A comprehensive investigation of copper binding properties of metformin using on-disc magnetic microbead agglomeration with real-time analysis

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A comprehensive investigation of copper binding properties of metformin using on-disc magnetic microbead agglomeration with real-time analysis

Authors & affiliations:

Rokon Uddin, Xueling Quan, Marco Donolato, Robert Burger and Anja Boisen
Department of Micro-and Nanotechnology, Technical University of Denmark
rokud@nanotech.dtu.dk

Introduction: Metformin is a widely used type-2 diabetes drug. Its copper-binding properties are known to be crucial but still not fully understood. We present a comprehensive investigation of its interaction with L-cysteine-Cu complex using a magnetic microbead (MB)-based assay on a microfluidic disc. The assay scheme is similar to the one presented in our previous study, where optomagnetic readout and magnetic nanobeads were used [1]. In this study, we have significantly simplified the detection by using an optical scanning method [2] and micrometer-sized beads. Additionally, we can measure the effect of 100-fold lower concentration of metformin than in our previous study. Our results clearly illustrate the strong metformin-Cu interaction and provide the opportunity of real-time analysis.

Methods:

The assay platform is a microfluidic disc made of Poly(methylmetacrylate) (PMMA) bonded together using pressure-sensitive adhesive (Fig.2a). For studying L-cysteine-Cu interaction, Cu solution and 1µm streptavidin-coated MBs (1mg/ml) functionalized with biotinylated L-cysteine (volume ratio 1:11) were incubated for 10 minutes with gentle shaking and then loaded into the disc. For studying interaction between metformin and L-cysteine-Cu complex, metformin was added to a separately pre-incubated L-cysteine-Cu (volume ratio 2:1) solution, further incubated for 5 minutes and loaded into the disc. Finally, all samples were incubated on-disc under permanent magnetic field with continuous shaking for 10 minutes before scanning by oCelloScope (Fig.2b).

Results: Scanning results of four different samples are illustrated in Fig.3.
We performed real-time analysis of cluster formation as illustrated in Fig4.

Fig3: (a) Magnetic beads functionalized with biotinylated L-cysteine used as the control sample (b) L-cysteine functionalized MBs incubated in Cu solution followed by 10min magnetic incubation. MB clusters form because of the formation of L-cysteine-Cu complex. (c) & (d) After the addition of 2.1µM and 21µM metformin into two separate mixtures of Cu and L-cysteine functionalized MB followed by magnetic incubation, the MB-clusters are broken in respective order for the breakage of L-cysteine-Cu complex and formation of metformin-Cu complex. (e) Measured MB cluster size vs amount of metformin in the sample: we conclude that samples with 21µM and 2.1 µM metformin have 57.9% and 34.6% reduced mean cluster size respectively than the sample with only Cu. Scale bar: 50µm.

Discussion: The presented results show that metformin, even at low concentration, is able to break the L-cysteine-Cu complex, which can affect mitochondrial function [3]. The investigation provides in-depth insight on the metformin-Cu interactions by quantifying the size of the clusters formed by molecular reaction. Thus, the novel detection strategy and kinetics analysis could be used for studying metal-binding properties of metformin-analogues.

References: