- *PC-capLC-μESI-IT-MS/MS*: las muestras se inyectaron en un sistema cromatográfico conectado *on-line* a un espectrómetro de masas de trampa iónica lineal (LTQ, ThermoFisher). El análisis se realizó en modo dependiente, adquiriéndose un espectro de barrido completo y 8 MS/MS de las señales más abundantes. En el caso de los fosfopéptidos que perdían el grupo fosfato, se realizó un tercer barrido de MS^3 sobre el ión derivado de dicha pérdida. Se utilizó el software SEQUEST para la búsqueda en bases de datos.

Resultados

El análisis de estos extractos nos ha permitido caracterizar 331 puntos de fosforilación (279 pSer, 49 pThr y 8 pTyr) en un total de 322 fosfopéptidos únicos correspondientes a 247 proteínas. Alrededor de la mitad de estos puntos de fosforilación no se encuentran anotados en SwissProt. Esta colección deriva de espectros de fragmentación de alta fiabilidad seleccionados en condiciones muy restrictivas en las que no se obtenía ningún falso positivo cuando la búsqueda se realizaba sobre el conjunto de las bases de datos SwissProt y Trembl humanas mezcladas con las mismas bases de datos invertidas. En el caso de fosforilación en Ser se han podido definir al menos 4 motivos claros de fosforilación. Esta colección es la única existente hasta el momento en linfocitos T primarios humanos.

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Phosphoproteomics: analytical strategies and computational data analysis

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In order to achieve high-sensitivity analysis of phosphoproteomes with a high dynamic range, it is crucial to couple complementary methods for separation and sequencing of (phospho) peptides. Large-scale phosphoproteomic strategies (Ficarro *et al.*, 2002; Gruhler *et al.*, 2005; Li *et al.*, 2007) typically include enrichments on phosphopeptides using IMAC or TiO_2 resin or Calcium phosphate precipitation (Connor *et al.*, 1999; Larsen *et al.*, 2005; Zhang *et al.*, 2007; Thingholm *et al.*, 2007) followed by reverse-phase liquid-chromatography (LC) coupled to ESI tandem mass spectrometry (MS^n). In addition, the combination of CID and ETD fragmentation in MS/MS is a very promising approach for phosphoproteomic studies (Gruhler *et al.*, 2005). Quantitative methods (i.e. SILAC and iTRAQ) in phosphoproteomics provide new insights into functional phosphorylation events.

Various strategies for functional phosphoproteomic analysis of signaling pathways are being established in biological and biomedical research, for example for the identification of novel drug targets and for the understanding of disease pathogenesis.

We will discuss several strategies for large-scale phosphoproteomics, focusing on critical steps which could increase the number of identified phosphorylated sites, especially those which belong to low abundance proteins. We will also discuss the importance of using bioinformatic tools and validation of MS/MS spectra in order to avoid errors in the assignments phosphorylation sites (Kjernø 2002, Mann *et al.*, 2002, Jensen 2006), e.g. using Mascot, MS Quant, VEMS, and ProteinCenter (Schulze *et al.*, 2003; Matthiesen 2007, Ingrell *et al.*, 2007).

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New insights in the study of s-nitrosylation & s-nitration: strategies and problems

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Introduction

The study of the physiological role of reactive nitrogen species (RNS) is attracting increasing interest in the biomedical community. The most common protein modifications by RNS are S-nitrosylation or covalent incorporation of a nitrosile radical to a thiol group, and nitration, or incorporation of a NO_2 group, generally to tyrosine residues. It is currently thought that nitrosative stress is one of the most relevant pathogenic factors in cardiovascular pathology, including atherosclerosis, hypertension, diabetes and cardiac insufficiency, and NO has become recognized as a key signaling molecule in plants. Moreover, there are evidences that exposing endothelial cells to the immunosupressor cyclosporin A (CsA) increases production of RNS, and particularly peroxinitrite, that stimulates nitration of proteins, being the protein MnSOD one of the candidates to CsA-induced nitration (Horcajo-Redondo *et al.*, 2005). Here we have developed and used proteomics techniques to identify specific sites of S-nitrosylation and S-nitration in protein extracts from different species. The growing interest in the study of these modifications have led to the creation of a Spanish working group, the "*Nitrosoteam*",