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Tabla 1

Observado[1]	*PM[2]*	*Secuencia[3]*
533.57 (4+)	2130.2391	V.PEIKPAIPLPGPEPKPKPEP.E
565.83 (4+)	2259.2950	V.PEIKPAIPLPGPEPKPKPEPE.V
590.40 (4+)	2259.2950	V.KVPEIKPAIPLPGPEPKPKPEP.E
622.62 (4+)	2486.4494	V.KVPEIKPAIPLPGPEPKPKPEPE.V

1 Peso Molecular observado en el LC-MS-MS.
2 Peso Molecular de los iones simplemente cargados observados en el MALDI-TOF.
3 Secuencia determinada tras la interpretación de los espectros de MS-MS en MASCOT.

Conclusiones

Cuatro fragmentos de titina han sido aislados e identificados por primera vez en un extracto de jamón curado, confirmándose la extensa proteólisis de esta proteína durante el procesado del mismo. Esta investigación contribuye a conocer más sobre los cambios bioquímicos que tienen lugar en el músculo post-mortem y, particularmente, aquellos que afectan a la degradación de la estructura muscular.

Bibliografía

Larrea V., Hernando I., Quiles A., Lluch M.A., Pérez-Munuera I. Meat Science, 2006; 74:586-593.

Toldrá F., Flores M., Sanz Y. Food Chemistry, 1997; 59(4):523-530.

In solution separation of membrane proteins using free flow electrophoresis (FFE)

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Introduction

Free-Flow Electrophoresis (FFE) in the isoelectric focusing (IEF) mode provides a carrier-free alternative to 2D-gel electrophoresis that is not limited in pH or size range. So far, the application of IEF-FFE for the separation of membrane proteins has been limited by the tendency of the membrane proteins to precipitate at their isoelectric point (pI). Here we present Interval Zone FFE (IZ-FFE) as a novel mode of FFE. In contrast to IEF, the separation is carried out at a constant pH relying on the net protein charges. The applied pH of the separation medium is selected such that it is different from the pI of the proteins to be separated thereby maintaining proteins in solution that would otherwise precipitate.

Material and methods

A total cell extract from HeLa cells was prepared by sonication in lysis buffer followed by centrifugation. Membrane proteins were extracted by including washing steps in HBS buffer and sodium carbonate buffer and dissolving the final pellet in lysis buffer. IZ-FFE was performed at pH 7.8 on a BD™ FFE System. The separation media contained a proprietary cleavable detergent to increase solubility of the membrane proteins. Collected protein fractions were digested and analyzed using LC-MS/MS. The LC-MS/MS setup consisted of Agilent 1100 binary HPLC system coupled to a Bruker HCTultra ion trap mass spectrometer.

Results

We observed precipitation in the separation chamber when the total cell extract from HeLa cells was separated in the IEF-FFE mode even when a detergent was included in the separation media. Application of the IZ-FFE mode significantly increased protein solubility. Separation of the total cell extract was performed in the IZ-FFE mode using cleavable detergent at high loading rates without precipitation of proteins in the separation chamber. This was also the case for the membrane protein extract of the HeLa cells. The separation media containing the cleavable detergent facilitated the coupling to LC-MS/MS since no cumbersome detergent removal was necessary. The identified membrane proteins from the LC-MS/MS analysis of the individual FFE fractions were analyzed based on the number of transmembrane domains using the TMHMM software (www.cbs.dtu.dk/services/TMHMM/).

Conclusions

FFE provides an alternative method for the separation of membrane proteins. Protein precipitation can be avoided by using the IZ-FFE mode. Coupling to LC-MS/MS is facilitated by the introduction of a cleavable detergent in the separation media.

Systematic analysis of protein interactions with tetraspanins by high-troughput, second generation proteomics technics

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Introduction

The tetraspanin superfamily of transmembrane proteins are clustered in compact structural groups forming specialized membrane microdomains (Tetraspanin Enriched Microdomains, TEM or TERM). Through heterolog and homolog interactions, tetraspanins regulate signalling processes mediated by cellular adhesion molecules, growth factor receptors and costimulatory proteins (Hemler, 2005). In spite of the growing interest for these proteins, the cytosolic interactions by which tetraspanins are involved in various receptor activation pathways, their cytoskeleton anchorage and in general the protein ligands that interact with these proteins are poorly known. In this work we have made a systematic analysis of interacting partners of different proteins that are presented in TERMs, including ICAM-1, VCAM-1, CD81, CD151 and EWI-2 (Barreiro and Yañez-Mo et al., 2005). This was accomplished by “pull-down” techniques and high-throughput protein identification of the captured ligands by mass spectrometry.

Material and methods

The schematic workflow is represented in Figure 1. Synthetic biotinylated peptides spanning the C-terminal cytoplasmic end of these proteins were incubated with extracts from different cell models, including HeLa cells and lymphocytes, and then captured using Streptavidin-sepharose microbeads. Proteins interacting with the peptides were subjected to digestion and the resulting peptides systematically analyzed by HPLC-linear ion trap MS/MS mass spectrometry. Proteins were automatically identified from the MS/MS spectra in a human database by using the pRatio software developed by our group (Navarro et al., 2007); error rate of protein identification was controlled by using decoy databases and was inferior to 5% (López-Ferrer et al., 2004).

Results

More than one hundred proteins from HeLa cells and a similar number from resting or CD3-activated