



2-Dimensional Gel Electrophoresis *versus* Quantitative Protein Sequence Tags (qPST™): a comparative proteomic study in yeast

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Summary

The global proteome profiling approach quantitative Protein Sequence Tag (qPST™) employs a peptide-labelling strategy using proprietary stable isotope tags for differential protein analysis. To assess the differential quantitative data obtained by qPST™ proteome samples from yeast grown on either 2% galactose or on 2% ethanol were analysed in parallel using two dimensional gel electrophoresis (2-DE) combined with MS. The two differential profiling approaches displayed, identified and quantified a similar number of proteins, namely 463 with qPST and 457 with 2-DE. Approximately one third of the proteins was identified with both approaches, one third was exclusively detected with qPST™ and one third was exclusively addressed with 2-DE. The qPST approach detected 56 regulated proteins (>2-fold) and the 2-DE approach detected 73 regulated proteins (>2-fold and p<0.005). Among those 47 regulated proteins were found with both approaches. Most of the other regulated proteins showed the same trend of regulation with qPST™ and 2-DE. Using a representative set of 18 proteins the coefficient of variation (CV) of the quantified protein expression ratios were measured with both qPST and 2-DE. With qPST CV's between 0.3% and 30% with an average CV of 11% and with 2-DE CV's between 19% and 68% with an average CV of 38% were found. This study demonstrates that differential protein profiling with 2-DE or qPST™ are complementary to a good extent and that proteins addressed by both systems show similar regulations. The quantitative reproducibility is similarly good with both systems and both approaches performed in parallel offers an attractive solution to profile differential protein expression toward biomarker discovery.

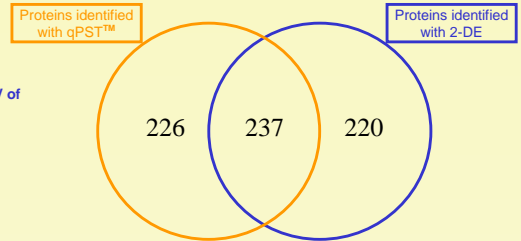


Figure 3: Venn diagram showing overview of the proteins identified with qPST™ and 2-DE approaches. 463 proteins were identified with qPST™ and 457 proteins were identified with 2-DE.

Introduction

Some time ago we introduced the Protein Sequence Tag technology (PST™) which was successfully applied to the characterisation of low abundant membrane proteins. More recently, we developed the quantitative PST approach (qPST™) for the differential quantitative profiling of complex proteomes. In order to assess the quantitative data obtained by qPST™, yeast protein samples grown on different carbon sources were analysed in parallel using 2-DE. Here, we present the results of this comparative proteomic study between 2-DE and qPST™.

Methods

Sample preparation

Cells were harvested from yeast utilising either 2% galactose or 2% ethanol as sole carbon source. The total cell lysate of both samples were isolated.

PST procedure

The qPST™ procedure is described in Prinz et al., [3] and a schematic representation is shown in Figure 1. Briefly, each protein sample was treated with CNBr, reduced, alkylated and labelled with light or heavy isotopic mass tags. After pooling the labelled mixtures and enzymatic cleavage, the peptide mixture was fractionated and each fraction was analysed by one LC/MS run (for peptide quantification) and a successive LC-MS/MS run (for peptide identification). The detection and quantification of peptide pairs were achieved by analysing the LC-MS run with our proprietary software. The peptide identification was performed by targeted MS/MS experiments and analysed with SEQUEST after generating include lists of regulated peptide pairs (2-fold regulation). The positive identifications were cross-matched to the regulation data. Only identified regulated peptides were taken into account in this study. In parallel, an undirected peptide analysis by LC-MS/MS was performed to achieve a broad protein identification list.

2D gel electrophoresis

Isoelectric focusing was performed using 24cm IPG strips (3-10NL, GE Healthcare) and total protein loaded was 80µg for analytical gels and 800µg for preparative gels. SDS-PAGE was performed using 10% acrylamide gels. After silver staining, gels were scanned and images were processed and spots analysed using the Progenesis software package (v2005). Figure 2 shows the image of a silver-stained gel. For spot identification purposes all detected protein spots in preparative Coomassie-stained gels were excised, destained, digested with trypsin and peptides were spotted onto MS-targets using the Spot Handling Work Station (GE Healthcare). Peptide mass fingerprints were prepared using MALDI-ToF MS (Voyager, Applied Biosystems). Protein identifications were performed using the Ms-Fit program and the Swiss-Prot database.

Results

Number of proteins displayed with qPST™ and 2-DE

As shown in Figure 3, the two approaches displayed, identified and quantified a similar number of proteins (463 proteins with qPST and 457 proteins with 2-DE). Altogether, both technologies identified 683 proteins. Approximately, one third of the proteins was identified with both approaches (237/683), one third was exclusively detected with qPST™ (226/683) and one third was exclusively detected with 2-DE (220/683).

