УДК 543.94

# KINETIC CAPILLARY ELECTROPHORESIS-UV-ION MOBILITY-MASS SPECTROMETRY FOR MONITORING ENZYME CONFORMATION, ACTIVITY, AND INHIBITION

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Kinetic Capillary Electrophoresis (KCE) is an electrophoretic separation of molecular species, which interacts inside a capillary during electrophoresis, and it establishes a new paradigm that separation methods together with MS can be used as comprehensive kinetic tools with mass and structure elucidation in proteomics. The spectrum of reported KCE applications for proteins includes: direct measuring equilibrium and rate constants for protein – protein, protein – DNA, protein – small molecule, protein – peptide interactions, studying thermochemistry of affinity interactions, kinetic selection of DNA aptamers to proteins with predefined binding parameters from combinatorial libraries and the use of the DNA aptamers in ultra-wide dynamic range quantitative analyses of proteins. Here, I describe the use of KCE-MS to monitor the ligand-induced conformational changes of human tissue transglutaminase (TG2, 79 kDa) correlated with its enzymatic activity. KCE separated and detected slowly interconverting open (the half-life of 5 min) and closed (14 min) conformations of human TG2. This represents an important step in assigning functional capacity to conformers of TG2 in addition to establishing KCE for on-line monitoring of protein dynamics in solution. Our KCE-UV-MS instrument was capable of both multiplexing and direct quantitative analysis of protein conformers and enzymatic activity. Three competitive irreversible inhibitors were analyzed and $EC_{50}$ for enzymatic activity and conformational changed were determined. KCE-MS serves as a unique method for simultaneous studying protein structure and function, including the in situ catalytic activity of conformers and their susceptibility to inhibitorbinding.



УДК 578.74

# APTAMER-FACILITATED PROTECTION OF ONCOLYTIC VIRUS FROM NEUTRALIZING ANTIBODIES

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Oncolytic viruses promise to significantly improve current cancer treatments through their tumor-selective replication and multimodal attack against cancer cells. However, one of the biggest setbacks for oncolytic virus therapy is the intravenous delivery of the virus, as it can be cleared from the bloodstream by neutralizing antibodies before it reaches the tumor cells. We have selected DNA aptamers against an oncolytic virus, vesicular stomatitis virus, using a competitive binding approach, as well as against the antigen binding fragment (Fab) of antivesicular stomatitis virus polyclonal antibodies, in order to shield the virus from the neutralizing antibodies and enhance its *in vivo* survival. We used flow cytometry to identify these aptamers and evaluated their efficiency to shield vesicular stomatitis virus in a cell-based plaque forming assay. These oligonucleotides were then modified to obtain multivalent binders, which led to a decrease of viral aggregation, an increase in its infectivity and an increase in its stability in serum. The aptamers were also incubated in nondiluted serum, showing their effectiveness under conditions mimicking those *in vivo*. With this approach, we were able to increase viral infectivity by more than 70% in the presence of neutralizing antibodies. Thus, this method has the potential to enhance the delivery of vesicular stomatitis virus through the bloodstream without compromising the patient's immune system.