ABRF-PRG06: Relative Protein Quantification

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Goal

The 2006 Proteomics Research Group (PRG) study was designed to assist ABRF members to evaluate their proficiency in identifying and quantifying proteins in a mixture. From a comparison of the results obtained by different strategies, participating laboratories will be able to gauge their own capabilities and establish realistic expectations for the approaches that were used.

Abstract

An increasingly important aspect of current biological investigations is the design of experimental systems that yield quantitative results. The drive towards quantitative studies is taking place on several levels and is made possible by the development of new technologies and chemistries. It is, therefore, not surprising that there is keen interest in making the analysis of proteins more quantitative. It is anticipated that comprehensive quantitative analysis of protein expression will greatly advance our understanding of metabolic processes, cellular systems and pathogenic mechanisms of disease.

The 2006 PRG study is particularly timely in that core laboratories are increasingly taking on proteomics-related projects, using a variety of different strategies for relative quantification. The present study was designed to evaluate different methodologies that are available for relative quantification of proteins between experimental samples.

The study sample consisted of two protein mixtures with the same eight proteins in each tube. Four of the proteins were present at a 1:1 ratio and the others were at varying ratios.

The participating laboratories were challenged to identify the proteins and determine their relative quantities in the two samples.

The primary goal of this study was to give laboratories an opportunity to evaluate their capabilities and methods of choice with regard to determining:

- Relative differences of protein quantities between two samples
- The influence of quantification technique on the ability to identify proteins
- The level of confidence and consistency in the quantitative data
- The ability of software to determine quantitative differences between samples

Table 1. Proteins in PRG06 study samples

Protein[1]	M.W. (kDa)	Quantity (pmol) A	Quantity (pmol) B	Ratio (A/B)
β-casein	24.0	598	150	4:1
Bovine serum albumin	66.6	195	195	1:1
Carbonic anhydrase I	28.9	28	90	1:3
Catalase	57.5	299	59	5:1
Glycogen phosphorylase	97.4	3	235	1:76
Lactoperoxidase	77.5	300	300	1:1
Horseradish peroxidase	43.3	298	298	1:1
Ribonuclease A	13.7	296	296	1:1

[1]Proteins were purchased from Sigma-Aldrich: β-casein, catalog no. C-6905; bovine serum albumin, A-0281; carbonic anhydrase I, C-6653; catalase, C-40; glycogen phosphorylase, P-1261; lactoperoxidase, L-8257; horseradish peroxidase, P-6782; ribonuclease, R-4875.

Sample Preparation

All proteins were purchased from Sigma-Aldrich Chemical Co. (St. Louis MO). Deionized water (18.2 MΩ; Milli-Q Gradient A10, Millipore) was used to prepare all solutions. For proteins supplied in small quantities (carbonic anhydrase I, 2.3 mg/vial; glycogen phosphorylase, 10 mg; horseradish peroxidase, 10 mg; and lactoperoxidase, 5 mg), the requisite volume of water was added to each vial to generate the individual stock solutions. Samples of the remaining proteins (β-casein, bovine serum albumin, catalase, ribonuclease A) were weighed on an analytical balance and separately dissolved in water. The protein content of each individual stock solution was assessed by amino acid analysis. Three stock mixtures were then prepared: (1) a mixture containing the 4 proteins that were present at the same proportion in both samples; (2) the remaining proteins for sample A; (3) the remaining proteins for sample B. Each sample A and B received an equal volume of (1) and a volume of stock mixture (2) or (3) as appropriate. Each vial was dried in a vacuum centrifuge and stored at room temperature prior to mailing.

Results

Data submission. Samples were requested by 92 laboratories (North America, 73; Europe, 14; Asia, 5); 50 participants (ABRF members, 29; non-members, 21) submitted 53 datasets, corresponding to a 54% return rate.

Methods used. Mass spectrometry was used for protein identification for all samples. Gel-based and gel-free approaches were employed for quantification.

Method[1]	Number
Gel-based (35.8%)	
1D Coomassie	4
2D Coomassie	4
2D silver-stain	2
1D fluorescence	4
2D fluorescence	1
2D DIGE	3
2D radioactivity	1
MS/isotope (41.5%)	
iTRAQ	12
$^{16}O/^{18}O$	5
ICPL	4
O-methylisourea	1
MS/non-isotope (22.6%)	
Ion current	11
Spectral count	1

[1]DIGE, difference gel electrophoresis; 2D radioactivity, $^{125}I/^{131}I$; iTRAQ, isobaric tags for relative and absolute quantitation; ICPL, isotope coded protein label

Calculations. As a numerical assessment of how close the submitted results for the relative quantities of a protein in samples A and B were to the expected ratio, the following calculations were made:

$$\%\ error\ of\ ratio = [\,|\,(expected\ ratio - observed\ ratio)\,|\,/\,expected\ ratio\,] \times 100$$

Conclusions

A summary of observations made from the study results is shown below. It is important to remember that the number of responses for each method was limited. Therefore, no firm conclusions can be drawn regarding the optimal method for assessing differences in protein quantities between different samples.

Most respondents were able to identify and report quantification data for all eight proteins.

For the majority of methods used, the greatest *% error of ratio* was seen for glycogen phosphorylase (expected ratio 1:76).

Mass spectrometry using ion current (number of respondents (N) = 9) or spectral count (N = 1) and 2D electrophoresis using radioactivity (N = 1) yielded ratios closest to expected for glycogen phosphorylase.

Overall, electrophoresis showed greater variability of *% error of ratio* than MS-based methods.

The results obtained by electrophoresis were closer to the expected values for proteins present at a 1:1 ratio compared to proteins that were in other ratios in the two samples.

