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'Back to basics' with the energy-absorbing matrix in SELDI-TOF MS

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The optimism created by the first results of the search for protein biomarkers with surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS) is tempered by its lack of reproducibility. Consequently, a 'back to basics' approach is required to understand the nature of SELDI-TOF MS, especially in order to verify if the lack of reproducibility has an analytical chemical component.

This study describes the effect of the presence of alkali cations in sinapinic acid (SPA). The background of this study is that in the majority of SELDI-TOF MS studies SPA is used as the energy absorbing matrix (1). As SELDI-TOF MS is actually a type of matrix assisted laser desorption/ionization time-of-flight-mass spectrometry (MALDI-TOF MS) technology and alkali metals ions are known to form cation adducts to the protein analytes in MALDI-TOF MS analysis (2), the presence of alkali cations in SPA could influence in a negative way the detection of protein biomarkers.

Materials and Methods

The quality of SPA of Ciphergen (SPA-c) and Fluka (SPA-f) was investigated by SELDI-TOF MS analysis (PBS IIc analyzer, Ciphergen Biosystems, Fremont, CA, USA) on NP20 arrays (Ciphergen) using equine apomyoglobin and bovine serum albumin as model proteins (Sigma, St Louis, MO, USA). Of each protein the relative mass accuracy (RMA) and signal-to-noise (S/N) ratio were determined (n=4). SPA-c was analyzed with and without the presence of NaCl and KCl combined or not with $(NH_4)H_2PO_4$ Ultrex® ultrapure (JT Baker, Deventer, The Netherlands). RMA is defined as $\Delta(m_{measured} - m_{theoretical})/ m_{theoretical}$, where $m_{measured}$ is the measured and $m_{theoretical}$ the theoretical *m/z* value of the signal of the respective model protein. SPA-f was analyzed only combined or not with $(NH_4)H_2PO_4$ Ultrex® ultrapure.

Results and discussion

The addition of simply $(NH_4)H_2PO_4$ to SPA-c or SPA-f resulted in an improvement of RMA of the signals of both model proteins (Figure 1A and C). The effect on the S/N ratio was less prominent, but the overall effect of the addition of $(NH_4)H_2PO_4$ was an increase of the

