ENZYME REACTION CHARACTERISATION BY CAPILLARY ZONE ELECTROPHORESIS WITH UV/VIS DETECTION

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In medicine and agriculture there is an insufficient development of new drugs by which infectious diseases and pests on agriculture plants cannot be treated sufficiently. The development of new, safer and cheaper drugs in this case is a challenge in research. The Leibniz-Research Cluster (LRC) biosynthetic micro-production units is a cooperative project of five different Leibniz-Institutes, tasked with the investigation of new ways for the discovery and development of bioactive compounds.

Malonyl coenzyme A (MalCoA) and acetyl coenzyme A (AcCoA) represent building blocks of polyketides, which can have antibiotic qualities. Therefore, it is of high interest to find new ways for a cost effective production of these building blocks. The approach of the LRC is to produce MalCoA, AcCoA and other bioactive compounds in flow-through cascaded bio/synthetic micro-production units with immobilised cell-free enzymes combined with in-flow product purification via micro Free-Flow Electrophoresis (µFFE). In a first step the enzyme malonyl coenzyme A synthase (MatB) catalyses the formation of MalCoA from sodium malonate (NaMal) and coenzyme A (CoA) under hydrolysis of adenosine triphosphate (ATP) to adenosine monophosphate (AMP) and free phosphate (P). In a second step the enzyme malonyl coenzyme A decarboxylase (MatA) forms AcCoA from MalCoA under elimination of carbon dioxide (CO_2). In a last step the enzyme CoA citrate synthase produces free CoA and citrate out of AcCoA and oxaloacetate.

We developed a capillary zone electrophoresis based method for the separation of cAMP, AMP, ADP, ATP, CoA, AcCoA and MalCoA with UV/Vis detection. We show that this method is capable of online analysis of single enzyme reactions and enzyme cascade reactions by automated direct injection from the reaction vessel. In this case negligible sample amounts (about 0.03 %) are removed from the reaction volume for the analysis, both educt and product concentration can be determined and long reaction times can be measured with high reproducibility.