



Contents lists available at ScienceDirect

Methods

journal homepage: www.elsevier.com/locate/ymeth

Advances in enzyme substrate analysis with capillary electrophoresis

Srikanth Gattu, Cassandra L. Carihfield, Grace Lu, Lloyd Bwanali, Lindsay M. Veltri, Lisa A. Holland*

C. Eugene Bennett Department of Chemistry, West Virginia University, Morgantown, WV 26506, United States

ARTICLE INFO

Article history:

Received 11 December 2017

Received in revised form 1 February 2018

Accepted 5 February 2018

Available online xxx

Keywords:

Capillary electrophoresis

Enzyme

Inhibitor

Michaelis-Menten constant

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.

© 2018 Published by Elsevier Inc.

Contents

1. Introduction	00
2. Background	00
2.1. Fundamental principles of capillary electrophoresis	00
2.2. Enzyme analysis using capillary electrophoresis	00
2.2.1 Determining K_M values	00
2.2.2 Constraints of the assay	00
3. Adapting the separation to determine K_M values	00
3.1. Modes of capillary electrophoresis separations to isolate product from substrate	00
3.1.1. Charge-to-size ratio	00
3.1.2. Secondary equilibria with cyclodextrin host:guest complexes	00
3.1.3. Alternative secondary equilibria and size-based separation	00
3.1.4. Modification of the capillary surface	00
3.2. Modes of detection of capillary electrophoresis	00
3.3. Off-line and on-line coupling the incubation with the capillary electrophoresis	00
3.3.1. Compatibility of the enzyme reaction and the separation	00
3.3.2. Off-line incubations	00
3.3.3. In-line incubations: overview	00

* Corresponding author at: 100 Prospect Street, 217 Clark Hall of Chemistry, Morgantown, WV 26506, United States.

E-mail address: Lisa.Holland@mail.wvu.edu (L.A. Holland).

3.3.4.	Role of pH with on-line enzyme incubations	00
3.3.5.	Partial filling for discontinuous reaction and separation pH	00
3.3.6.	Transverse diffusion for discontinuous reaction and separation pH	00
3.3.7.	Mixing in capillary	00
4.	Evaluating enzyme inhibitors using capillary electrophoresis	00
4.1.	Background	00
4.1.1.	Determining K_i for competitive inhibition	00
4.2.	Inhibition analyses by capillary electrophoresis	00
4.2.1.	K_i Determination: competitive inhibition	00
4.2.2.	Mechanisms of inhibition	00
4.2.3.	Determining K_D to evaluate K_i	00
4.2.4.	Screening for enzyme inhibitors	00
5.	Enzyme immobilization techniques	00
5.1.	Attributes of enzyme immobilization	00
5.1.1.	Open tubular immobilization design and application	00
5.1.2.	Particle immobilization design and application	00
5.1.3.	Monolith immobilization design and application	00
5.1.4.	Microfluidic immobilization design and application	00
6.	Emerging techniques and future directions	00
6.1.	Enzymatic capillary electrophoresis assays to identify substrate structure	00
6.1.1.	Structural identification of glycans	00
6.1.2.	Structural determination integrated with mass spectrometry	00
6.2.	Future directions	00
	Acknowledgements	00
	Appendix A. Supplementary data	00
	References	00

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.

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

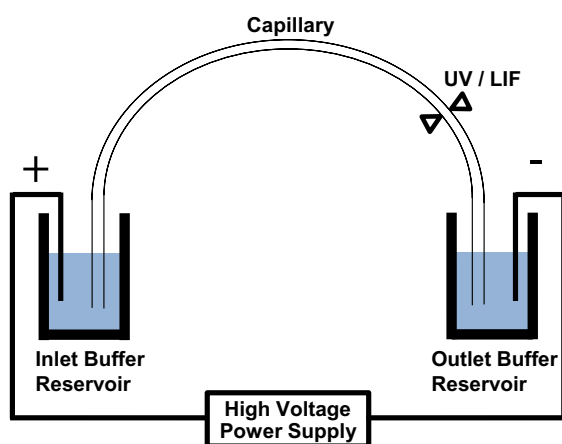


Fig. 1. Schematic of capillary electrophoresis setup. It consists of capillary, buffer reservoir containing background electrolyte and a detector. Analytes are separated in capillary under the influence of the electric field supplied by high voltage power supply. A color version of this figure is available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

Download English Version:

<https://daneshyari.com/en/article/8339979>

Download Persian Version:

<https://daneshyari.com/article/8339979>

[Daneshyari.com](https://daneshyari.com)