Proteomics of pupal brains in *Sarcophaga crassipalpis*: first database of diapause-associated proteins

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ABSTRACT

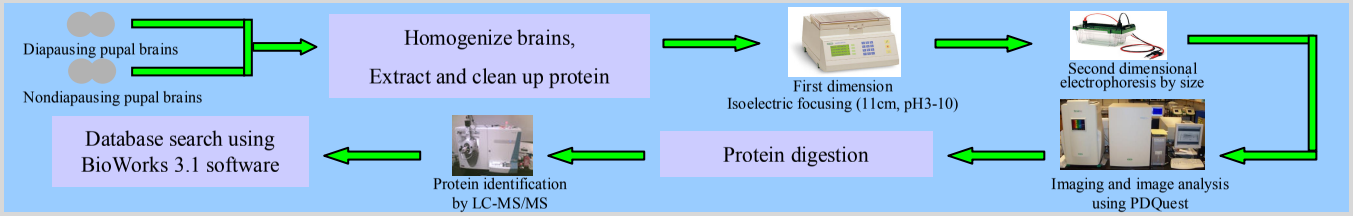
Most molecular work on insect diapause has focused on the expression of unique diapause transcripts, rather than the protein products. Here we present our first results from a proteomic comparison of diapausing and nondiapausing pupal brains. Proteins extracted from diapausing pupal brains in the flesh fly *Sarcophaga crassipalpis* were separated by two-dimensional gel electrophoresis and compared with those from nondiapausing pupal brains. Unique proteins and proteins expressed at different levels in diapausing and nondiapausing brains were identified by Nano-LC/MS/MS (capillary-liquid chromatography-nanospray tandem mass spectrometry). With this approach and Coomassie staining we detected 17 unique or upregulated (⩾3x) spots, and 16 spots that were missing or downregulated in diapause. Most of the brain proteins present in higher amounts during diapause were heat shock proteins (members of the HSP70 and small HSP families). Brain proteins that were less abundant in diapause include phosphoenolpyruvate synthase, fatty acid binding protein, endonuclease, retinal pigment epithelium 65-protein,Ylt1, 16S rRNA pseudouridylate synthase, pupative S-transferase, and EG0003.7. Our 2-D proteome maps include many additional unknown proteins. While the mRNAs encoding certain of these proteins (e.g.HSPs) were previously known to be associated with diapause, many of the other proteins were not known to be linked to diapause, thus suggesting that the proteomic approach nicely supplements work done at the transcript level.

INTRODUCTION

Diapause is a genetically determined developmental arrest in response to unfavorable environmental conditions. Our present knowledge of diapause is mainly based on studies showing that some related genes are transcriptionally modified in diapause (Denlinger, 2002, Denlinger, 2005). However, the amount of mRNA transcripts do not represent the actual protein levels; translation and post-translational regulation play important roles as well (Gygi et al., 1999; Renaut et al., 2006).

The brain was chosen for this study due to it critical role in diapause regulation -- photoreception and storage of diapause information (Richard et al., 1986; Denlinger, 2002). Previous research indicated that some proteins appear to be specific to diapause in the flesh fly, *Sarcophaga crassipalpis,* yet, those proteins were not identified nor assigned functions (Joplin et al. 1990) due to lack of techniques available at that time. The objective of this study is to identify brain proteins that are differentially expressed during diapause using a proteomic approach combining 2-DE, LC-MS/MS and data bank searches.

METHODS



RESULTS

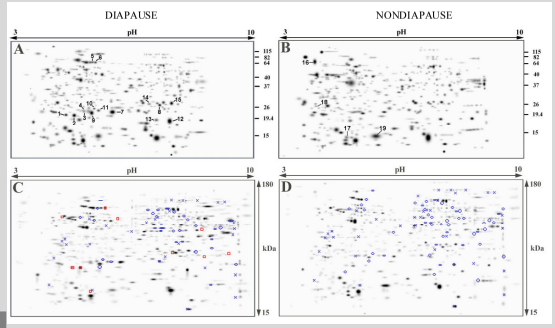


Figure 1. 2DE maps of pupal brain proteins from diapause and nondiapause *S. crassipalpis*. All the proteins were separated by IEF in the first dimension, then by size in the second dimension. Maps were analyzed with PDQuest software. Selected spots from Coomassie stained gels (A and B) were labeled with number 1-19. Labeled spots in Sypro ruby stained gels(C and D) are differentially expressed proteins in diapause and nondiapause: diapause unique (□), diapause 3 fold upregulated (○), diapause 3 fold downregulated (×).

