Bangl. J. Vet. Med. (2007). 5 (1 & 2): 01– 07

A REVIEW ON SUBCELLULAR OR ORGANELLAR PROTEOMICS WITH SPECIAL REFERENCE TO APICOMPLEXAN PARASITES

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ABSTRACT

Despite several well-known limitations, mass spectrometry-based proteomics is still performing important role for post-genomic investigations. As large-scale proteomic investigation of whole organism or cell has been found more complex with available analytical tools, subcellular fractionation prior to mass spectrometry is becoming more useful approach now-a-days. In this review, an attempt has been made to summarize all such subcellular or organellar proteomic investigations performed to date with its implications for apicomplexan parasites.

Key words: Subcellular proteomics, parasite, mass spectrometry, fractionation

INTRODUCTION

The Apicomplexa consists of a large number of parasitic species, including some zoonotic important protozoa like causal agents of malaria, leishmaniasis, chagas disease, africal trypanosomiasis, toxoplasmosis etc. Significant molecular biological research is underway on different aspects of these parasites which is largely due to their unique organelles and structures as well as biology that hinders their control initiatives. It is also assumed that some of the members of this group have evolutionary relationship with that of eukaryotes and their recent genome sequence projects also provided evidence in its favour. With the development of numerous technologies for analysing the genome and proteome of an organism, proteomics is now more powerful than before to answer many biological questions that were not possible before. While global proteomic experiments is producing large amount of proteome data, the fractionation and subcellular proteomics are now becoming more and more useful to explore stage-specific organellar proteome in more explicit manner.

SUBCELLULAR OR ORGANELLAR PROTEOMICS

The classical approach in proteomics couples two-dimensional gel electrophoresis (2-DE) with post-gel identification by mass spectrometry. While this technique has proved quite efficient, its limitations are now well described (Wilkins *et al.,* 1998). Large-scale proteomics studies indicate that complete analysis of whole cells or tissues is too complex for available technology. There is currently no single proteome analysis strategy that can sufficiently address all levels of the organization of the proteome (Gygi *et al.,* 2000; Santoni *et al.,* 2000). Therefore, to achieve a more complete analysis of a proteome it is desirable to focus on subcellular proteomes (Rabilloud *et al.,* 1998).

Cells are compartmentalized, thus providing distinct environments for biochemical processes such as protein synthesis and degradation, provision of energy-rich metabolites, protein glycosylation and DNA replication. The compartmentalized structure of a cell is supported by subsets of gene products that are specifically targeted to particular subcellular structures. Therefore, protein localization is linked to cellular function that requires proteome analysis with subcellular resolution (Dreger, 2003a,b).

Although many years have passed since most cellular organelles were initially characterized by microscopy and subcellular fractionation, a complete catalogue of the proteins in each organelle has yet to be obtained. The complexity of eukaryotic cells hinders a single step characterization of the complete proteome which necessitates alternative approaches. While the classical proteomics approach using 2-DE was successful for analyzing the proteome of different organisms, it was also evident that the number of proteins expressed in complex eukaryotic cells largely exceeds the resolving power of 2-DE. To overcome this limitation, subcellular fractionation is of choice and is used by a number of researchers (Jung *et al.,* 2000).

Subcellular or organelle proteomics have been recently reviewed extensively by a number of authors (Jung *et al.,* 2000; Schirmer and Gerace, 2002; Brunet *et al.,* 2003; Dreger, 2003a,b; Huber *et al.,* 2003; Taylor *et al.,* 2003; Warnock *et al.,* 2004). The approach has been used for analysis of synaptic proteins (Walikonis *et al.,* 2000; Phillips *et al.,* 2001), synaptic vesicles (Hartinger *et al.,* 1996) and yeast plasma membranes (Navarre *et al.,* 2002). This also led to a number of comprehensive global organellar proteomic studies (Table. 1). In all those cases, either one or two-dimension gel electrophoresis separation of proteins was used prior to mass spectrometric protein identification. However, for analysis of membrane proteins, gel-based separation was not fully successful and alternative techniques were devised.

