Abstract

Application of capillary electrophoresis for the study of enzymes

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Enzymes are biological catalysts that play an important role in biochemical reactions necessary for normal growth, maturation and reproduction through the whole living world. They catalyze virtually all chemical reactions in living systems and the assay of enzyme activity is probably one of the most frequently encountered procedures in biochemistry and molecular biology. This work aimed to develop capillary electrophoresis (CE)-based assay for different enzyme, thereby exploring the capabilities of CE-UV regarding this application. The enzymatic systems included in this project are all clinically relevant but differential with respect to the type and number of substrates, products and cofactors. In order to investigate these enzymes, model substrates that are converted into UV detectable products were used. CE is a powerful and relatively new analytical tool. The small dimensions of the CE separation systems utilized are a primary advantage for the bio-research. A recent trend is the use of CE in the area of immunoassays and enzyme assays, due to its fast analysis time and the minute amounts of sample. Especially in-capillary approaches to study of enzyme activity, such as electrophoretically mediated microanalysis or EMMA, are of particular interest since the assay and all its necessary operations, completely occur within the capillary, thereby reducing the volume of the assay from the microliter level to the nanoliter level. For each enzymatic system included in this project, the electrophoretic parameters, which are convenient to perform EMMA assays, were investigated: the composition of the background electrolyte (type and concentration), protocol of injection and incubation, applied voltage, capillary dimensions (length and diameter) and temperature. The first enzyme to be studied is rhodanese, followed by angiotensin converting enzyme (ACE) and phenol sulfotransferase (PST). The developed in-capillary assays were then used to investigate whether kinetic parameters can be derived for the enzyme-catalyzed reactions. In a next step, different inhibitors of rhodanese and ACE were studied. The capabilities of CE regarding integrated enzymatic reactions were explored in this project and general conclusions were drawn with respect to the integration of enzyme reaction and separation by CE with UV detection. The miniaturized and integrated technique, EMMA, can be characterized by minimal reagent use, the absence of manual procedures and by complete automation. This minimizes the risk of cross contamination and strongly reduces the assay costs.

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