Proceedings of the ***4th International Peptide Symposium*** *in conjunction with the* ***7th Australian Peptide Conference*** *and the* ***2nd Asia-Pacific International Peptide Symposium, 2007***

Jackie Wilce (Editor) on behalf of the Australian Peptide Association

Isotope Coded Protein Labelling (ICPL) in combination with Liquid Chromatography MALDI-TOF/TOF mass spectrometry analysis (LC-MALDI) used for the quantification of complex protein mixtures

Mark R. Condina[1], Johan O.R. Gustafsson[1] and Peter Hoffmann[1]*

[1]Adelaide Proteomics Centre, School of Molecular and Biomedical Science, The University of Adelaide, Adelaide, SA 5005, Australia
E-mail: peter.hoffmann@adelaide.edu.au

Introduction

Quantification of protein expression levels in complex protein mixtures like cell lysates or tissue extracts is still a challenge in proteomics. This is particularly the case when low amounts of proteins are available or the protein mixture contains mainly membrane proteins, such that 2D gel electrophoresis in combination with fluorescence dye labeling would be unable to be used for quantification [1-6]. In this case, isotopic labeling in conjunction with Liquid Chromatography and mass spectrometry is the method of choice. Here we have validated a method utilizing an Isotope Coded Protein Label (ICPL) reagent with Liquid Chromatography and mass spectrometry (LC-MALDI) for the quantification of protein expression levels in complex protein mixtures.

Results and Discussion

We have used an Agilent 1100 Series HPLC system with a micro well-plate auto sampler and a capillary pump and separated injected peptide samples on a LC PACKINGS Dionex Acclaim PepMap100 C18 (180 µm I.D. x 15 cm, 3 µm, 100Å) column. Separated peptides were then automatically spotted by a Proteineer fc spotting robot on AnchorChip MALDI targets, both from Bruker Daltonics (Bremen, Germany). Spotted targets were processed with an ultraflex III MALDI-TOF/TOF mass spectrometer (Bruker Daltonics, Bremen, Germany) with Flex control 3.0 and WARP-LC software (both from Bruker Daltonics, Bremen, Germany) for quantification and identification.

In order to validate the system we have labeled three proteins (Bovine serum albumin, Ovalbumin, Bovine Carbonic Anhydrase II) in different ratios with the light and the heavy isotope tag (SERVA electrophoresis, Heidelberg, Germany). The six labeled proteins were subsequently mixed together and digested in-solution with trypsin. This sample was then used to determine both the sensitivity and quantification capability of the above described system. We were able to quantify and identify these three proteins at a level of ~100 ng total protein amount with this system. Figure 1A shows a representative ICPL labelled pair with a 6 Da difference (corresponding to the light and heavy forms of the isotope tag), which was chosen using WARP-LC software.