Table 1. Comprehensive global organellar proteomic investigations

Structure	No of proteins	Methods	References
ER: Microsomes (mixed sample)	491	ICAT/MudPIT	Han *et al.,* 2001
Spliceosome	311	Affinity capture/1D-PAGE/ GPF LC-MS/MS	Rappsilber *et al.,* 2002
Nucleolus	271	1D-PAGE/ESI-MS/MS	Andersen *et al.,* 2002
Nuclear envelope	148	2-DE/PMF/PSD	Dreger *et al.,* 2001
Peroxisome	181	GPF LC-MS/MS	Yi *et al.,* 2002
Yeast mitochondrion	179	1D-PAGE/LC-MS/MS	Pflieger *et al.,* 2002
Yeast mitochondria	436	1D-SDS/ LC-MS/MS 2-DE/ MALDI-MS/ MudPIT	Sickmann *et al.,* 2003
Phagosome	140	LB/2-DE/ESI-MS/MS	Garin *et al.,* 2001
Golgi	81	1D-PAGE/ED/PMF/ ESI-MS/MS	Bell *et al.,* 2001
Chloroplast	81	2-DE/EDMAN/PMF/ ESI-MS/MS	Peltier *et al.,* 2000
Lysosomes	27	2-DE/PMF/ ESI-MS/MS	Journet *et al.,* 2002
Exosomes	21	1D-PAGE/PMF/ ESI-MS/MS	Thery *et al.,* 2001
Endosome	???	2-DE/	Fialka *et al.,* 1997
Centrosome	70	1D-PAGE/ nanoLC-MS/MS	Andersen *et al.,* 2003
Rat liver mitochondrion	192	2-DE/ MALDI-MS	Fountoulakis *et al.,* 2002
Human placenta mitochondrion	78	2-DE/ MALDI-MS	Lescuyer *et al.,* 2003
Human placenta mitochondria	46	Blotting, NTS, 2-DE/ MALDI-MS	Rabilloud *et al.,* 1998
Human heart mitochondria	82	1D-PAGE/ PMF	Taylor *et al.,* 2002
Human heart mitochondria	615	1D-PAGE/ LC-MS/MS	Taylor *et al.,* 2003b
Chloroplast of *Arabidopsis thaliana*	36	2-DE/ MALDI-MS	Schubert *et al.,* 2002
Tonoplast or vacuole of *Arabidopsis thaliana*	163	1D-PAGE/ LC-MS/MS	Shimaoka *et al.,* 2004

Table 1. Comprehensive global organellar proteomic investigations (contd.)

Structure	No of proteins	Methods	References
Mitochondria of *Arabidopsis thaliana*	56	2D-BN-SDS/ MALDI-MS and ESI-MS/MS	Kruft *et al.*, 2001
Mitochondria of *Arabidopsis thaliana*	77	2-DE/ MALDI-MS	Millar *et al.*, 2001
Mitochondria of *Arabidopsis thaliana*	14	2-DE/ ESI-MS/MS	Werhahn and Braun, 2002
Pea leaves mitochondria	37	ED, 2-DE/ ESI-MS/MS	Bardel *et al.*, 2002
Yeast mitochondria	546 (477)	RP-LC-MS/MS; LC-FTICR-MS	Prokisch *et al.*, 2004
Rat liver mitochondria	~100	2-DE/ MALDI-MS	Lopez *et al.*, 2000
Rat liver mitochondria	13	2D-BN-SDS-PAGE/ MALDI-MS	Brookes *et al.*, 2002
Rat heart mitochondria	22	2D-BN-SDS-PAGE/ MALDI-MS	Brookes *et al.*, 2002
Yeast mitochondria	253	2-DE/ MALDI-MS	Ohlmeier *et al.*, 2004

ER - endoplasmic reticulum; ESI - electrospray ionization; GPF - gas phase fractionation; ICAT - isotope coded affinity tagging; LB - latex bead encapsulation; 2-DE - two-dimensional electrophoresis; LC-MS/MS - liquid chromatography- tandem mass spectrometry; MudPIT - multidimensional protein identification technology; PAGE - polyacrylamide gel electrophoresis; PMF - peptide mass fingerprinting; PSD - post source decay; RP - reverse phase; FTICR - Fourier transform-ion cyclotron resonance; BN - blue native; NTS - n-terminal sequencing; ED - edman degradation.

