

Tubulin-tyrosine ligase catalyzes covalent binding of 3-fluoro-tyrosine to tubulin: kinetic and [19F]NMR studies

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The use of 3-fluoro-tyrosine as an alternative substrate for the enzyme tubulin:tyrosine ligase which catalyzes the incorporation of tyrosine into the α -tubulin subunit was investigated. The incorporation of tyrosine into tubulin was inhibited competitively by 3-fluoro-tyrosine with an apparent K_i of $25 \mu\text{M}$. The affinity for this analog was similar to that of tyrosine, confirming that the hydrogen at position 3 of the aromatic ring is not essential for the reaction catalyzed by TTLase. The incorporation of 3-fluoro-tyrosine into the C-terminus of the α -tubulin subunit was demonstrated through [19F]NMR spectroscopy. The 3-fluoro-tyrosine signal at -58.6 ppm (trifluoroacetic acid as external standard), with a bandwidth of 24.7 Hz presented a chemical shift of 0.75 ppm upfield and an enlargement in the bandwidth (30.5 Hz) when incorporated into tubulin. These results strongly suggest that this amino acid is exposed to the solvent in tubulin. Tubulin covalently labeled with 3-fluoro-tyro