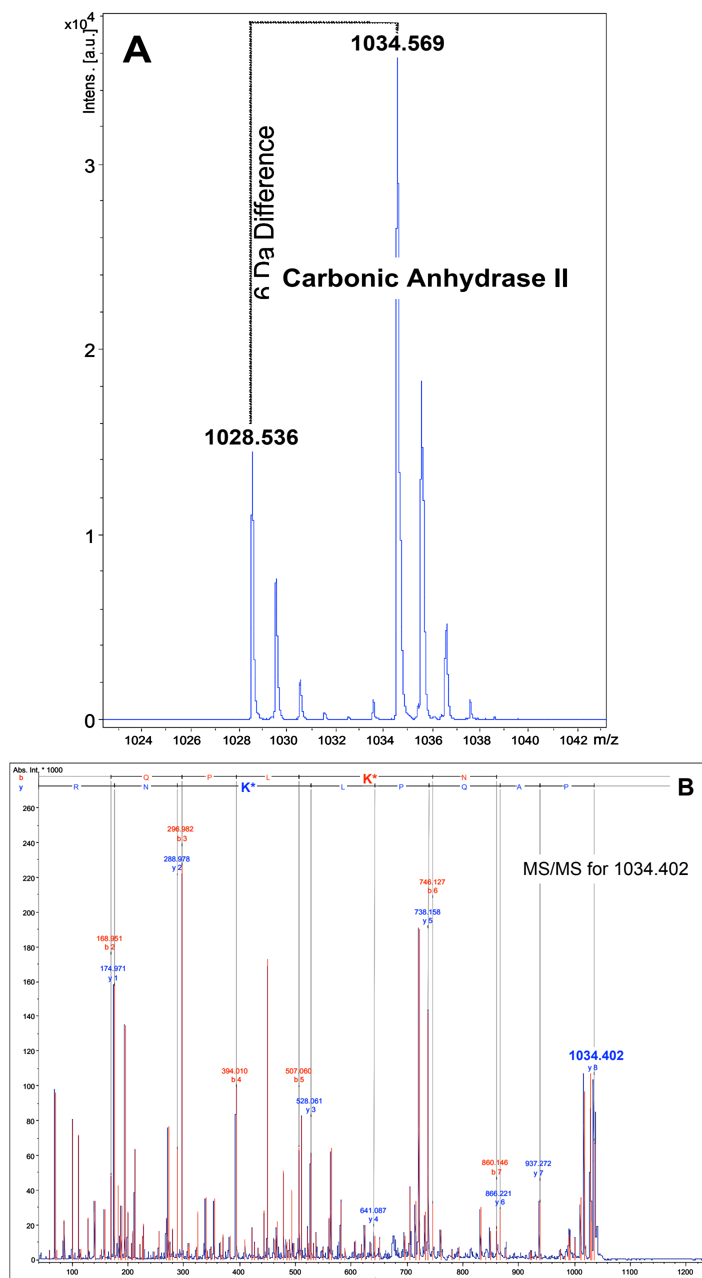
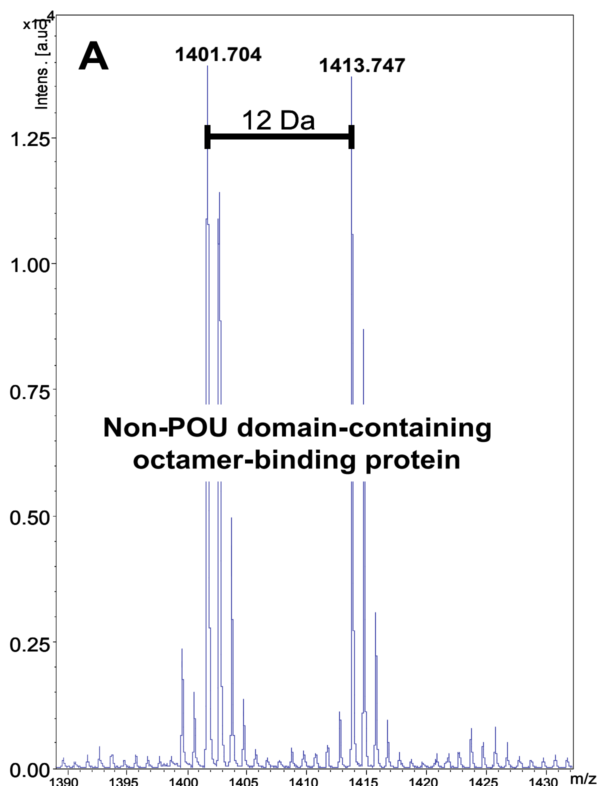


Fig. 1. ICPL labelled control sample.

A: Representative MALDI-TOF MS spectrum of a ICPL a labelled pair.

B: MALDI-TOF/TOF tandem MS spectrum of the peptide labelled with the heavy form of the isotope tag

Subsequent MS/MS analysis identified the peptide as Bovine Carbonic Anhydrase II, as shown in Figure 1B.



In addition, complex protein lysates obtained from resting mouse CD4+ T lymphocytes were run over our system, with some representative results shown here (Figure 2). Analysis of one fraction obtained from the lysate obtained around 1500 labelled pairs, consequently identifying 10 proteins, including Non-POU domain-containing octamer-binding protein (Figure 2A and B).

