Docking Studies of the Binding Mode of Dictyostatin and Its Analogues to the Taxoid Binding Site on β-Tubulin

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Introduction

Microtubules are an essential part of the molecular apparatus that enables the division of a cell's contents during mitosis. These fibers, consisting of polymerized heterodimers of α-tubulin and β-tubulin subunits, form the mitotic spindle that facilitates the separation of replicated chromosomes during mitotic anaphase. In order for microtubules to function in this capacity, however, it must be possible for their length to be adjusted dynamically. They must grow in order to form the mitotic spindle and then disassemble in order to move the replicated chromosomes to opposite poles of the cell. This dynamic behavior is made possible by the binding of GTP to each β-tubulin subunit and its eventual hydrolysis, which converts it to GDP. If the growing end of a microtubule, which is "capped" with β-tubulin, bears unhydrolyzed GTP, then it is resistant to depolymerization of its heterodimer subunits. However, if the growing end bears GDP, the bonds between subunits become weak and the microtubule is prone to disassemble. Thus, one mechanism by which this dynamic process of growth and disassembly of microtubules can be disrupted (thereby stopping the cell cycle at metaphase of mitosis) is to stabilize the growing end so that it will not depolymerize regardless of whether it bears GTP or

GDP.[7] Because the rapidly dividing cells which comprise tumors are especially sensitive to such disruption of mitosis, a considerable and increasing number of drugs exploit this mechanism to stop the proliferation of various cancers.[5]

For some years, the microtubule-stabilizing alkaloid paclitaxel and its analogue docetaxel have been some of the most important chemotherapeutic drugs for the treatment of certain cancers. However, researchers and physicians have long since realized that there are a number of problems with these particular agents, particularly challenges related to delivery of the drugs, and resistance that has been observed in several carcinoma cell lines and in humans. For example, these substances are essentially insoluble in water and often must be dissolved in harmful solvents in order to be administered effectively. Also, in experiments with certain human colon and ovarian carcinoma cell lines overexpressing P-glycoprotein efflux pumps and/or exhibiting mutations that affect the taxoid binding site, the effectiveness of taxoid compounds have been shown to be diminished by up to 2800 times compared to their effectiveness against non-resistant lines.[2,5] Thus, a major current area of research focuses on attempts to discover and synthesize anti-mitotic agents that are potent, effective against taxoid-resistant tumors, and more easily deliverable. One relatively new drug that seems to meet these criteria is a marine sponge-derived macrolactone compound known as dictyostatin[4], which will be the subject of this research.

Methods

The structure of dictyostatin was confirmed, along with the structures of certain dictyostatin analogues, through total synthesis by Shin, et al, in 2004.[8] MOE (and perhaps other

software as needed) will be used to dock these structures in the taxoid binding site on β-tubulin (for which dictyostatin competes with the taxanes[3]) in order to find each compound's most energetically favorable conformation in the binding pocket. Geometry optimizations will then be performed on these docked ligands. Possible intermolecular interactions between the ligand and nearby amino acids in the binding pocket will be analyzed to give insight into the mechanisms by which dictyostatin and its analogues interact with the binding pocket. A similar procedure will be carried out with the microtubule-stabilizing compounds paclitaxel and epothilone (both of which occupy, but interact with different amino acid side chains in, the same binding pocket on β-tubulin as dictyostatin[2,6]) as a control to evaluate the validity of the computational results, since the structures of these two compounds in the binding pocket have been determined through cryoelectron microscopy.[1,6] The wild-type tubulin structure will be obtained from a high-resolution cryoelectron crystallography-determined structure by Nogales, et. al., in the Protein Data Bank.[7] A mutation in β-tubulin known to impact the effectiveness of dictyostatin and certain analogues, substitution of valine for Phe270, will be introduced to study the interactions of the dictyostatin ligand with the amino acid in this position in the binding pocket and provide a rationalization for the impact of these mutations on the effectiveness of the compounds under study.

Anticipated Results

It is hoped that the results of this study, which will be the various interaction energies computed for the proteins bound by the ligands under study, will correlate with the results of

experiments that have recently been conducted on the effectiveness of certain dictyostatin analogues in several different cell lines and help rationalize the current structure-activity relationship for dictyostatin.[3] In particular, it is expected that our modeling approach will clarify the protein-ligand interactions that give rise to the decreased effectiveness of dictyostatin and certain analogues in mutant cell lines expressing mutant β-tubulin with valine substituted for Phe270, presumably by exposing a significant energetically favorable interaction between the ligand and the Phe270 amino acid in the binding pocket of wild-type β-tubulin. Further, it is conceivable that hypotheses will be formed regarding new molecules to synthesize.

References

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