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Oligonucleotide-based fluorescent probe for sensing of cyclic dia denylate monophosphate in bacteria and diadenosine polyphosphates in human tears

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Cyclic dia-denylate monophosphate (c-di-AMP) and P1,P5-dia-denosine-5' penta-phosphate (Ap5A) have been determined to play important roles in bacterial physiological processes and human metabolism, respectively. However, few, if any, methods have been developed that use fluorescent sensors to sense c-di-AMP and Ap5A in the real world. To address this challenge, this study presents a fast, convenient, selective and sensitive assay for quantifying c-di-AMP and Ap5A fluorescence based on the competitive binding of dia-denosine nucleotides and a polyadenosine probe to coralyne. The designed probe consists of a 20-mer Adenosine base (A20), a fluoro-phore unit at the 5'-end and a quencher unit at the 3'-end. Through A2-coralyne-A2 coordination, coralyne causes a change in the conformation of A20 from that of a random coil to a folded structure, thus enabling the fluorophore to be close to the quencher. As a result, fluorescence quenching occurs between the two organic dyes. When the A20 coralyne probe encounters the dia-denosine nucleotide, the resulting complex of coralyne and dia-denosine nucleotides forces the removal of coralyne from the probe. Such conformational change in the probe leads to the restoration of fluorescence. Within a short analysis time of 1 min, the proposed probe provides high selectivity toward c-di-AMP and Ap5A over other common nucleotides. The probe's detection limit at a signal-to-noise ratio of 3 for both c-di-AMP and Ap5A were estimated to be 0.4 and 4 μM, respectively. The practicality of the proposed probe was demonstrated by quantifying c-di-AMP in bacteria lysates and Ap5A in human tears.

Biography

Ming Mu Hsieh has completed his PhD from National Taiwan University and Postdoctoral studies from National Taiwan University. He is currently working as a Professor of Applied Science at National Taitung University. He has published more than 40 papers in reputed journals. His current research includes the development of analytical techniques for small molecules by capillary electrophoresis and green methods for synthesizing fluorescent carbon dot and its applications.

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