A rapid transglutaminase assay for high-throughput screening application

Wu YW and Tsai YH

Abstract

Transglutaminases (TGs) are widely distributed enzymes that catalyze posttranslational modification of proteins by Ca(2+)-dependent cross-linking reactions. The family members of TGs participate in many significant processes of biological functions such as tissue regeneration, cell differentiation, apoptosis, and certain pathologies. A novel technique for TG activity assay was developed in this study. It was based on the rapid capturing, fluorescence quenching, and fast separation of the unreacted fluorescent molecules from the macromolecular product with magnetic dextran-coated charcoal. As few as 3 ng of guinea pig liver transglutaminase (gpTG) could be detected by the method; activities of 96 TG samples could be measured within an hour. The K(m) of gpTG determined by this method for monodansylcadaverine (dansyl-CAD) and N, N-dimethylcasein was 14 and 5 muM, respectively. A typical competitive inhibition pattern of cystamine on dansyl-CAD for gpTG activity was also demonstrated. The application of this technique is not limited to the use of dansyl-CAD as the fluorescent substrate of TG; other small fluor-labeled TG substrates may substitute dansyl-CAD. Finally, this method is rapid, highly sensitive, and inexpensive. It is suitable not only for high-throughput screening of enzymes or enzyme inhibitors but also for enzyme kinetic analysis.