The German Society of Proteome Research

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www.proteomic-forum.de

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Results
With our workflow we successfully separated antibody proteoforms and analyzed the underlying molecular differences. Additionally we were able to separate stable as well as transient native protein complexes from human cell lysate.

Conclusion
HIC-RPC-MS is a powerful alternative for fractionation of proteins and multi-protein complexes, making use of their distinct hydrophobic properties for bottom-up proteomics experiments. The workflow developed is suitable for characterization of native soluble protein complexes as well as peptide mapping of individual purified proteins and biologics.

P215
Potential effects on the variation of the venom for species of the vipers family caused by environmental influences
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The broad geographic distribution of venomous snakes (vipers) with their diverse habitats and different environmental conditions make vipers ideal for analyzing substantial variation in venom composition and assess potential causes of venom variation. Hereinafter we focused on the venom composition of three viper species with different habitats. The nose-horned desert viper (Cerastes cerastes) native to the deserts in North Africa, in turn the mangrove pit viper (Cryptelytrops purpureomaculatus), which can be found in South/Southeast Asia and the Pelias spp. native to the Caucasus. Investigations on the venom compositions could lead to new pharmacological drugs with interesting bioactivity.

The proteome of all venom (C. cerastes/ C. purpureomaculatus/ Pelias spp.) was analyzed by intact mass profiling as well as bottom-up mass spectrometry and components were identified via de-novo sequencing. The comparison of the venoms showed similarities in the composition regarding the characteristic protein families, but the relative occurrence of these toxins for each species is variable. Additionally we found unique components in the venom of the C. cerastes and the C. purpureomaculatus. The cytotoxicity of the crude venoms was tested on a panel of cancer cell lines together with normal cell lines by MTT and showed IC50 values between 0.12 and >50 µg/mL. The cytotoxicity analysis of the isolated venom components against different cell lines are still in progress.


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The study of intact casein as a model system for the separation of intact phosphorylated proteins by Capillary Electrospray Ionization (CESI)
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Introduction
CESI is the integration of capillary electrophoresis (CE) and electrospray ionization (ESI) into a single process in a single device. CESI-MS operates at low nL/min flow rates offering several advantages including increased ionization efficiency and a reduction in ion suppression. In this work we describe the use of CESI in the study of intact casein in its various forms (alpha, beta and kappa).

Objective
The objective of this study was to investigate the use of CESI-MS for the separation of intact phosphoproteins with a view to its application for the top down analysis of this class of intact proteins.

Materials & methods
Protein standards were obtained from Sigma Aldrich and prepared in a variety of different solvents and injected by pressure onto a 30 µm ID, 91 cm long positively coated capillary. The CE separation used reverse polarity with a background electrolyte (BGE) consisting of a mixture of volatile acids with water/organic solvent mixtures. The QTOF system used was fitted with a NanoSpray® III source and used in full scan mode with an ionspray voltage set in the range of 1600 – 2200 V depending on the BGE. The CESI results were compared with a standard C4 reverse phase HPLC separation of the same sample.

Results
A CESI-MS method has been developed with the capability of separating alpha, beta and kappa casein and the baseline separation for alpha casein from its dephosphorylated form. The CESI separations were done at room temperature and produced sharper peaks than the corresponding LC separation and also provided a different migration compared to elution order of the LC separation.

Conclusions
This study has shown the complementary nature of CESI compared to LC-MS and has provided a brief insight into the possibilities of using this technology for the study of intact proteins and has highlighted the ability of CESI-MS to separate proteins by differences in charge in this case levels of phosphorylation.
Potential effects on the variation of the venom for species of the Viperidae family caused by environmental influences

