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Optimization of gold nanoparticle-based real-time colorimetric assay of dipeptidyl peptidase IV activity

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Abstract

Dipeptidyl peptidase IV (DPP-IV also referred to as CD-26) is a serine protease enzyme with remarkable diagnostic and prognostic value in a variety of health and disease conditions. Herein, we describe a simple and real-time colorimetric assay for DPP-IV/CD-26 activity based on the aggregation of gold nanoparticles (AuNPs) functionalized with the peptide substrates: Gly-Pro-Asp-Cys (GPDC) or Val-Pro-ethylene diamine-Asp-Cys (VP-ED-DC). Cleavage of the substrates by DPP-IV resulted in aggregation of the AuNPs with accompanying colour change in the solution from red to blue that was monitored using either a UV-visible spectrophotometer or by the naked eye. Factors, such as pH, ionic strength and the structure of the substrate that influence the cleavage reaction in solution were investigated. The effects of potential interference from serum proteins (lysozyme, thrombin and trypsin) on the analytical response were negligible. The detection limits when GPDC or VP-EN-DC functionalized AuNPs were used for DPP-IV assay were 1.2 U/L and 1.5 U/L, respectively. The VP-EN-DC method was preferred for the quantitative determination of DPP-IV activity in serum because of its wide linear range 0 - 30 U/L compared to 0-12 U/L for the GPDC assay. Recoveries from serum samples spiked with DPP-IV activity, between 5-25U/L, and using the VP-EN-DC modified AuNPs method ranged between 83.6-114.9%. The two colorimetric biosensors described here are superior to other conventional methods because of their simplicity, stability, selectivity and reliability.

Keywords

gold nanoparticles, colorimetric assay, peptide substrates and DPP-IV enzyme activity.