Capillary electrophoresis-mass spectrometry based metabolomics approaches for volume-restricted applications

Mever, M. van

Summary

Metabolomics is a powerful tool that can provide a comprehensive insight into the complexity of human biology and the pathophysiology of diseases. By analyzing the metabolome, which refers to the complete set of small (endogenous) molecules (with a mass <1500 Da) present within a given biological system, a direct functional read-out of an organism's physiological status can be obtained. In particular, metabolomics has emerged as a promising approach for neuroscience research, as it helps to shed light on the molecular mechanisms underlying neurodegenerative and neuropsychiatric diseases. One of the main challenges in (brain) metabolomics studies is the reliable and sensitive analysis of volume-limited biological samples. Therefore, it is crucial to develop reliable and sensitive analytical workflows to enable the study of biomedical questions intrinsically dealing with low sample amounts. In this thesis, innovative microscale analytical workflows based on capillary electrophoresis (CE) coupled to mass spectrometry (MS) are developed in order to generate metabolic profiles in volume-restricted samples. Until now, MS-based analytical workflows have already shown potential for brain metabolomics studies that deal with low sample volumes (**chapter 2**). However, CE-MS is still underrepresented compared to other chromatographic techniques like liquid chromatography (LC)-MS and gas chromatography (GC)-MS, which is primarily due to misconceptions that the technique is less robust, less sensitive, and less user-friendly. Therefore, the ultimate aim of this thesis is to showcase that the technical reproducibility, detection sensitivity, and metabolic coverage of CE-MS are sufficient to allow use for different material-limited matrices of interest for neuroscience research.

First, an interlaboratory study is conducted that overcomes the well-known issue of migration time variability in CE-MS and thereby increases its reproducibility, by converting migration times to the effective mobility scale (**chapter 3**). Furthermore, the study showcases the suitability of the effective mobility parameter as a universal and reliable criterion for peak identification in metabolomics. In **chapter 4**, a method is presented that employs on-line preconcentration with sample stacking based on pH junction to improve detection limits in CE-MS. The method is systematically optimized using a design of experiments (DoE) strategy, and demonstrates to be applicable for the direct profiling of endogenous metabolites in volume-limited rat brain microdialysis samples of only 10 µL. Detection limits in the low nanomolar range are achieved for amino acid neurotransmitters, which demonstrates its potential for trace-sensitive neurological studies.

The next chapter of this thesis focuses specifically on CE-MS-based metabolomics studies for the analysis of polar anionic compounds, which are metabolites that

are typically difficult to analyze using CE-MS (**chapter 5**). First, **chapter 5A** covers recent methodological developments to improve anionic metabolite profiling by CE-MS, along with an overview of recent applications in different research areas. As a follow-up, **chapter 5B** reports on a novel chemical derivatization procedure that provides a permanent positive charge to acidic metabolites. This allows their analysis using the same conditions as used for basic metabolites, thereby significantly improving their detection limits. The method is demonstrated for the analysis of energy metabolism-related metabolites in low numbers of HepG2 cells.

In **chapter 6**, the focus is on utilizing CE-MS for non-targeted metabolomics. The chapter presents a CE-MS workflow for metabolic profiling of extracts from individual zebrafish larvae as well as pools of small numbers of larvae. The workflow is used in order to study the specific role of the mineralocorticoid receptor during stress on metabolite levels, and which mechanisms might be important in this association. Multivariate data analysis is employed to identify differential metabolites, and reveals distinct metabolic profiles for all groups. Furthermore, the data suggests that the mineralocorticoid receptor may be a key regulator of enzymes involved in cystathionine metabolism and subsequently impact key physiological processes.

Finally, in **chapter 7**, a general conclusion and discussion are provided on the developed CE-MS workflows for material-limited biological samples. It highlights the importance of developing reliable and sensitive analytical methods and workflows to enable the study of biomedical questions using a metabolomics approach. Further possible developments and potential directions are also discussed.