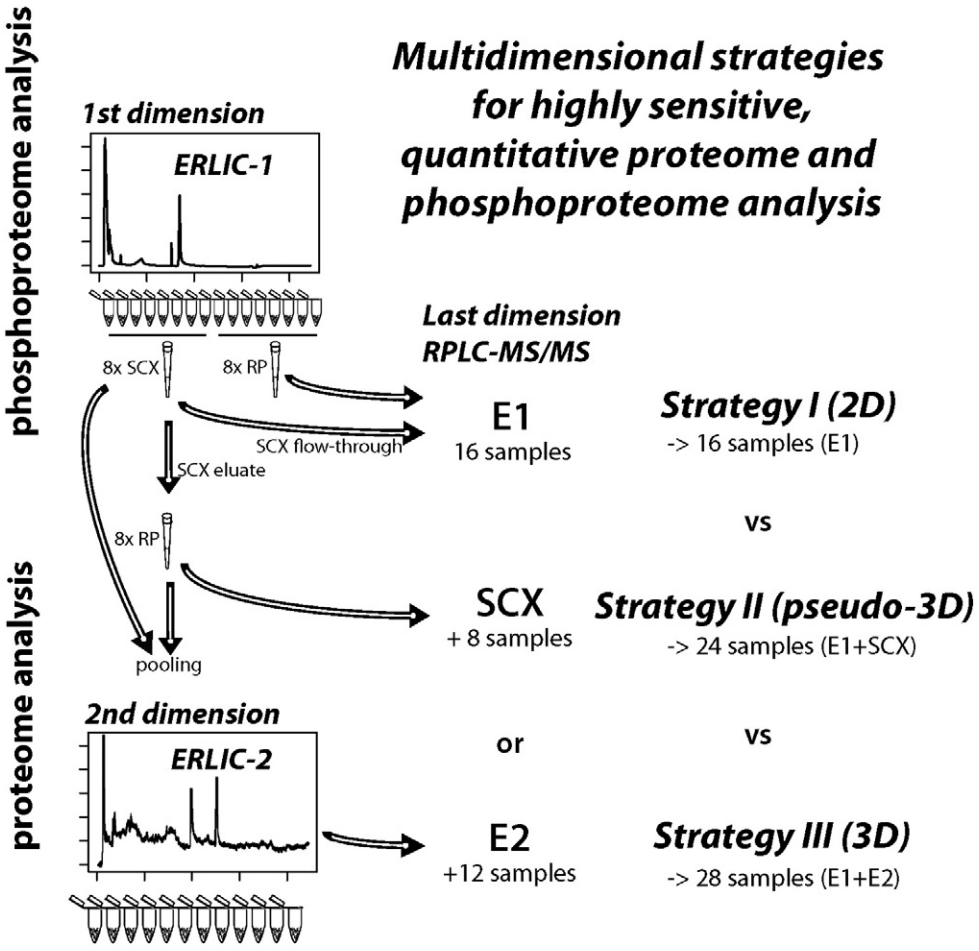

Fig. 1. Multidimensional, ERLIC-based strategies for highly efficient proteome and phosphoproteome analysis. Starting from a two-dimensional ERLIC-1 strategy (E1) we systematically integrated a proteomics workflow (SCX, E2) by recovering the non-phosphorylated peptides. The resulting 3D strategy (E1 + E2) allows an in-depth analysis of both, the proteome and the phosphoproteome.