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Advances in enzyme substrate analysis with capillary electrophoresis

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ABSTRACT

Capillary electrophoresis provides a rapid, cost-effective platform for enzyme and substrate characterization. The high resolution achievable by capillary electrophoresis enables the analysis of substrates and products that are indistinguishable by spectroscopic techniques alone, while the small volume requirement enables analysis of enzymes or substrates in limited supply. Furthermore, the compatibility of capillary electrophoresis with various detectors makes it suitable for K_M determinations ranging from nanomolar to millimolar concentrations. Capillary electrophoresis fundamentals are discussed with an emphasis on the separation mechanisms relevant to evaluate sets of substrate and product that are charged, neutral, and even chiral. The basic principles of Michaelis-Menten determinations are reviewed and the process of translating capillary electrophoresis electropherograms into a Michaelis-Menten curve is outlined. The conditions that must be optimized in order to couple off-line and on-line enzyme reactions with capillary electrophoresis separations, such as incubation time, buffer pH and ionic strength, and temperature, are examined to provide insight into how the techniques can be best utilized. The application of capillary electrophoresis to quantify enzyme inhibition, in the form of K_I or IC_{50} is detailed. The concept and implementation of the immobilized enzyme reactor is described as a means to increase enzyme stability and reusability, as well as a powerful tool for screening enzyme substrates and inhibitors. Emerging techniques focused on applying capillary electrophoresis as a rapid assay to obtain structural identification or sequence information about a substrate and in-line digestions of peptides and proteins coupled to mass spectrometry analyses are highlighted.

Contents

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1. Introduction

The annual global market of industrial enzymes is reported to be billions of USD [1–7], and impacts commercial sectors that include energy, animal feed, household products, food processing and pharmaceuticals [1,2,5,6]. Enzymatic processing is even recognized as a critical component for sustainable chemical manufacturing [8]. For this reason significant effort is made to discover new enzymes [9,10] as well as to improve the stability, specificity, or efficiency of existing enzymes [7,11]. These endeavors require analytical approaches to characterize the enzyme performance in order to advance manufacturing.

Current approaches of monitoring enzymes require analytical tools to quantify the amount of product generated by incubating the enzyme with a particular substrate. A barrier to most approaches is that the product and substrate are indistinguishable by optical spectroscopy. This has led to the development of a limited set of substrates that undergo significant change in the spectroscopic profile upon conversion to product. An alternative approach is the use of separation-based assays that provide a means to sort the product and substrate in space and then detect them individually.

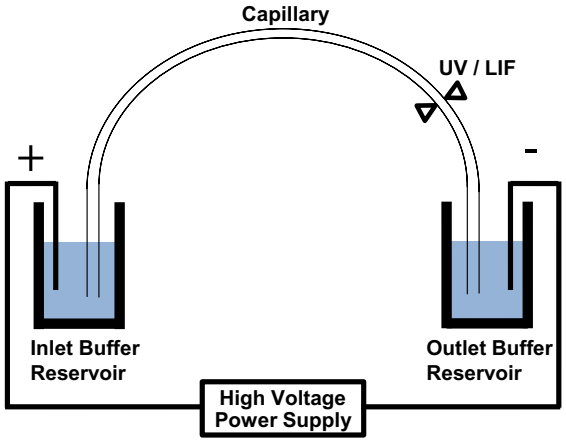

Fig. 1. Schematic of capillary electrophoresis setup. It consists of capillary, buffer reservoir containing background electrolyte and a detector. Analytes are separated in capillary under the influence of the electric field supplied by high voltage power supply. A color version of this figure is available on-line.

Capillary electrophoresis separations offer many advantages over chromatographic separations that make the method an attractive technique for enzyme analysis. Capillary electrophoresis consumes nano- to picoliter sample volumes for each run. Electrophoresis runs are rapid, requiring minutes to complete. The high separation efficiency of capillary electrophoresis enables the separation of complex samples. Commercially available instruments are equipped with robotics, which automate the sample analyses. The method is amenable to total miniaturization and can ultimately be translated into portable microfluidic systems. These benefits make capillary electrophoresis an excellent platform to study enzyme kinetics, specificity, and inhibition, as well as to expand the possibilities to use enzymes as tools in analytical techniques.

The purpose of this paper is to shed light on the capabilities of capillary electrophoresis for the evaluation of enzyme performance. In order to understand how the technique is adapted to different enzyme and substrate systems, fundamental principles of the method are described. The process of converting capillary electrophoresis separations into Michaelis-Menten constants (K_M) is also delineated. Applications reported from 2012 to 2017 are summarized. Areas of focus include the calculation of K_M, inhibition studies, optimization of enzyme turnover and approaches to screen or compare different enzymes. Different strategies for enzyme immobilization for in-capillary analyses are described. Future directions in this field, especially in strategies for in-capillary sequencing as well as structural identification are addressed.

2. Background

2.1. Fundamental principles of capillary electrophoresis

Separation in capillary electrophoresis is based on the charge-to-size ratio of analytes in an electric field. A simple schematic of