Among electrophoresis methods, the lowest *% error of ratio* was seen for 2D DIGE (N = 3) and 2D radioactivity (N = 1) while 2D Coomassie (N = 3) and 2D fluorescence (N = 1) exhibited relatively high error ratios.

Ratios obtained by MS/ion current (N = 9) or spectral count (N = 1) were as close to the expected values as those obtained by stable isotope labeling (N = 20).

The *% error of ratio* was evenly distributed for all proteins except glycogen phosphorylase when MS/stable isotope labeling was used.

MS/ion current or spectral count exhibited an even distribution of *% error of ratio* for all proteins, including glycogen phosphorylase.

Quantification by mass spectrometry was not affected by prior separation of intact proteins by electrophoresis.

Repetition of an analysis yielded results that were closer to expected compared to analyses performed only once (particularly for electrophoresis). Additional analyses (triplicate and quadruplicate) did not yield further improvement.

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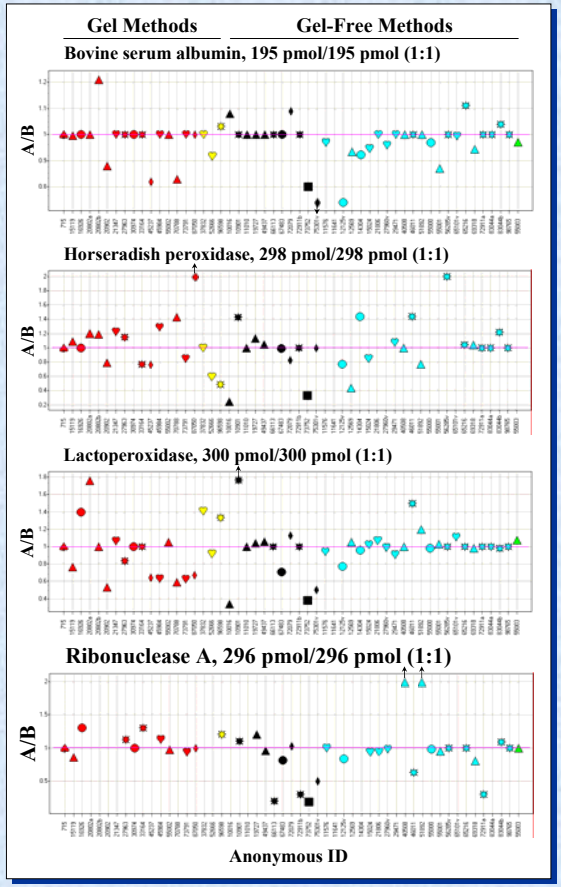
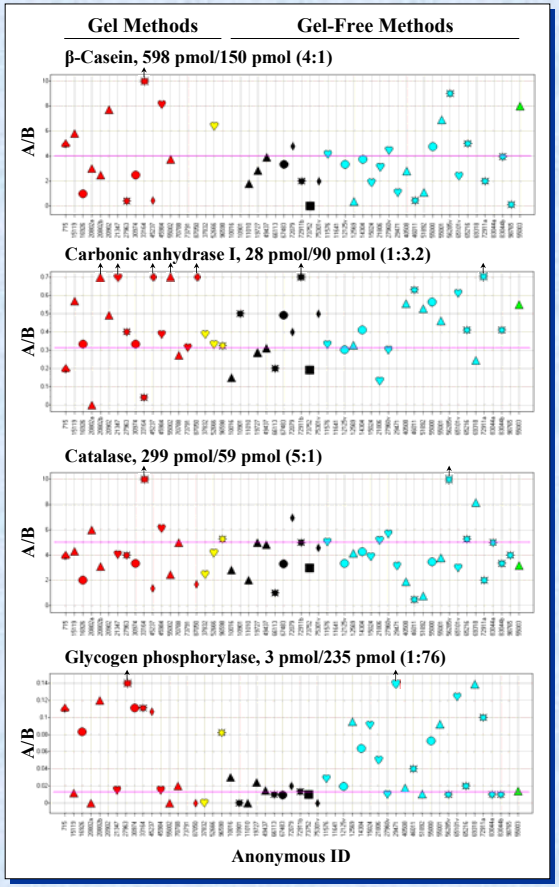
For additional information, please visit http://www.abrf.org/PRG





Scatter plots of results submitted by participating laboratories. The color of each entry corresponds to the general method utilized for relative quantification; the shape of the entry indicates the type of mass spectrometer used. For submissions in which gel-based methods were employed for quantification, the shape of the symbol represents the mass spectrometer used for protein identification. The absence of an entry for a particular protein/participating laboratory signifies that no results were submitted. The scatter plots were generated using Spotfire Decision Site (v. 8). Note that the participants were informed that bovine serum albumin and three of the other proteins were present in samples A and B at a 1:1 ratio.

Method for Quantification

- Densitometry
- DIGE
- Ion current
- Isotope MS
- Spectral count

Type of mass spectrometer

- 3D ion trap
- Linear ion trap
- Linear ion trap/FT
- Quadrupole-TOF
- TOF
- TOF/TOF
- Combination

The PRG strongly points out that the data received from the study participants are not intended to promote any particular method or type of equipment. The study and survey were undertaken with the goals of helping member laboratories and others to both improve and expand the range of their own capabilities and to provide them with a means of testing the techniques that they use. Furthermore the number of submitted responses was insufficient to afford a statistically significant measure of the ability of any method to "get the correct answer." We also point out that in some cases it is likely that the results represent the current experience levels of the scientists who performed the analyses and not the absolute capabilities of the methods used because some of the participating laboratories were conducting these analyses for the first time. Any representation to the contrary of what is stated above is the responsibility of the entity making that representation, and the PRG explicitly does not endorse any such representation.