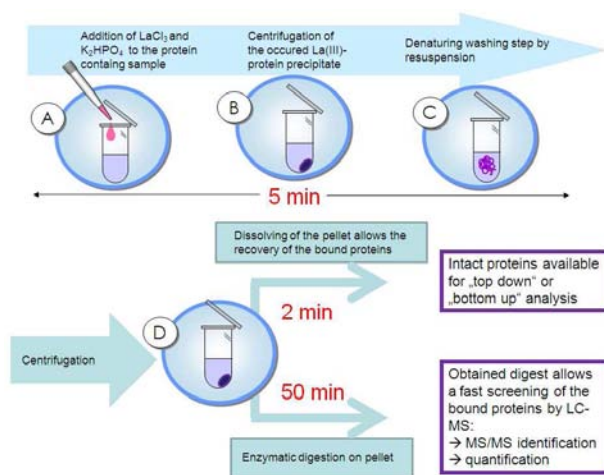


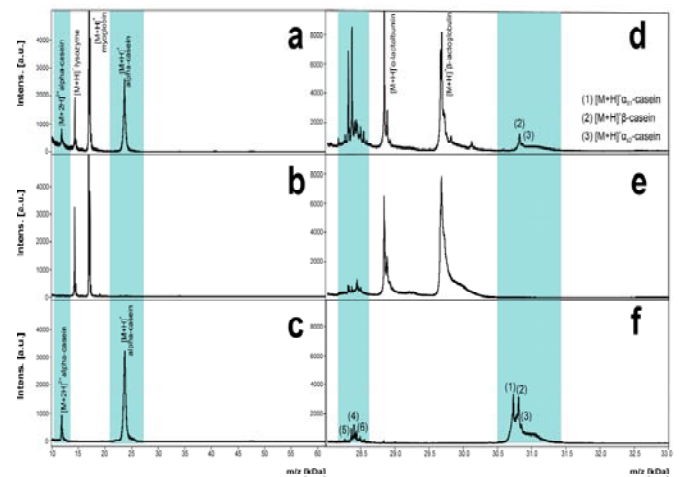
Lanthanum (III) Precipitation – A New Highly Selective Method for Phospho- and Glycoprotein Isolation and Analysis

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The knowledge of the very small solubility product of lanthanum phosphate ($3.7 \times 10^{-23} \text{ kmol}^2/\text{m}^6$) and the high affinity of La^{3+} to carbohydrate was transferred to the analysis of phospho- and glycoproteins. Through the developed, highly selective and easy reproducible method, it is possible to precipitate at the same time phosphorylated- and glycosylated proteins by La^{3+} , isolate them and analyse them by mass spec within ten minutes. The method was tested for several different biological fluids such as a HeLa cell lysate, fresh milk, and human liquor cerebrospinalis and our results (e.g. 16 proteins mentioned in the literature as potential biomarkers for Alzheimer's disease were detected in one analytical run) qualify this method as a valuable tool in the field of protein analysis, especially for the gaining more and more importance "top down" approach. Alternatively it is also possible to digest the proteins directly on the pellet for a "bottom up" analysis. In a further step the phosphopeptides still bound on the pellet after the digest can be recovered by dissolving the pellet for phosphosites determination. Other than the mentioned simple and fast sample preparation another important advantage is the absence of a stationary phase. On this account unspecific binding is avoided and a high selectivity achieved. Moreover, due to the tight binding of lanthanum to the phosphoproteins, stringent washing steps can be carried out by resuspending the pellet in a washing solution followed by centrifugation, without loss of bound proteins, which also increases the selectivity.



Schematic overview of the newly developed method for the simultaneous analysis of glycoproteins and phosphoproteins. The method enables the recovery of the specific bound proteins in less than ten minutes or the identification of the isolated proteins after an on pellet microwave-assisted digest via mass spectrometry within 50 minutes.



MALDI-TOF spectra indicate the high selectivity and efficiency of the precipitation method by comparing the mixture before (a), after the precipitation by La^{3+} (b) and after dissolving the pellet (c). For the measurement sinapinic acid (SA) was used as matrix. The mixture contains lysozyme, myoglobin, BSA ($1 \mu\text{g}/\mu\text{l}$ each) and α -casein ($0.1 \mu\text{g}/\mu\text{l}$). No signal was found for α -casein after the precipitation process, as it was completely precipitated as lanthanum-phosphoprotein complex. After dissolving the pellet α -casein was recovered and detected with the same intensity. The same experiment was carried out with fresh milk. MALDI-TOF spectra the milk before (d) and after the precipitation (e). The spectrum of the supernatant (e) shows the effectivity of the method as no one of the caseins was detected after the precipitation. The recovered α_{s1} -casein $[\text{M}+\text{H}]^+$ (1) and $[\text{M}+2\text{H}]^{2+}$ (4); β -casein $[\text{M}+\text{H}]^+$ (2) and $[\text{M}+2\text{H}]^{2+}$ (5); α_{s2} -casein $[\text{M}+\text{H}]^+$ (3) and $[\text{M}+2\text{H}]^{2+}$ (6) could be measured without any interference of other proteins which explains the increased intensity (f).