Comparison of the protein regulations found with qPST™ and 2-DE

With qPST™, 56 identified proteins were found to be regulated at least 2-fold (1.6 peptides identified / protein). With 2-DE, 73 identified proteins were found to be regulated at least 2-fold and with p<0.005. These proteins are listed in Table 1. 47 regulated proteins identified with qPST™ were identified with 2-DE as well. Among these proteins, 83% (39/47) displayed the same trend of regulation as shown in Figure 4. Eight protein spots (ENO1, EF1A, HS78, DHE5, HS72, CACP, EFTU and KAD1) displayed an opposite trend of regulation (Figure 4). For 3 spots (ENO1, EF1A and HS72), the identified proteins correspond to a fragment of the full-length protein. Figure 5 presents an example of an identified protein corresponding to GCY1 gene product and found to be regulated both in qPST™ and 2-DE. On the spectrum displayed, a 5Da mass difference is observed (600.0 and 601.7 Th for [M+3H]³⁺) which is in agreement with the mass difference between the heavy- and light PST tags. Finally, 9 regulated proteins identified with qPST™ were not identified with 2-DE (Table 1). Most of them correspond to basic proteins with pI near the limit of the IPG strips used or to proteins with low molecular weight. Of the 73 regulated proteins identified with 2-DE, 57 proteins were identified with qPST™ (Table 1) and 30 are regulated as well. Finally, 16 regulated proteins identified in 2-DE were not identified in qPST™. For a set of 18 proteins, CVs of expression ratios were calculated (Table 1). With qPST™, CV are between 0.3% and 30% with an average of 11%. With 2-DE, CV are between 19% and 68% with an average of 38%.

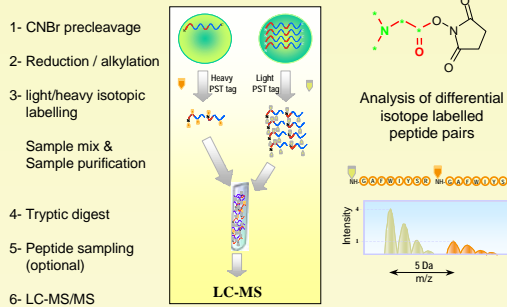


Figure 1: Schematic representation of the qPST™ procedure.

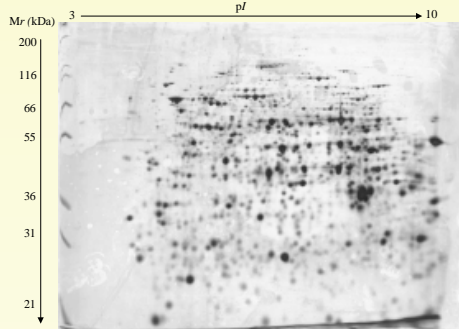


Figure 2: 2-DE map of the S. cerevisiae proteome. The pattern shown corresponds to a 2-D PAGE image of silver-stained proteins extracted from yeast strain grown on galactose as carbon source.

Conclusions

We found that qPST™ and 2-DE displayed, identified and quantified a similar number of proteins. This result confirms that these differential protein profiling approaches are complementary to a good extent with respect to the subset of proteins surveyed and analysed.

83% of the proteins regulated and identified with qPST™ show a similar trend of regulation with 2-DE. This observation highlights the robustness and reproducibility of the differential quantitative procedure of PST™ approach.

The reproducibility of the quantitative changes in protein expression (CV's) as shown with a representative set of 18 proteins are similarly good with both approaches and are in agreement with published results.

Finally, both approaches PST™ and 2-DE performed in parallel provide an approximate 50% increase in proteins identified and a powerful strategy towards the discovery of biologically relevant biomarkers in complex proteomes of cells, tissues and body fluids.

References

1. Kuhn, K., Thompson, A., Prinz, T. et al., J. Prot. Res. 2003, 2: 598-609.
2. Prinz, T., Müller, J., Kuhn, K. et al., J. Prot. Res. 2004, 3: 1073-1081.
3. Kuhn, K., Prinz, T., Schäfer, J. et al., Proteomics 2005, 5: 2364-2368.
4. Gygi, S. P., Rist, B., Gerber, S. A., et al., Nat. Biotechnol. 1999, 17: 994-999.
5. Eng, J. K., McCormack, A. L., Yates, J. 3rd, J. Am. Soc. Mass Spectrom. 1994, 5, 976-989.
6. Choe, L.H., Aggarwal, K., Franck, Z., Lee, K. H., Electrophoresis. 2005, 26: 2437-2449.

Table 1: List of proteins found regulated with 2-DE and qPST™ approaches. For the regulated proteins, the protein expression ratio Ethanol / Galactose and Coefficient of Variation (CV) are indicated.

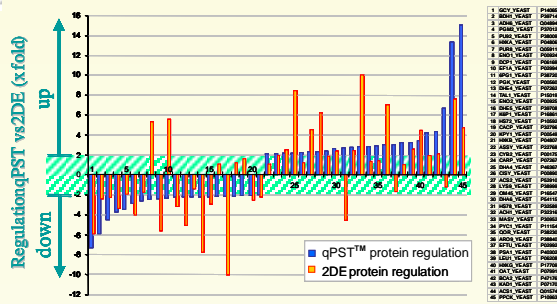


Figure 4: Schematic representation of the regulation of identified proteins observed both with qPST™ and 2-DE. Two proteins found regulated with qPST™ and present only with galactose with 2-DE are not indicated.

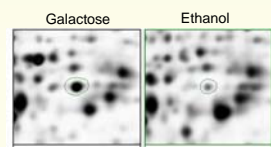


Figure 5: Example of protein regulation quantified by 2-DE and qPST™ and identified as the GCY1 gene product.