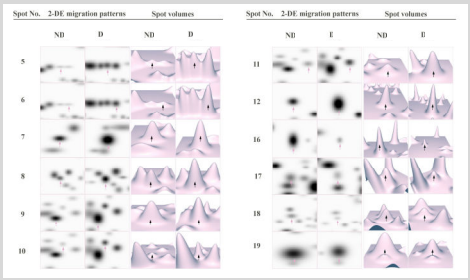


Figure 2 Selected area of two-dimensional gels and corresponding spot volumes for selected spots identified in brains from nondiapausing (ND and diapausing (D) pupae. Protein spot migration patterns were detected in Coomassie stained gels using BioRad VersaDoc model 1000 imaging system. Image pairs and detection of protein spots with relative spot volumes were achieved using PDQuest software. 2-DE, two dimensional electrophoresis.

Table 1. Number of spots from the qualitative and quantitative analysis of 2D gels

Stain	ND Total	D total	D unique	D 3-fold up	D missing	D 3-fold down
Coomassie blue	449	434	3	14	5	11
Sypro ruby	656	725	10	33	0	28

Table 2. Identification of unique or upregulated brain proteins in diapausing fly pupae

Spot No.	Accession No.	Species	Protein ID	Predicted mass(kDa)/PI	Mowse Score
5	gi|13560880	*Drosophila melanogaster*	heat shock protein Hsp70Bc	70.1/5.5	192
	gi|1037174	*Elmeria maxima*	immunoglobulin heavy chain binding protein	38.1/5.4	91
6	gi|13560880	*Drosophila melanogaster*	heat shock protein Hsp70Bc	70.1/5.5	130
	gi|5570	*Aplysia californica*	BiP/GRP78	73.7/4.8	90
	gi|1037174	*Elmeria maxima*	immunoglobulin heavy chain binding protein	38.1/5.4	90
7	gi|2058737	*Sarcophaga crassipalpis*	23kDa heat shock protein ScHSP23	22.9/6.0	553
8	gi|2058737	*Sarcophaga crassipalpis*	23kDa heat shock protein ScHSP23	23.0/6.0	435
9	gi|2058737	*Sarcophaga crassipalpis*	23kDa heat shock protein ScHSP23	23.0/6.0	142
	gi|66548185	*Apis mellifera*	PREDICTED: similar to thiol peroxiredoxin	18.1/5.6	114
	gi|67083335	*Ixodes scapularis*	thioredoxin-dependent peroxide reductase	25.8/9.1	98
	gi|54288811	*Leishmania guyanensis*	trypanothione peroxidase 2	22.9/6.2	82
	gi|7292862	*Drosophila melanogaster*	CG1633-PB, isoform B	22.0/5.5	67
	gi|74353992	*Bos taurus*	PRDX2 protein	22.2/5.4	65
10	gi|2058737	*Sarcophaga crassipalpis*	23kDa heat shock protein ScHSP23	23.0/6.0	517
11	gi|2058737	*Sarcophaga crassipalpis*	23kDa heat shock protein ScHSP23	23.0/6.0	817
12	gi|2058737	*Sarcophaga crassipalpis*	23kDa heat shock protein ScHSP23	23.0/6.0	134
	gi|29344078	*Enterococcus faecalis V583*	hypothetical protein IF 2105	39.6/4.7	55

Table 3. Identification of downregulated brain proteins in diapausing fly pupae

Spot No.	Accession No.	Species	Protein ID	Predicted mass(kDa)/PI	Mowse Score
16	gi|1591246	*Methanocaldococcus jannaschii DSM 2661*	phosphoenolpyruvate synthase	134.6/6.2	59
17	gi|160843	*Schistocerca gregaria*	fatty acid binding protein	15.0/6.2	179
18	gi|3757564	*Drosophila melanogaster*	EG:EG0003.7	24.3/4.4	214
19	gi|74310551	*Epidermophyton floccosum*	endonuclease	34.2/9.53	74
	gi|4001821	*Ambystoma tigrinum*	retinal pigment epithelium 65-protein	61.6/6.0	68
	gi|50552978	*Yarrowia lipolytica*	Ylt1	29.1/6.3	63
	gi|39721833	*Onion yellows phytoplasma OY-M*	16S rRNA pseudouridylate synthase	28.4/9.9	63
	gi|13639844	*Escherichia coli O157:H7*	putative S-transferase	22.7/5.1	55

CONCLUSIONS

- We demonstrate that 2DE analysis of pupal brain proteins by mass spectrometry is useful in confirming protein changes and illustrating physiological events associated with diapause.
- This proteomic study complements our work done at DNA and mRNA levels.
- Like our work at the mRNA level, the proteomic results show a major role for heat shock proteins in diapause, as well as several proteins not previously linked to diapause.

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