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REVIEW

Application of Capillary Electrophoresis in Enzyme Inhibitors Screening

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Abstract: Capillary electrophoresis plays an irreplaceable role in the field of enzyme inhibitors screening with unique features of high-efficiency separation, short analysis time, minimal sample consumption, high sensitivity, ease to realize automatization etc. This review gives an overview of the applications of capillary electrophoresis in the field of enzyme inhibitors screening, and summarizes both the widely used off-line (pre-capillary) enzyme assay and on-line (in-capillary) enzyme assay. Finally, some future trends are briefly addressed.

Key Words: Capillary electrophoresis; Enzyme inhibitor; Pre-capillary enzyme assay; In-capillary enzyme assay; Review

1 Introduction

Human diseases are often associated with abnormal expression of various enzymes. The body functions are disturbed if the enzyme expression level is higher than normal value, and some diseases may occur. For examples, acetylcholine esterase (AChE), which plays a key role in central nervous system, digest the neurotransmitter acetylcholine excessively if its activity is higher than normal level and diseases such as Alzheimer's disease and myasthenia gravis would occur[1]. Tyrosinase (TRS), the key and rate-limiting enzyme for synthesis of melanin, may cause melanin pigmentation and human skin pigmentation diseases further at an abnormally high level[2]. Therefore, it is very important to screen enzyme inhibitors and the method of curing diseases by screening key enzyme inhibitors has become an attractive strategy. Nowadays, a considerable part of the clinical drugs are based on enzyme inhibitors[3].

Traditional methods of the screening enzyme inhibitors include radiometric assay, thin-layer chromatography method, electrochemical method, ultraviolet spectrophotometry method and HPLC assay. These methods are well developed but still suffer from some defects[4]. Compared with the traditional methods in enzyme inhibitors screening, capillary electrophoresis (CE) possesses unmatched advantages such as short analysis time, minimal sample consumption, ease of automatization and especially the high-efficiency separation mechanism. Furthermore, various detection techniques can be combined with CE to detect the targets, including ultraviolet detection (UV), laser induced fluorescence (LIF), radiation, mass spectroscopy (MS) and electrochemical method[5]. UV detection is the most widely used detection strategy due to the advantages of low cost, convenient operation and environmentally benign characteristics.

The sensitivity of UV detection is limited by the concentrations of the targets, however, it can be improved by increasing the detection path-length by using bubble, Z-shaped and multi-reflection detection cells, or pre-concentration of targets by zero potential effect, sample stacking, field-amplified sample stacking and field-amplified sample injection[6]. In monitoring nucleotide pyrophosphatase/phosphodiesterase reactions, Iqbal *et al*[7] used polybrene, a polycationic polymer as a buffer additive to form a dynamical coating on the fused-silica capillary wall by electrostatic

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interaction. The product migrated in the direction of the electroosmotic flow (EOF), whereas the positively charged polybrene molecules moved in the opposite direction, resulting in product stacking and enhancing the detection sensitivity. The targets can also be stacked by dissolving sample in water or using the diluted buffer[8].

The methods for screening enzyme inhibitors by CE include four different modes: (1) the pre-capillary enzyme assay in which the enzyme reaction takes place off-line and the capillary is used just as the separation channel; (2) at-inlet reaction which include longitudinal diffusion and immobilized enzyme microreactors (IMER); (3) electrophorecially mediated microanalysis (EMMA) and (4) transverse diffusion of laminar flow profiles (TDLFP). Among these methods, at-inlet, EMMA and TDLFP belong to in-capillary enzyme assay. These methods are summarized in this review.

2 Pre-capillary enzyme reaction

In the off-line enzyme assay, the reactants are mixed off line, and the reaction is initiated by adding substrate, enzyme or coenzyme in the last step. After the reaction mixture is incubated for a specific time, the enzyme reaction is stopped by appropriate method, the reaction mixture is injected into the CE system for subsequent separation and detection. The enzyme kinetics, inhibition kinetics are studied and enzyme inhibitors are screened by monitoring the changes of the peak areas of products or substrates[9,10]. In the pre-capillary mode, the capillary is used just as the separation channel.