Whilst proteomics have the potential to define the composition of organelles, it is limited by organellar cross-contamination that can arise during subcellular fractionation. Thus the precise localization of proteins can be hindered by difficulties in preparing pure organelles (Brunet *et al.*, 2003; Dunkley *et al.*, 2004). However, comparative proteomics of organellar subfractions can mitigate these problems, as demonstrated by a recent study involving the nuclear envelope (Schirmer and Gerace, 2002).

There are also analytical difficulties associated with the monitoring of dynamic changes in the proteome at the subcellular level. This is because the organelles are not fixed entities but rather dynamic structures interacting with each other and remodeling themselves in response to various stimuli. Therefore, analysis of cell organelles in various conditions is required to understand the dynamic nature of integrated cell function (Brunet *et al.*, 2003).

Although organelles are thought to be a discrete entities with particular cellular functions, complex mechanisms of intracellular communication and contact sites between the organelles makes it difficult to evaluate the biological significance of proteins that are usually associated with one organelle, but are detected in the proteome of another organelle (Taylor *et al.*, 2003).

SUBCELLULAR FRACTIONATION OF APICOMPLEXAN PARASITES

Subcellular fractionation strategies represent the centerpiece of subcellular proteome analysis. There are relatively few reports on the subcellular fractionation of structures and organelles from parasitic protozoa especially Apicomplexa. The reason behind this includes the difficulty in obtaining enough cells to start the fractionation procedure and effective disruption methods where all the structures are well preserved (reviewed by de Souza and Cunha-e-Silva, 2003). There is a particular need for well-defined markers to characterize these isolated structures.

A number of reports have been published regarding isolation of apical complex organelles by subcellular fractionation of apicomplexan parasites (reviewed by Blackman and Bannister, 2001). They include secretory organelles like micronemes, rhoptries and dense granules from different stages of parasites. The micronemes were isolated by subcellular fractionation from *Sarcocystis tenella* (Dubremetz and Dissous, 1980), *Sarcocystis muris* (Strobel *et al.*, 1992), *Eimeria nieschulzi* (Dubremetz *et al.*, 1989), *Crytosporidium parvum* (Harris *et al.*, 2004) and *Eimeria tenella* (Tomley, 1994). The dense granules were reported to be isolated from *Sarcocystis tenella* (Dubremetz and Dissous, 1980), *Toxoplasma gondii* (Leriche and Dubremetz, 1991; Foussard *et al.*, 1991),

Sarcocystis muris (Entzeroth *et al.*, 1986; Pohl *et al.*, 1989) and *Plasmodium falciparum* (Trager *et al.*, 1992) The rhoptries were successfully isolated from *Eimeria nieschulzi* (Dubremetz *et al.*, 1989), *Toxoplasma gondii* (Leriche and Dubremetz, 1991; Foussard *et al.*, 1991; Garcia *et al.*, 2004), *Eimeria tenella* (Kawazoe *et al.*, 1992; Tomley, 1997), *Babesia bigemina* (Machado *et al.*, 1993), *Plasmodium yoelii*, *Plasmodium berghei and Plasmodium chabaudi* (Sam-Yellowe *et al.*, 1998, 1999, 2004). There is also a report of separating rhoptry fraction contaminated with dense granule structures from erythrocytic schizonts of *Plasmodium falciparum* (Etzion *et al.*, 1991; Jaikaria *et al.*, 1993; Sam-Yellowe *et al.*, 1995). A number of techniques have also been reported for the isolation of apical organelles from *Eimeria tenella* sporozoites (Tomley, 1997).

