Data Article

Proteomics datasets of developing rat brain: Synaptic proteome and SUMO2/3-ylome

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A B S T R A C T

During brain development, synapses undergo structural rearrangements and functional changes mediated by many molecular processes including post-translational modifications by the Small Ubiquitin-like MOdifier (SUMO). To get an overview of the endogenous SUMO-modified proteins in the developing rat brain synapses, our first aim was to characterize the synaptic proteome from rat at 14 postnatal days (PND14), a period that combines intense synaptogenesis, neurotransmission and high levels of SUMO2/3-ylation. In this purpose, we isolated the synaptosomal fraction by differential centrifugation on sucrose percoll gradient and characterized the synaptosomal proteome by denaturing immunoprecipitation using specific anti-SUMO2/3 antibodies prior to proteomics analysis. The information presented in this article complement the publication “Proteomic Identification of an Endogenous Synaptic SUMOylome in the Developing Rat Brain” [1], by focusing on the characterization of the synaptic proteome of PND14 rat brain. Altogether, these data can inform future experiments focused on studying the functional...
consequences of synaptic SUMOylation regarding synapses structure and function. In addition, they can provide the basis for future mechanistic studies investigating brain pathologies involving altered SUMOylation levels.

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Specifications Table

| Subject            | Neuroscience: Cellular and Molecular |
|--------------------|--------------------------------------|
| Specific subject area | Synaptic proteomics, post-translational modifications by SUMO |
| Type of data       | Tables/Figures/Raw data               |
| How the data were acquired | The data were acquired using nanoLC-MS/MS analysis; nanoLC (U3000, ThermoFisher Scientific) using a reversed-phased analytical column 75 μm i.d. x500mm (3 μm, 100 Å) Acclaim PepMap 100 C18 (ThermoFisher Scientific), coupled to a Q-exactive plus mass spectrometer (ThermoFisher Scientific). The raw data were analyzed using SEQUEST integrated into the Proteome Discoverer v2.2 (ThermoFisher Scientific) by searching the data against the Uniprot Rattus norvegicus Reviewed and Unreviewed FASTA database (accessed on 18 July 2018, 35,575 entries). |
| Data format        | Raw/Analyzed/Filtered                 |
| Description of data collection | Synaptosomal fractions were prepared from PND14 rat brains upon differential centrifugation on Percoll-sucrose density gradients. Synaptosomal proteins were separated by SDS-PAGE and subjected to in-gel trypsin digestion prior to LC-MS/MS analysis. Synaptic SUMO2/3-modified protein were purified by specific anti-SUMO2/3 immunoprecipitation on synaptosomal fraction prior to SDS-PAGE separation, in gel trypsin digestion and LC-MS/MS analysis. Concomitant immunoprecipitations using mouse IgG were performed as negative control. |
| Data source location | The raw data has been collected in the Proteomics/Lipidomics Facility from the Institut de Pharmacologie Moléculaire et Cellulaire, (Valbonne Sophia-Antipolis, France). The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE repository with the dataset identifier PXD028804. |
| Data accessibility | Repository name: ProteomeXchange Data identification number: PXD028804 Direct URL to data: http://proteomcentral.proteomexchange.org/cgi/GetDataset?ID=PXD028804; Mendeley Data, V1, doi: 10.17632/bf99y7dsnh.1 |
| Related research article | Pronot M, Kieffer F, Gay AS, Debayle D, Forquet R, Poupon G, Schorova L, Martin S, Gwizdek C. "Proteomic Identification of an Endogenous Synaptic SUMOylome in the Developing Rat Brain." Front Mol Neurosci. 2021 Nov 23;14:780,535. doi: 10.3389/fnmol.2021.780535. eCollection 2021. DOI: 10.3389/fnmol.2021.780535. |
Value of the Data

- The datasets presented here and in the related article [1] provide relevant information regarding the synaptic SUMO2/3-ylated candidate proteins.
- The present analysis focuses on the characterization of the synaptic proteome of PND14 rat brain, with GO terms enrichment analyses and prediction of synaptic protein/protein clusters highlighted for the SUMO2/3-ylation status of its components.
- Both datasets provide a unique resource to the scientific community to thoroughly assess the SUMO-mediated regulation of synapses structure and function.
- These data could be used/reused to adduce further insights into brain pathologies involving altered SUMOylation levels.

1. Data Description

We recently published two proteomics datasets [1]: one identifies synaptic proteins from PND14 rat brain and the other provides a list of SUMO2/3-ylated protein candidates in the synaptic compartment. In the present article, we provide a further characterization of the synaptic proteome of the PND14 rat brain with enrichment analyses for GO terms using generic (Fig. 2 and Supplementary Table S1) or synapse specific tools (Fig. 3 and Supplementary Table S1) as well as a presentation of the main synaptic protein/protein sub-networks highlighting the SUMO2/3-ylation status of its components (Fig. 4 and Supplementary Table S2) as referenced in the SUMO2/3-ylome we have established. The overall workflow for samples preparation, datasets acquisition and data analysis is depicted in Fig. 1. To map synaptic protein content, PND14 rat forebrains were subjected to differential centrifugation on four-steps Percoll-sucrose density gra-