Xin *et al*[11] determined the inhibition activity of Captopril to angiotensin converting enzyme (ACE) by off-line assay. The enzymatic reaction conditions and separation conditions were optimized to achieve an accurate, simple and rapid assay. That study provided a screening model for further screening ACE inhibitors. Li *et al*[12] determined the activity of dihydrofolate reductase and the half-maximal inhibitory concentration (IC_{50}) of the known inhibitor by CE. The consistency of the experimental results with literature proved that this system could be used for dihydrofolate reductase inhibitors screening. Jia *et al*[13] screened dihydrofolate reductase inhibitor by pre-capillary assay and EMMA. The experimental results showed that the pre-capillary enzyme assay exhibited the advantages of high sensitivity, good reproducibility of migration time and peak area and ease to control compared with EMMA. Zhong *et al*[14] screened neuraminidase inhibitors from *Phascolosoma esculenta*, among of which 7 components were found to have inhibitory activity on neuraminidase. The enzymatic reaction and separation conditions were optimized and the established model was used for screening anti-influenza virus drugs neuraminidase inhibitors from complex systems. Iqbal *et al*[15] screened and characterized inhibitors and substrates of adenosine kinase by off-line assay. In that study, the substrate and product were separated efficiently and the obtained inhibition constant (K_i) of the standard inhibitors was identical with that measured by the standard radiometric method, the results proved the reliability and accuracy of the established screening model. In the study of 20S proteasome, Chen *et al*[16] detected the hydrolysis of peptide substrate MG132 and MG115 in real time and investigated the inhibitory effects of a series of compounds on the 20S proteasome. The enzymatic reaction was initiated by mixing substrate and emzyme at 37 °C followed by immersing the reaction mixture in an ice bath for 10 min to quench the reaction. Then the supernatant obtained by centrifugation was injected into the capillary for further analysis. Li *et al*[17] screened monoamine oxidase (MAO) inhibitors by using protein-lipid complexes. The reaction was quenched by adding $HClO_4$ solution containing EDTA and $Na_2S_2O_5$. That method had the advantages of simplicity, rapidity, saving of enzyme and less interference factors compared with traditional methods for MAO inhibitors screening. By using the same strategy, Bryant *et al*[18] studied acetyl coenzyme A carboxylase holoenzyme by pre-capillary enzyme assay. The reaction mixture was injected into the CE system for separation after incubation for a period. The biotinylated carboxylase and carboxyltransferase could be monitored and screened simultaneously with this method. The kinetics and inhibition studies of carbonic anhydrase were performed by Iqbal *et al*[19] with the pre-capillary enzyme assay. Carbonic anhydrase and substrate 4-nitrophenyl acetate were mixed and incubated at 37 °C for 10 min and the reaction was terminated by freezing the reaction mixture. Then the reaction mixture was hydrodynamically injected into the capillary for separation. Malina *et al*[20] investigated the enzymatic kinetics and inhibition kinetics of phosphofructokinase-1 by pre-capillary enzyme assay. The spectral interference from inhibitors was greatly reduced, which thus reduced the possibility of false positive results in studies of enzyme inhibition through CE separation of the enzyme and substrate. The measured IC_{50} matched values reported in the literature obtained using coupled spectrophotometric assays.

In pre-capillary enzyme assay, both the enzymatic reaction and separation are independent. So the enzymatic reaction and separation conditions can be optimized respectively and do not interfere with each other. However, there are some limitations to this mode. Firstly, the enzymatic reaction which is very fast must be terminated by adding quenching reagents or changing the reaction conditions before injecting into the CE system. Secondly, although only nanoliter-scale sample is consumed in CE detection, pre-capillary enzyme assay requires a large number of reactants to initiate the reaction which would cause the waste of reagents especially for the expensive enzymes. Promisingly, the in-capillary enzyme assay can make up for the deficiency of the pre-capillary enzyme assay.