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## CHLOROACETYLATION OF CYCLOSTREPTIN INFLUENCES ITS INTERACTION WITH TUBULIN

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Cyclostreptin (Cs) is a natural product from *Streptomyces* sp.9885 that irreversibly stabilizes cellular microtubules by covalent binding to tubulin, causes cell cycle arrest, evades drug resistance in MDR tumor cells and inhibits paclitaxel-binding to microtubules. In a previous work (1) we demonstrated that cyclostreptin irreversibly binds to  $\beta$ -tubulin through Thr220 and Asn228 (the type-I pore binding site). To gain further information about this binding site, two reactive derivatives of cyclostreptin were synthesized and studied.

In this work we characterize the interaction binding sites of monochloroacetylated cyclostreptin in position 15 (15CA-Cs), or in position 17 (17CA-Cs), which were also cytotoxic in MDR cells and accumulate cells in G2+M phase of the cell cycle in the same way as cyclostreptin, within microtubules. As performed in (1), we have used a hybrid triple-quadrupole mass spectrometer to analyze the filtered precursor ions by the detection of AC-Cs-derived fragments in the third quadrupole. We observed a change in the specificity of CA-Cs-interacting sites within the tubulin molecule both in the formed microtubules and in unpolymerized tubulin. Although the tubulin interacting-domain was the same we had previously found (219-LTTPTYGDLNHLVSATMSGVTTCLR-243), neither Thr220, nor Asn228 residues were CA-Cs-labeled in microtubules, while the CA-Cs binding site was detected in Cys241. However, in the dimeric, unpolymerized tubulin, we detected the Thr220 interacting site with CA-Cs as well.

Interpretation of the reaction mechanisms of the CA-CS derivatives with Thr and Cys side chains in the type-I pore binding site of microtubules is discussed.

1. Buey RM, Calvo E, Barasoain I, Pineda O *et al.* Cyclostreptin binds covalently to microtubule pores and luminal taxoid binding sites. *Nature Chemical Biology* 3, 117-125 (2007).