A Whispering Gallery Mode based sensor platform for single enzyme real time conformational changes.

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Abstract

Understanding the conformational changes that occur in an enzyme during turnover is crucial for understanding biomolecular processes and for new rational drug discovery. Many single-molecule techniques have been developed in the past three decades, such as Förster resonance energy transfer, although powerful, these techniques have their limitations, for example, limited temporal resolution, or necessity for fluorescent labelling. In this study a new class of highly sensitive optical devices based on the whispering gallery mode sensor have been investigated and employed to track the conformational changes of a kinase enzyme in real time. A thermostable Geobacillus stearothermophilus phosphoglycerate kinase enzyme has been used as model enzyme to prove that the optoplasmonic whispering gallery mode sensor can detect the conformational changes involved in enzyme turnover. The enzyme was expressed and purified to a high degree of purity that was suitable for crystallographic studies as well as biochemical investigation. The purified enzyme has been used for kinetics and biophysical enzymatic characterisation. A reproducible protocol has been established to immobilise the enzyme onto gold nanoparticles in a defined orientation to track the conformational changes occurring on the sensor. The enzyme has been immobilised onto the gold nanoparticles using two different methods: through the his-tags introduced onto the recombinant enzyme and directly onto the gold through a surface cysteine residue introduced by site-directed mutagenesis. The kinase enzyme was found to retain activity using both methods. To rationalise the observed conformational changes occurring during enzymatic turnover, the crystal structures of the native enzyme and its complexes with the substrate, and with substrate together with non-hydrolysable ATP, have been determined to high resolution, between 1.2 Å and 2 Å. Finally, for the first-time a real-time visualisation of the movement of the phosphoglycerate kinase during enzymatic turnover has been recorded. Repeating signals from the sensor were registered and only observed when both the substrate 3-phosphoglycerate and ATP are introduced with the enzyme into the sensor chamber. No signal is observed with the 3-phosphoglycerate alone or ATP alone or when 3-phosphoglycerate and a non-hydrolysable ATP analogue were used with the enzyme. The sensor system presented in this thesis shows potential for future fast, real-time, rapid throughput, lab-on-chip measurements for studying single enzymes.