Docking of Dictyostatin, 16-normethyldictyostatin, and Discodermolide, 14-normethyldiscodermolide, to β-Tubulin via Molecular Dynamics Modeling

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INTRODUCTION

Microtubules are multi-functional cytoskeletal elements crucial for the proper execution of mitosis. If a cell is unable to undergo mitosis, it is unable to multiply and may eventually die by apoptosis – an unpleasant possibility for a healthy cell, but a highly desirable outcome for cancer cells.

At the basis of microtubules are α and β-tubulin monomers each containing an N-terminal nucleotide-binding domain, generally bound by GTP. Microtubules are composed of αβ-dimers, which bind together in a three-start left handed helix to form microtubules. When examined, microtubules appear to be made up of long, straight protofilaments of the heterodimers. These protofilaments are bound to each other through homogenous lateral contacts (α-α and β-β) to form microtubule walls. The bound nucleotide is at the heart of the interface for longitudinal (intra-dimer and inter-dimer) interactions. As a microtubule grows, the exposed GTP on the β-subunit becomes hydrolyzed to GDP, which is embedded within the interface. Microtubules therefore have a plus (= growing) end capped by β-tubulin and a minus end capped by α-tubulin. The nucleotide bound to the α tubulin of the minus end of the microtubule is engaged in longitudinal interactions, and therefore, lateral interactions at the minus end are strong and independent of the state of the nucleotide. On the other hand, the GTP bound to the β-tubulin of the plus is exposed to the cell environment. A GTP cap produces strong lateral contacts, but if
the GTP cap is hydrolyzed to GDP, the β-tubulins interact weakly laterally. The result is a stable minus end and a dynamic plus end where the microtubule can rapidly grow and shrink (1).

The action of a class of chemotherapeutic compounds known as taxanes (i.e., paclitaxel (Taxol), and docetaxel (Taxotere)) has been found to be the strengthening of the lateral contacts between β-tubulin (1) by the binding of these agents to a specific site on the β-subunit. This stabilization inhibits the dynamic nature of the plus end of the microtubule, disabling it from functioning properly in the mitotic spindle during mitosis, and thereby stopping sister chromatid exchange and cell division.

A number of other compounds have been found that act in a similar fashion, i.e. microtubule stabilization, and competitively inhibit taxane binding to tubulin. Two families of these compounds are known as epothilones and discodermolides. Before high resolution cryoelectron microscopy (a.k.a. electron crystallographic) studies had established the binding mode of epothilone, studies based on resistant cancer lines with β-tubulin mutations led to postulation of a common pharmacophore for epothilones and taxanes (2). After the electron crystallography was performed, it was found that while these two compounds shared the same binding pocket, there was no common pharmacophore but rather that “tubulin displays a promiscuous binding pocket” for microtubule stabilizers (3).

No crystallographic studies of tubulin/microtubules and the discodermolides have been reported. Discodermolide is a polyketide natural product, a polyhydroxylated, polymethylated carbamate-bearing C24:4 fatty acid lactone, with potent microtubule stabilizing properties and the highest known affinity for the taxane binding site. Recently, another natural product with great structural similarity to discodermolide, dictyostatin, which is the macrolactone, carbamate-less analogue of discodermolide, has been shown to have essentially the same activity.
Interestingly, the 16-normethyl analogue of dictyostatin, although similar in potency against cells with wild type tubulin, is many-fold less potent in cells resistant to taxanes by virtue of expression of β-tubulin with a Phe270->Val mutation, a residue known to be in close contact to the taxanes in the ligand binding site of the protein. This is the first agent found to be affected by this particular mutation, and these results strongly suggests that the C-16 region of dictyostatin and its analogues occupy that region of the binding site. The corresponding analogue of discodermolide, 14-normethyldiscodermolide, is a known synthetic agent with activity similar to that of discodermolide but as yet untested against these mutant β-tubulin expressing cells. Therefore, it is the purpose of this research to examine the molecular models of the discodermolides and dictyostatins computationally docked to β-tubulin, based upon the known structure of β-tubulin and experimental data between analogues of these compounds and cancer lines (4, 5).

**METHODS**

Because it has been discovered that different families of compounds have different contacts within the β-tubulin binding pocket, the compounds in question cannot simply be superimposed upon compounds (taxanes, epothilones) whose binding modes have already been established. Therefore, molecular dynamics (MD) calculations will be performed to test for the most energetically favorable conformation of each compound within the β-tubulin binding pocket. The program that will be used will be AutoDock, but other programs will be investigated and possibly used to test for consistency of results. First, a trial run will be performed with epothilone A and paclitaxel, whose binding modes are already known. The simulated binding conformations will be compared to those discovered by electron crystallography in order to evaluate the accuracy of the computations. After the program settings have been adjusted to
obtain the best result on the crystallographically determined agents, dictyostatin, 16-normethylidictyostatin, and discodermolide, 14-normethylidiscodermolide, will be docked accordingly. The results obtained will then be compared to the experimental data available.

POSSIBLE RESULTS
It is expected that after iterative adjustment of the program settings in the docking module of AutoDock, MD models for epothilone A and paclitaxel will be obtained that agree highly with the known binding modes of these agents with β-tubulin. Furthermore, when the same settings are applied to the ligands under investigation, the MD models produced are expected to help account for the experimental observations based on tests in cells with and without mutations in β-tubulin.

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