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A novel protein digestion method with the assistance of alternating current denaturation for high efficient protein digestion and mass spectrometry analysis

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ABSTRACT

Protein denaturation has always displayed a huge necessity for mass spectrometry (MS)-based protein identification methods in proteomics. In this research, a novel protein digestion method with the assistance of alternating current (AC) denaturation has been proposed and evaluated. In this method, merely, 200 mM ammonium bicarbonate buffer solution (pH, 8.2) was used to dissolve proteins and act as the electrolyte, and protein denaturation could be achieved in several seconds. For apo-transferrin, ovalbumin and bovine serum albumin that are resistant to digestion in their native states, confident amino acid sequence coverage by matrix assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) analysis were obtained after 200 v AC denaturation. The applicability of this method was further investigated via analyzing a rat liver proteome sample using nano reversed phase liquid chromatography-electrospray ionization-tandem mass spectrometry (nanoRPLC-ESI-MS/MS). As a result, 458 proteins were identified which is comparable to the in-solution digestion via 8 M urea denaturation (375 proteins). All these results demonstrated that AC denaturation could offer an efficient assistance for a clean and high-throughput digestion in the individual level and proteome level.