![Fig. 1. Experimental workflow for the proteomics analysis of the synaptic proteome and SUMO2/3-ylome.](image-url)
dients in order to isolate the synaptic compartment. Four independent synaptosomal prepara-
tions were analyzed by LC-MS/MS. The MS data were analyzed using Proteome Discoverer v2.2
against the Rattus norvegicus UniProt Knowledgebase. Only "Master Proteins" passing a cut-off
of 1% False Discovery Rate for both peptides and proteins (Combined Protein FDR Confidence:
"High") were considered for further analysis (Supplementary Table S3). We selected among the
filtered proteins those detected in at least 3 of the 4 preparations to establish a PND14 rat brain
synaptic proteome thus comprising 4379 proteins. To isolate synaptic SUMO2/3-modified pro-
teins, denaturing immunoprecipitation using a specific anti-SUMO-2/3 antibody combined with
a peptic elution were performed on PND14 rat brain synaptosomal lysates. Four independ-
ent synaptic SUMO2/3 immunoprecipitation assays, with concomitant IgG negative controls,
were also subjected to LC-MS/MS analysis. For each immunoprecipitation, proteins identified in
IgG eluate were withdrawn from the attendant SUMO2/3 identified proteins list. The proteins
present at least in 3 of the 4 filtered lists were selected as synaptic SUMO2/3 modified pro-
teins, leading to a final list of 803 candidate SUMO2/3 targets. More than 97% of the synaptic
SUMO2/3-ylome overlap with the synaptic proteome [1]. The repository data files located at
https://www.ebi.ac.uk/pride/archive/projects/PXD028804 contain: LC-MS/MS raw files generated
for these datasets, msf files generated by Proteome Discoverer analysis and results for identified
proteins as xlsx files. The lists of proteins assigned to each dataset are available in the Supple-
mental Information of the original publication [1]. The PND14 synaptic proteome was subjected
to GO terms enrichment analysis using the clusterProfiler tool available on the ProteoRE interface
[2] followed by dimensionality reduction using Revigo [3] (Fig. 2 and Supplementary Table S1).
The top 20 enriched GO terms for Cellular Components (CC), Molecular Functions (MF) or Bio-
logical Processes (BP) are represented in Fig. 2. In line with a synaptic enrichment, terms related
to synapses specificities or referring to pathways or organelles tightly connected to synaptic
organization and function, such as vesicle-mediated trafficking or mitochondrial processes, are
overrepresented. In addition, the identified synaptic proteins were classified based on their asso-
ciation to the synapse specific Gene Ontology terms using SynGO [4]. Sunburst plots of all
the significant Synaptic GO terms for CC and BP as well as hierarchical trees representing the
top 20 enriched terms in each category are shown in Fig. 3 and detailed in Supplementary Ta-
bles S1. We observe a high enrichment in pre- and post-synaptic components as well as in many
synaptic functions such as vesicle cycle, neurotransmission or synaptic signaling. Last, Fig. 4 il-
lustrates the eight most important protein clusters associated to the proteins we identified in
the developing rat brain synapses, along with their associated processes, as predicted by the Cy-
toscape platform [5] implemented with StringApp [6]. The complete details of predicted protein-
protein interactions are provided in Supplementary Table S2. Proteins identified in our synaptic
SUMO2/3-ylome are colored in red while other synaptic components appear in gray. We found
SUMO2/3-modified proteins in all the clusters, showing the implication of the SUMOylation in
all the represented synaptic processes.
Fig. 2. Terms enrichment analysis of the synaptic proteome according to the Gene Ontology, knowledge base. The list of synaptic proteins was subjected to enrichment analysis against the rat proteome followed by redundancy reduction and top 20 terms are represented in bubble plot for GO Cellular Components (A), GO Molecular functions (B), GO Biological Processes terms (C).
Fig. 3. Terms enrichment analysis of the synaptic proteome according to the synapse specific SynGO database. The list of synaptic proteins was subjected to enrichment analysis the against “brain expressed” background and organized in sunburst plot for all the significant terms (A-B) or in top 20 terms hierarchical tree (C-D) for location/CC (A-C) or function/BP (B-D).
2. Experimental Design, Materials and Methods

2.1. Experimental design

The experimental workflow we set up, combining synaptosomes isolation, specific SUMO2/3-ylated proteins immunoprecipitation and LC-MS/MS analysis, aims to: (i) map the synaptic proteome from postnatal day 14 rat (PND14) brains and (ii) delineate a list of endogenous SUMO2/3-ylated candidate proteins in this compartment. Each protein dataset arises from four biological replicates. In the present study, the synaptic protein dataset was further characterized by generic or synapse specific GO terms enrichment analysis. Besides, a mapping of predicted protein clusters including the SUMOylation status of their components was generated from the identified synaptic proteins.
2.2. Materials and methods

2.2.1. Biological material and experimental procedures

Synaptosomes from PND14 Wistar rat brain were obtained by differential centrifugation on four-steps sucrose-Percoll gradients as described in the related article [1]. To characterize the PND14 rat brain synaptic proteome, 10 μg of proteins of four independent synaptosomal preparations were separated on gradient SDS-PAGE, in-gel reduced/alkylated by a treatment with DTT/IAA, digested by trypsin and analysed by LC-MS/MS. Regarding the synaptic SUMO2/3-ylome, four independent immunoprecipitations were performed on 8 mg of proteins from denatured synaptosomal lysates using 160 μg of immobilized monoclonal anti-SUMO2/3 antibodies or mouse IgG as negative control. Proteins eluted by peptidic competition were concentrated by TCA precipitation, separated on gradient SDS-PAGE and subjected to in gel reduction/alkylation and trypsin digestion prior to LC-MS/MS analysis.

