**A novel Raman reporter for the nanosensing of proteins through their disulfide bond structure**

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Introduction.

Surface – enhanced Raman spectroscopy (SERS) is a sensitive analytical method for the detection of biological targets such as protein, DNA, antibody, aptamer, etc. Insulin is a small protein which is a key biomarker for the clinical diagnostics of diabetes, insulinoma, and trauma. Radioimmunoassay (RIA), chromatography and enzyme-linked immunosorbent assay (ELISA) are common analytical methods for the quantification of insulin in the blood. However, they are time-consuming, costly, and laborious. Therefore, the development of novel nanosensor for the rapid determination of insulin levels is required [1].

Aims.

This work, aims to develop a novel nano-sensing method for the detection of protein biomarkers. A new benzothiazole Raman reporter and cost-effective nanomaterial utilise for the rapid determination of insulin by SERS.

Methods.

5×10-7 M of the new benzothiazole dye was loaded onto a gold nanoparticles (average size = 50 nm) and allowed to stand for 1 hour for the complete adsorption of the dye. Insulin standards in the concertation range 10-7-10-14 M (in PBS, pH = 7.4) were reduced by Tris(2-carboxyethyl)phosphine (TCEP, 0.2 mM). The reduced insulin was added to the dye-coated nanoparticles, centrifuged after 20 min and screened by SERS.

Results.

The Raman spectra of the new dye probe (Raman reporter) in the presence of different concentrations of reduced insulin are shown by the figure. The linear relationship between the SERS signal intensity at 1331 cm-1 and log the concentration of reduced insulin is depicted by the figure inset and followed the equation Y= -1048.4 X- 6134.8 (R2 =0.98).

Discussion.

The reduction of insulin disulfide bond structure generates free sulfhydryl (SH) groups that have highly affinity to the gold nanoparticles. Therefore the reduced insulin molecules compete with the pre-adsorbed dye probe. This results in the displacement of the dye probe molecules and the reduction of Raman signal intensity at 1331 cm-1. The reduction in the Raman signal intensity at 1331 cm-1 was proportional to the concentration of the reduced protein and therefore was used for its rapid and sensitive indirect SERS quantification in the concentration range 10-7-10-14 M.

Conclusion.

A new SERS method was developed for the ultra-trace analysis of insulin. The new method utilises a benzothiazole dye as a Raman reporter for the detection of the protein after modification of its disulfide bond structure. The SERS quenching of the Raman reporter signal at 1331 cm-1 allowedfor the indirect detection of the protein down to 10-14 M. The new Raman reporter can be utilised for rapid SERS detection many proteins that have disulfide bonds structure.

**References**

1. Tan, S, Han, R, Wu, S, Liang, H, Zhao, Y, Zhao, H & PengLi. (2019). A novel fluorescent sensing platform for insulin detection based on competitive recognition of cationic pillar[6]arene. Talanta, 197, 130-137.