

Inhibition of Tubulin Self-Assembly and Tubulin-Colchicine GTPase Activity by Guanosine 5'- γ -Fluorotriphosphate)

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The inhibitory effects of guanosine 5'- γ -fluorotriphosphate [GTP(γ F)] on both the polymerization and the colchicine-dependent GTPase activity of calf brain tubulin have been studied. The results demonstrate that this analogue of GTP, with a fluorine atom on the γ -phosphate, is a reversible competitive dead-end inhibitor of the colchicine-induced GTPase activity with a K_i value of $(1.8 \pm 0.6) \times 10^{-4}$ M. GTP(γ F) did not promote assembly of tubulin from which the E-site guanine nucleotide had been removed. It binds to the exchangeable nucleotide site competitively with respect to GTP, diminishing both the rate and extent of tubulin polymerization. Treatment in terms of the Oosawa-Kasai model of the inhibitory effect of GTP(γ F) on the assembly led to a value of $K_{dis} = 1.1 \times 10^{-6}$ M for the complex GTP(γ F)-tubulin. This analogue does not bind to the postulated third site. The growing of tubulin polymers at 37 °C was arrested by GTP(γ F), and only limited depolymerization was induced by the a