Development and biological application of a novel fluorescent probe for ratiometric imaging of protein tyrosine phosphatase activity

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We have designed and synthesized a novel ratiometric fluorescent probe 1 for protein tyrosine phosphatase (PTP) activity based on spectral overlap integral switching of FRET. The fluorescence spectra of 1 exhibited a large shift in their emission wavelength after reaction with PTPs in vitro. Within the 1 labeled cells, the increase in the emission ratio was observed, and it was suppressed by the addition of a PTP inhibitor orthovanadate and hydrogen peroxide. So it was shown that 1 could detect PTP activity in living cells. Next, we applied this imaging method to biological studies on the regulation of PTP activity during contact growth inhibition of normal cells. In normal cells, PTP activity of dense cells was markedly enhanced compared to that of sparse or medium-density cells. The increased PTP activity was also observed in sparse cells treated with a reducing agent, lipoxygenase inhibitor, and PLA2 inhibitor, and resulted in cell growth inhibition. These observations suggest that PTP activity could be regulated by ROS produced in lipoxygenase-mediated arachidonate metabolism and play a key role in density-dependent growth inhibition.