EVALUATION OF PLASMA PROTEIN PREPARATION METHODS FOR PROTEOMICS STUDIES

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The choice of the best sample preparation method is essential for blood plasma proteomics, because, although there are many studies with this kind of sample, the methods used are quite variable and can define the possible findings. The objective of this work is to compare three methods of plasma proteins processing for high-resolution mass spectrometry studies, aiming to define the method that depletes the most abundant proteins (mainly albumin) and enriches the least abundant ones. Blood from ten healthy participants was collected, the plasma was separated by centrifugation and submitted to three different preparation methods: 1) Chromatographic depletion (COL); 2) Precipitation with acetonitrile (ACN) and 3) Precipitation with trifluoroacetic acid (TCA). All samples were denatured, reduced, alkylated trypsinizated, desalted and analyzed on LCMS/MS Quadrupole Q Exactive Plus Hybrid Orbitrap (Thermo Fisher Scientific). The m/z spectra generated were processed by the PatternLab V software and later in String. The COL method enabled the identification of 212 proteins; the ACN method, 147 proteins, and the TCA, 264 proteins. Qualitatively, both COL and TCA methods depleted the most albumin from the samples. By observing interactions between proteins derived from the COL method, relevant pathways such as blood coagulation, hemostasis, immune response, inflammation, lipid transport and metabolism were found. Proteins with multiple connections were observed, such as FGG (Fibrinogen Gamma Chain), ApoA1 (Apolipoprotein A1), suggesting their functional importance in plasma. Interactions between proteins obtained through the ACN method showed that low abundance proteins are important for specific biological processes, such as cell signaling, metabolism and immune response. Interactions were observed between growth factors (IGFBP), receptors and signaling molecules (APOA1, APOC1, APOC2). With the TCA method, the interactions observed showed fundamental proteins in controlling the response to cellular stress, helping to maintain protein homeostasis and correct protein folding (HSP90, HSP70). Some important proteins in the innate immune response were observed (C3, C4, C5), coagulation factors (FGA, FGB, FGG) and metabolic enzymes (GAPDH and PKM) that are involved in glycolysis, these enzymes play critical roles in cellular energy production. Despite being the same samples, the use of different methods results in significant variations in protein interaction networks. All methods showed a high density of interactions in the center of the network, however, the density of interactions, the presence of specific clusters and the distribution of peripheral nodes varies depending on the method used. TCA presented a highly dense and diverse protein network, including immunological and inflammatory proteins, encompassing protein functional groups observed in the other methods. This emphasizes the importance of choosing the analysis method regarding costs, execution time and the focus of the study.

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