



Detection and Quantification of Intracellular Nitrogen Dioxide(NO₂) and Hydrogen Sulphide(H₂S)

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Abstract

Detection of small gaseous molecules have been carried out in the past using various techniques including colorimetry, electron paramagnetic resonance, electrochemistry, gas chromatography, metal-induced sulfide precipitation. Whereas the fluorescent probe based detection has many advantages such as high sensitivity, selectivity and versatility to yield information about the gaseous moieties. To couple with the quantitative information obtained using fluorescent probes, high quality confocal imaging was carried out to unravel the intracellular localization of the gaseous molecules.

This study was based on the two fluorescent probes namely Ni(II) dithiocarbamate complex based fluorescent probe sensitive towards nitrogen dioxide (NO₂) and BODIPY-Cyclen(BDP-Cy) based fluorescent probe sensitive towards hydrogen sulfide (H₂S). Both these probes are hydrophobic in nature, hence a novel cationic liposome mediated delivery method was utilised for subcellular detection. The NO₂ specific probe was investigated in cellular environment to unravel the fate of nitric oxide (NO)(known gaseous messenger). The external NO donor namely DEANO was administered in RAW 264.7 cells to determine the formation of NO₂ due to the rapid reaction NO and oxygen in the cellular system. The positive results allowed us to further look into endogenously produced NO₂ using inducible Nitric Oxide Synthase (iNOS) pathway. Lipopolysaccharides (LPS) was used as stimulant and increase in fluorescent intensity was observed with increase in stimulation time. These results were in accordance to previous results suggesting a possible conversion of NO produced in the cell to NO₂.

The H₂S specific probe was analysed for its selectivity and sensitivity in RAW 264.7 cells using external donors such as Na₂S and NaHS. A dose dependent increase in fluorescent intensity was observed, which allowed us to further monitor the endogenous H₂S in HEK 293 cells by using gene knockdown and overexpression of inherent cystathionine γ -lyase (CSE) gene. The fluorescent probe was further applied in determining the H₂S produced by natural and synthetic polysulfides in MCF-7 cells. Finally, a combination of NO₂ sensitive probe and H₂S sensitive probe was utilised in analysing the quenching activity of H₂S on nitrogen radicals.



Synergistic Inhibition Activity of Dracoflavan B and Epigallocatechin-Gallate on α -Amylase

By Ms. **Adeline Ik Chian Wong**

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Abstract

Type II diabetes is an escalating problem worldwide where the number of cases is forecasted to double by the year 2030. Diabetes can be modulated via the retardation of starch digestive enzymes like α -amylase which have been targeted by commercially available drugs like acarbose. Nonetheless, these drugs cause undesirable side-effects like flatulence and diarrhoea. With respect to this, we postulate that drug combination would be a plausible means of reducing these undesirable side-effects by decreasing the dosage of the active inhibitors while maintaining its efficacy for glycaemic control.

In this study, we studied the combined inhibitory effect of Epigallocatechin Gallate (EGCG) with Dracoflavan B (DFB) in vitro. Our study shows that the combination of EGCG and DFB produced a synergistic effect in inhibiting α -amylase. This was confirmed by Combination Index (CI) values where CI < 1 demonstrates synergistic behaviour. The IC₅₀ of DFB and EGCG tested individually against α -amylase was 53.8 \pm 3.5 and 247.5 \pm 43 μ g/mL, respectively. The combination of DFB:EGCG at ratios 4:1, 2:1, 1:1, 1:4 resulted in subsequent decrease in IC₅₀ values. Their IC₅₀ values were 48.2 \pm 1.3, 44.4 \pm 2.8, 38 \pm 2.0 and 32.5 \pm 2.4, respectively. In order to understand its mechanism of inhibition, docking studies were done and results showed that both EGCG and DFB bind non-competitively to the two secondary binding sites (SBS) on α -amylase with one inhibitor having a preference towards one binding site over the other. EGCG has a preference of binding at SBS1 with a binding energy of -11.25 whereas DFB has a binding energy of -5.88 at SBS2. We propose that there was 'Bliss Independence' occurring where mutually non-exclusive binding results in cooperative inhibition of α -amylase.

These data provide valuable implications for an alternative therapy for type 2 diabetics using a combination of inhibitors. Furthermore, it also provides new insight into the use of naturally occurring enzyme inhibitors in foods rich in EGCG like green tea as a supplement to aid in the treatment of diabetes.

Host: Dr. Liu Mei Hui **Date:** 28th March 2014, Friday
Time: 12 to 1 pm **Venue:** Seminar Room S14-06-19

ALL ARE WELCOME!