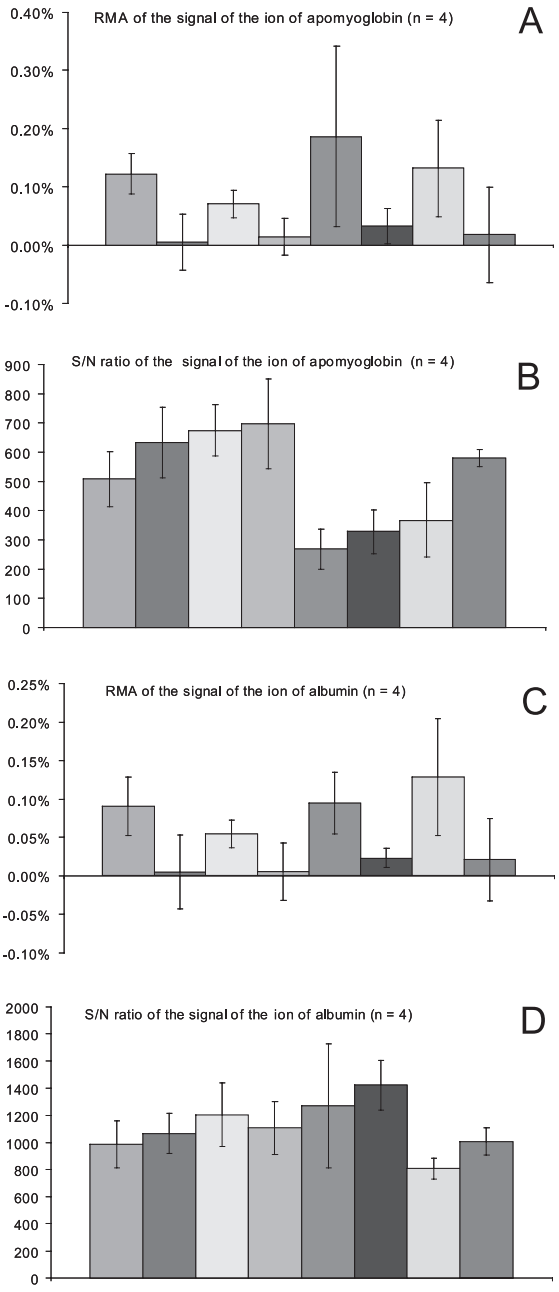

Figure 1. Effect of the presence of$(NH_4)H_2PO_4$ on the relative mass accuracy (RMA) (A and C) and signal-to-noise (S/N) ratio (B and D) of the signal of the ions of model proteins (apomyoglobin, A and B and albumin, C and D) using sinapinic acid of Ciphergen (SPA-c) and Fluka (SPA-f) combined or not with the presence of NaCl and KCl.
■ SPA-c; ■ SPA-c (NH4)H2PO4; □ SPA-c + NaCI; ■ SPA-c (NH4)H2PO4 + NaCI; ■ SPA-c + KCI; ■ SPA-c (NH4) H2PO4 + KCI; □ SPA-f; ■ SPA-f + (NH4) H2PO4.

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S/N ratio (Figure 1B and D). Because $(NH_4)H_2PO_4$ is potent alkali cation adduct ion suppressor (3), these outcomes indicate that the SPA of both sources might contain significant amounts of alkali cations.
The background of this phenomenon is that proteins in MALDI-TOF MS and thus also in SELDI-TOF MS analyses generally are ionized through protonation, i.e. formation of a $[M+H]^+$ ion. In addition to protonation also alkali cationization can occur, in which alkali cations such as Na^+ and K^+ are bound instead of a proton, i.e. formation of $[M+Na]^+$ and $[M+K]^+$ ions. Both processes are in competition of each other and may result in extra peaks in the mass spectrum. As the mass resolving power of the PBS IIc analyzer is insufficient to separate these extra peaks from that of the $[M+H]^+$ ion, the overall result of the presence of alkali cations is peak broadening and decrease of peak intensity, i.e. deterioration of RMA and decrease of S/N ratio.
The addition of NaCl and KCl to SPA-c significantly deteriorated the RMA of the signals of both proteins, of which the decline could be compensated by the co-addition of $(NH_4)H_2PO_4$ as an alkali cation adduct ion suppressor (Figure 1A and C). The overall effect on the S/N ratio showed a trend of improvement when $(NH_4)H_2PO_4$ was added (Figure 1B and D).

Conclusions

The relative mass accuracy and signal-to-noise ratio of the signals as obtained by the SELDI-TOF-MS analysis of apomyoglobin and albumin were deteriorated by controlled addition of alkali cation salts to SPA and improved by addition of an alkali cation adduct ion suppressor. Thus, non-controlled presence of alkali cation ions in the EAM could affect the reproducibility of SELDI-TOF MS analysis.

Literature

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Standardization of calibration and quality control using surface-enhanced laser desorption/ionization time-of-flight mass spectrometry

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Proteomic pattern analysis by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS) is one of the most promising new approaches for the discovery and identification of potential biomarkers for various types of cancer, e.g., ovarian (1), prostate (2), lung cancer (3) or for other diseases, like inflammatory diseases (4). Notwithstanding using identical types of biological specimens and the same analytical platform (5), several groups identified different patterns for the same types of cancers. Differences between the pre-and post-analytical strategies are responsible for the different results (6). Therefore, a stringent standardized protocol is needed, not only for pre- and post-analytical aspects, but also for calibration and quality control (QC) performance.
The aim of our study was to establish a well-defined protocol for calibration of the Protein Biosystem IIc (PBS IIc) instrument (Ciphergen Biosystems Inc. Fremont, CA, USA), to implement QC samples with independent certified standards and to determine acceptance criteria for the QC samples. Because the QC samples were spotted on a NP-20 array (Ciphergen Biosystems Inc.), which is a normal phase array, without washing or selective binding steps, only the MALDI-TOF MS part of the PBS IIc instrument was checked.

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Methods

Calibration samples
Instrument calibration was performed externally with the All-in-1 peptide and All-in-1 protein standards. Both standards as well as the SPA solution as energy absorbing matrix (Ciphergen Biosystems, Inc.) were prepared according to the recommendations of the manufacturer, with the exception that TFAH as a solvent component was used instead of TFA. On a