Until now, there is no published report at organellar or subcellular proteomic analysis of *C. parvum* sporozoites. In addition to global proteomic investigations, the integration of high throughput proteomics with modern cell fractionation strategies can provide the higher resolution needed to analyse fully the proteome picture of this important protozoan. Subcellular components of *Crytosporidium parvum* sporozoites have been fractionated by Petry and Harris (1999). Here, sporozoites were subjected to cell disruption using a French press and subcellular fractionation by ultracentrifugation using a sucrose density step gradient was applied. Petry and Harris were successful in separating highly enriched preparations of the parasite membrane, the micronemes, dense granules and amylopectin granules. However, they could not find a separate fraction containing rhoptries. For a greater understanding of the biology and biochemistry of *Cryptosporidium*, further study is essential to characterize the complete organellar proteome of this structure. This will ultimately help us to reconstruct the various metabolic features of this parasite.

The field of genomics provides a list of potential proteins encoded by an organism's genome, while data derived from proteomic analysis can provide further information that allows assignment of specific proteins to different subcellular structures. In recent years, organellar proteomics has profiled mitochondrial, chloroplast, nucleolar proteomes, uncovered minor Golgi proteins (Taylor *et al.*, 2000), and compared functional states of the Golgi complex (Wu *et al.*, 2000). Future comparative proteomic studies can provide a better insight towards a complete map of all the cellular proteins in each organelle, in each tissue, at each stage of development.

QUALITY CONTROL FOR EFFECTIVE CELL FRACTIONATION

One of the major limitations of the organelle proteomics is the difficulty in assessing the degree of purity of the enriched fraction. The efficiency of fractionation is critical for the information content of the whole study, such as the accuracy with which proteomics data will enable one to assign potential newly discovered gene products to subcellular organelle or structures. Therefore, one of the essential requirements for successful cell fractionation is the evaluation of the isolation procedure. In most cases this is usually achieved using morphological and/ or biochemical methods. The morphological approach applies light and electron microscopy of purified materials, while the biochemical approach is based on determination of any enzyme activity or antigen or markers (e.g. lipid, antigen biomarker). However, simultaneous use of both approaches is recommended for more reliable evaluation of organelle purification.

A number of classical enzyme markers have been used to evaluate fractionation procedures (reviewed by de Souza and Cunha-e-Silva, 2003). For instance, marker enzymes of protozoan organelles includes galactosyl transferase (golgi complex), hexokinase (glycosome), glucose-6-phosphate and NAPH-cytochrome c reductase (endoplasmic reticulum), adenyl cyclase (plasma membrane), H^+ pyrophosphatase (acidocalcisome), malic decrboxylase (hydrogenosome) and sucinate-cytochrome c reductase (mitochondria). Assessments primarily based on enzyme assay by specific marker enzymes are thus very important tools for evaluating effective fractionation.

Morphological analysis based on microscopy is another significant aid to assess the success of separation and enrichment. Moreover, with the advancement of modern technologies (e.g. DIGE and ICAT labelling), it is now possible to quantitatively measure the level of expected proteins in a particular fraction and comparative analysis with the starting material can help assess the level of enrichment attained (Brunet *et al.*, 2003).

CONCLUSIONS

Subcellular fractionation is a flexible approach resulting in reduced sample complexity and is most efficiently combined with high-resolution 2-DE gels and MALDI analysis as well as with gel-independent techniques like MudPIT or HPLC (Huber *et al.*, 2003). The enrichment of subcellular compartments followed by the identificat-

ion of their protein contents by proteomics is a powerful method for protein localization and functional studies. This will ultimately increase our capability to comprehensively understand the organellar as well as global proteome and thereby will be a significant tool for molecular biological research.

ACKNOWLEDGEMENTS

We are grateful to Dr. J. M. Wastling of University of Liverpool, UK for useful discussion about the manuscript. The work was funded by the Commonwealth Scholarship Commission, UK.

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