2.2.2. Data acquisition

To perform sample analysis, the nanoHPLC ultimate 3000 (Thermo Fisher Scientific) was coupled via a nanoelectrospray ionization source to a Hybrid Quadrupole-Orbitrap High Resolution Mass Spectrometer (Thermo Fisher Scientific). 5 μl of peptidic solution was injected and concentrated on a μ-Precolumn Cartridge Acclaim PepMap 100 C18 (i.d. 5 mM, 5 μm, 100 Å, Thermo Fisher Scientific) using a H2O/ACN/FA 98%/2%/0.1% solution at a flow rate of 10 μL/min. Peptides separation was next achieved on a 75 μm i.d. x 500 mM (3 μm, 100 Å) Acclaim PepMap 100 C18 column (Thermo Fisher Scientific) at a flow rate of 200 nL/min with the solvent system: (A) 100% water, 0.1%FA, (B) 100% acetonitrile, 0.08% FA, according to the following gradient: t = 0 min 4% B; t = 3 min 4%B; t = 170 min, 35% B; t = 172 min, 90% B; t = 180 min 90% B (temperature set at 35 °C). MS spectra were acquired at a resolution of 70 000 (200 m/z) using the following settings: scan range of 150–1800 m/z, AGC target value of 5e5 and maximum injection time of 50 ms. The 10 most intense precursor ions were selected and isolated with a window of 2 m/z and fragmented by Higher energy C-Trap Dissociation with normalized collision energy of 27. MS/MS spectra were acquired in the ion trap using the following settings: resolution of 17 500 (200 m/z), AGC target value of 2e5 and maximum injection time of 100 ms.

2.2.3. Data analysis

Raw data were reprocessed using Proteome Discoverer v2.2 (ThermoFisher Scientific) implemented with the SEQUEST HT module and searched against the UniProtKB Rattus norvegicus Reviewed and Unreviewed FASTA database with the following settings: enzyme specificity fixed to trypsin with two missed cleavages allowed, carbamidomethylation of cysteines set as a fixed/static modification and only oxidation of methionine considered as dynamic/variable modification. A mass accuracy of ±10 ppm was used to precursor ions and 0.02 Da for product ions. Results were filtered at 1% FDR for both peptides and proteins as estimated by target-decoy method. To establish the synaptic proteome of PND14 rat brain, only Master Proteins detected in at least 3 of the 4 synaptic preparations were selected. For the synaptic SUMO2/3-ylome, proteins identified for each immunoprecipitation in the mouse IgG eluate were removed in the concurrent SUMO2/3 eluate. Then, proteins present in at least 3 of the 4 filtered lists were selected as synaptic candidates for SUMO2/3 modification.

2.2.4. Bio-informatics analysis

Gene Ontology (GO) terms enrichment analyses for Cellular Components (CC), Molecular Functions (MF) or Biological Processes (BP) were performed against the rat proteome using clusterProfiler (ontology level 2) on the web interface ProteoRE [2] (https://proteore.org/). Hits were selected according to their adjusted p value using the Benjamini–Hochberg method (adjusted p-value ≤ 0.01). To reduce redundancy in the GO terms, the clusterProfiler output was fed into Revigo [3] (Reduce + Visualize Gene Ontology, http://revigo.irb.hr/) and p-values were used to select and cluster GO terms with a similarity score of 0.5 (small) for BP and 0.7 (medium) for CC and MF. Enrichment analyses using the synapse specific online data analysis platform SynGO 1.1
Ethics Statements

The animal study was complied with the ARRIVE guidelines and were carried out in accordance with EU Directive 2010/63/EU for animal experiments. Protocols were reviewed and approved by the Animal Care and Ethics Committee (Comité Institutionnel d’Ethique Pour l’Animal de Laboratoire N28, Nice, France).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Proteomic identification of an endogenous synaptic SUMOylome in the developing rat brain (Original data) (ProteomeXchange).
Supplementary files: Proteomics datasets of developing rat brain: synaptic proteome and SUMO2/3-ylome (Original data) (Mendeley Data).

CRediT Author Statement

Félicie Kieffer: Investigation, Methodology, Writing – original draft; Marie Pronot: Investigation, Writing – original draft; Anne-Sophie Gay: Investigation, Writing – original draft; Delphine Debayle: Investigation, Writing – original draft; Carole Gwizdek: Conceptualization, Methodology, Investigation, Writing – original draft.

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