Comparison of Sample Extraction Protocols for Two-dimensional Gel Electrophoresis of Rat Brain Tissue.

Veiga-Souza, F.H.[1,2], Cruz, GCN[1], Sousa, M.V. [1]
[1]Laboratório de Bioquímica e Química de Proteínas, Depto. de Biologia Celular, Universidade de Brasília, Brasília, DF, Brazil; [2]Universidade Estadual de Goiás, Anápolis, GO, Brazil

Rats have been used in many experimental models in neuroscience research. The present study reports a comparison of several sample extraction protocols for two-dimensional gel electrophoresis (2DE) of rat brain tissue. The study is part of our ongoing research aiming at identifying the pattern of protein expression and post-translational modifications (PTM) involved in operant learning in rats. The frozen brain of rats were placed in liquid nitrogen, ground thoroughly to a very fine powder with a mortar and pestle, and transferred to tubes containing lysis buffer A or B. The samples suspended in each buffer were submitted to six different conditions: 1) incubation at room temperature (RT) for 1 h; 2) incubation at RT for 1 h followed by trichloroacetic acid/acetone (TCA/A) precipitation; 3) sonication and incubation at RT for 1 h; 4) sonication and incubation at RT for 1 h followed by TCA/A precipitation; 5) extraction with sample grinding kit and 6) extraction with sample grinding kit and TCA/A precipitation. Samples containing 5 μg of total protein were separated by one-dimensional SDS-PAGE and subjected to silver staining. Buffer A allowed the detection of more protein bands. Best resolution was found in association with condition 3. Subsequently, a sample prepared with buffer A and condition 3 was submitted to 2DE, showed a reproducible pattern with high-resolution. The optimized protocol is currently being used as an efficient method for preparing rat brain samples for proteomic analysis by 2DE followed protein identification and PTM characterization by mass spectrometry.

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