The effectiveness to quantify and identify these samples highlights the sensitivity and ability of this system to be used for protein expression analyses.

Acknowledgments

We would like to thank Dionex Australia for providing the Acclaim PepMap100 C18 column used for LC-MALDI.

References

1. Bisle, B., Schmidt, A., Scheibe, B., Klein, C., Tebbe, A., Kellermann, J., Siedler, F., Pfeiffer, F., Lottspeich, F., and Oesterhelt, D. (2006). Mol Cell Proteomics *5*, 1543-1558.
2. Hu, J., Qian, J., Borisov, O., Pan, S., Li, Y., Liu, T., Deng, L., Wannemacher, K., Kurnellas, M., Patterson, C., Elkabes, S., and Li, H. (2006). Proteomics *6*, 4321-4334.
3. Patton, W.F., Schulenberg, B., and Steinberg, T.H. (2002). Curr Opin Biotechnol *13*, 321-328.
4. Sarioglu, H., Brandner, S., Jacobsen, C., Meindl, T., Schmidt, A., Kellermann, J., Lottspeich, F., and Andrae, U. (2006). Proteomics *6*, 2407-2421.
5. Schmidt, A., Kellermann, J., and Lottspeich, F. (2005). Proteomics *5*, 4-15.
6. Wu, W.W., Wang, G., Baek, S.J., and Shen, R.F. (2006). J Proteome Res *5*, 651-658.

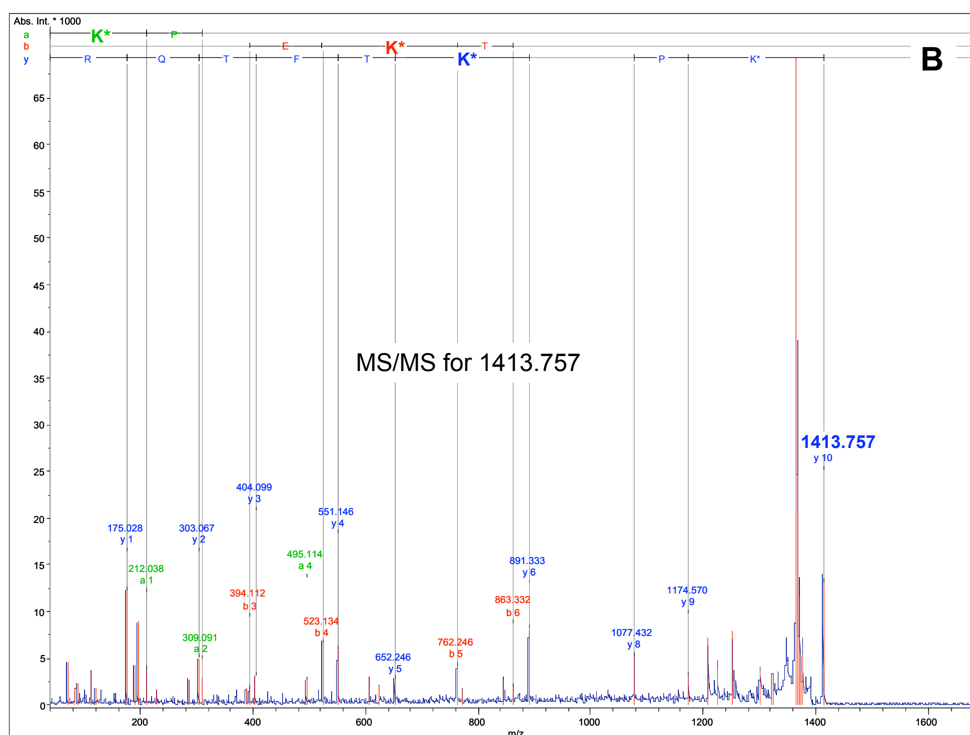


Fig. 2. ICPL labelled mouse CD4+ lysate fraction.

A: Representative MALDI-TOF MS spectrum of an ICPL an labelled pair obtained from fraction 12 of 30.

B: MALDI-TOF/TOF tandem MS spectrum of the peptide labelled with the heavy form of the isotope tag