A comparative investigation of the C-terminal tails mobility in the $\beta\ I and \beta\ III isoforms of tubulin.$

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April 20, 2016

Abstract

Your abstract.

1 Introduction

Tubulin, a structural protein $\alpha/\beta heterodimer, is the building block of microtubules (MT). Each subunit \alpha and \beta is$
$to-tail fashion to form 13 protofilaments constituting the MTs. Mts exhibit a dynamic instability which is essen$

As a consequence of this key role, tubulin is a well known target for some of the most successful anti-tumor drugs, such as taxanes, vinca alkaloïds or colchicin (refs). After cellular uptake, microtubule network will be the target to distrupt chromatid separation thus preventing the meiosis. Cytotoxic agents will interfere with MT dynamics by inhibiting the polymerisation or depolymerisation of dimers of MT, leading to inhibition of the cancer cell division and proliferation (ref). Paclitaxel (Taxol), for example, binds to MTs and stabilizes the MTs already formed preventing the depolymerisation (ref). At the opposite, vinca alkaloïds (vinblastine) and colchicin prevent the polymerisation (ref).

Despite the fact that most clinical tubulin binding drugs target the $\beta subunit of the dimer, an important issu$
$tubulin, which is associated with highly malignant tumor phenotype. This overexpression is also related with a pa$

Until now, most of the studies on the tubulin do not take into account the isotype, mainly because the major changes occurs in the C-terminal tails (CTT) of the subunits (ref). These CTTs are still missing parts of the crystal structures found in the databases, thus making it complicated to study. Considering recent studies, CTTs exhibit primoridial roles for the MTs functionality, such as formation of MT, interaction with microtubule associated proteins and kinesin, acting as a lasso (ref). It is also a hotspot for posttranslational modifications (polyglutamylation, tyrosination, phosphorylation, etc) (ref).

We present a modelisation process of two complete human tubulins of $\alpha 1 \beta 1 and 1 \beta 3 isotypes. Furthermore,$
$termini of \beta subunit structural organisation and interaction with the core part of the tubulin, using multiple mol$

2 Material and methods

2.1 Human complete tubulin modelization

For this study, we have modeled two different tubulin isotypes, the $\alpha 1 \beta 1 and the \alpha 1 \beta 3. Each subunit was modeled$
$1SA0), chosen for the highest identity (cf table x), most recently reviewed and more complete among all crystal str$

$template selection, template-target alignment, model building and finally model evalutaion. For the templates$
$template matching method (pariwise alignement with known structure only, present in an associated database). T$
$bonded contacts, etc) in the first place followed by a minimization of all the violations of the restraints. For the tail$
$in scoring functions : molPDF, DOPE and GA341 scores. molPDF and DOPE score are energies, the lower the$
$1JFF, the PDB we used in our previous study$).

$\beta 3(Q13509)$	$\alpha 1(Q71U36)$	$\beta 1(Q9H4B7)$
1SA0 % sim	% sim	% sim
1JFF		
1TUB		

2.2 Molecular dynamics simulation

After this step, we submitted all models previously obtained to classic molecular dynamic simulation (five models for each isotypes modeled) in order to relax the structure and to explore the structural variability of the C-termini newly modeled. The simulation were made with Gromacs 5.0.4 with the OPLS-AA forcefield in, periodic boundary condition. The procedure consist of two steps of minimization, the first one is an *in vacuo* during 1000 steps without any constraints using steepest descent algorithm, followed by the addition of a water box of 2 nm around the tubulin filled with TIP3P molecule model and neutralization of the system with randomly added NaCl ions in the box while maintaining a 150mM concentration. The total system contain around 13 700 atoms for the tubulin dimer, 136 000 water molecules, and 300 ions (approx. 60% Na 40% Cl due to the high negative charge of the C-termini).

The second minimization step is done under the same set of parameters during 5000 steps to prevent any water clashes with the tubulin. The next stage before production phase consists of a two-steps equilibration procedure, an NVT equilibration followed by an NPT equilibration. Each stage lasted 100 ps, with an integration step of 2 fs. Temperature was fixed at 300 K using velocity rescale method, all bonds were constrained with the LINCS algorithm, electrostatic interactions were computed with the Particular Mesh Ewald method. For the pressure coupling during the NPT equilibration, we used Parinello-Rahman method at the value of 1 atm.

The production run were then performed with the same set of parameters and algorithms during 100 ns. Trajectories were saved every 10 ps, and the analysis were made on the last 95 ns, considering the first 5 ps as equilibration period.

2.3 Trajectory analysis

After obtaining the simulation data, we processed them with the Gromacs 5.0.4 utilities for basics observations and with VMD 1.9 for the graphical part. Most of the more complexes analysis were made with homemade scripts/programs.

For the clustering part, it was a two step process. Due to the high flexibility of the C-termini (mostly the one of the $\beta subunit$), $which are the parts of interest in this study, classicals methods$

3 Results

3.1 Model building

3.2 $\beta - subunitC - terminaltailorientationandfolding$

3.3 Contacts $\beta - subuntC - terminalwiththetubulincore$

4 Discussion