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INTRODUCTION

The broad geographic distribution of venomous snakes (Viperidae) with their diverse habitats and different environmental conditions make vipers ideal for analyzing substantial variation in venom composition and assess potential causes of venom variation[1]. Here we focused on the venom composition of three viper species with different habitats. The horned desert viper (Cerastes cerastes) native to the deserts in North Africa, in turn the mangrove pit viper (Cryptelytrops purpureomaculatus), which can be found in South/Southeast Asia and the Vipera (pelias) darsakvi which was found in the north-eastern of Anatolia, Turkey. Therefore differences in the compositions of the venoms could lead to new components with positive bioactivity, which may lead into some new pharmacological drugs. The proteome of all venoms were analyzed by intact mass profiling as well as bottom-up mass spectrometry and components were identified de-novo sequencing. Additionally, the cytotoxicity of the crude venom for these species was tested on a panel of cancerous and non-cancerous cell lines by MTT (3-4,5-dimethylthiazolyl-2)-5-diphenyltetrazolium bromide).

RESULTS AND DISCUSSION

Figure 1: Crude venom analysis. (from top to bottom: C. cerastes, C. purpureomaculatus, V. darsakvi). A) Chromatograms of used venom. The separation was accomplished by using a semi-preparative HPLC. The linear gradient was set at 5–40% B for 95 min, 40–70% for 20 min and finally 70% B for 10 min (A=water, B=acetonitril). Peak detection was performed at λ=214 nm using a diode array detector (DAD). B) Crude venom intact mass profiling. Determination of peptide masses. LC-ESI-HR-MS/MS experiments were performed on a LTQ Orbitrap XL mass spectrometer (Thermo, Bremen, Germany).

Figure 2: Crude venom analysis. (from top to bottom: C. cerastes, C. purpureomaculatus, V. darsakvi) SDS-PAGE-gels containing stained protein bands from chromatographic fractions. Bands were separated from the gel and subjected to in-gel tryptic digestion. The digested fractions were investigated by a LTQ Orbitrap XL mass spectrometer (Thermo, Bremen, Germany) and identified via de-novo sequencing.

Figure 3: Geographical distribution of the vipers. The horned desert viper (C. cerastes) prefers the dry and sandy areas of North Africa (red) and is conspicuous found around oasis. It may be easily recognized by its pair of subocular horns and its whole-body impressions from moving sideways. The mangrove pit viper (C. purpureomaculatus) has a 9-11 supralabials, the first united with the nasal and has a high variability of its body color. It can be found in the mangroves of South Asia (green). The mountainous vipera darsakvi prefers the alpine grasslands in the regions of eastern Turkey and Georgia (blue) and has a dark brown to black zigzag pattern along the back. (Regions according to the TDWG standard, not precise distribution maps. Photos by: Prof. Bayram Göcnem)

Figure 4: Venom composition of investigated vipers. The vipers are composed of various components listed in the following order of the snakes: C. cerastes, C. purpureomaculatus/V. darsakvi peptides (11.59%/20.34%/35.43%), metalloproteinases (9.41%/12.43%/27.24%), PLA2's (15.86%/22.21%/6.85%), C-type lectins (a 1.74%/3.98%/n/a), serine proteases (26.64%/1.77%/10.32%), other (11.61%/7.54%/n/a) and not annotated (17.22%/18.31%/8.10%). Additionally there were found LAAO's (5.42%) in the venom of C. purpureomaculatus and CRISP's (11.13%) in the composition of V. darsakvi.

Figure 5: Crude venom tests on cancer cell lines. Cytotoxicity tests via MTT assay with crude venom extracts. Viability of cancer and other cancer cell lines after crude venom treatment. Human cell lines from left to right: neuroblastoma, breast, lung, colon, kidney, breast, bladder, glioblastoma. Analysis against different cell lines are still in progress.

CONCLUSION

The comparison of the venoms showed the occurrence of the main protein families, which lead back to the affiliation of the species to the same family of venomous snakes. The notably differences were shown in the proportion of these proteins, which could most likely be caused by their environment and geographical distribution. In turn there exist protein families, which do not appear in other of the investigated species. The bioactivity screening showed promising activity against human cancer cells and still have to be investigated.

REFERENCES


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