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Hypothesis

Differential colchicine-binding across eukaryotic families: The role of highly conserved Pro268β and Ala248β residues in animal tubulin

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Abstract Colchicine–tubulin interaction, responsible for the disruption of microtubule formation, has immense pharmacological importance but is poorly understood in terms of its biological significance. The interaction is characterized by a marked higher affinity of colchicine for animal tubulins compared to tubulins from plants, fungi and protists. From an analysis of tubulin sequences and colchicine–tubulin crystal structure, we propose that Pro268β and Ala248β (270β and 250β in the crystal structure 1SA0) in animal tubulin are crucial for the observed differential binding. We also suggest that mediated by the binding of endogenous molecules to the colchicine-binding site, microtubule assembly in eukaryotes may be modulated in a family specific manner.

Keywords: Tubulin; Colchicine; Binding site; Principal component analysis; Proline; Beta-strand

1. Introduction

Microtubules play a central role in several basic cellular functions in all eukaryotes, of which cell division is the most important [1]. The building block of microtubules, the hetero-dimeric protein tubulin [2] (see Fig. 1a), is known to bind several molecules of pharmacological value [3]. Colchicine, a plant alkaloid, is one such molecule that can bind tubulin and inhibit tubulin polymerization in animal cells, leading to mitotic arrest [4]. Although colchicine itself is not used as an antimitotic drug due to its severe side effects, several synthetic antimitotic drugs, derived from colchicine, have immense pharmacological importance [5–8]. Despite being a well-characterized binding site, for molecules both related to and unrelated [9] to colchicine, the biological role of the colchicine-binding site in eukaryotes is not clearly understood.

A unique feature of the colchicine-binding site is its striking animal-specificity. Among the four eukaryotic families (plants, animals, protists and fungi), colchicine is known to bind most strongly to animal tubulins, with an affinity constant $\sim 10^6\ M^{-1}$. In comparison, the colchicine-binding affinity of *S. cerevisae* (fungus) and *T. pyriformis* (protist) is $\sim 10^2\ M^{-1}$, weaker by ~10,000 fold [10–14]. Plant tubulins also bind tubulin only weakly as has been shown from comparative binding assays [15]. The origin of this animal-specific tubulin affinity of colchine is not known. An understanding of the molecular origin of the animal-specificity of tubulin is important not only because it will broaden our understanding of this pharmacologically important drug–protein interaction, but also because it might shed light on any biological relevance of the colchicine-binding site. In this work we have addressed the issue of differential colchicine-binding to tubulin across eukaryotes from a careful analysis of tubulin sequences across a large number of eukaryotes and the crystal structure of colchicine-bound animal tubulin [2].

2. Criterion for identifying amino acids responsible for animal-specific colchicine-binding

2.1. Distinct clustering of a subset of animal tubulin sequences

In order to identify residues responsible for animal-specific colchicine binding to tubulin, we need a working hypothesis that can be used as a criterion for judging whether the identity of a selected set of residues is sufficient to account for the experimental observation. We proceed with the hypothesis that if the "collective nature" of a subset of residues in animal tubulins is distinct from that of a similar subset in non-animal tubulins, then one or more of the residues in the animal tubulin subset is responsible for the animal-specific colchicine-binding. We use principal component vectors to represent the "collective nature", as has been successfully applied in predicting protein functional residues [16].

2.2. Definition of primary and extended colchicine-binding sites

A second criterion is that the subset of residues must, directly or indirectly, be involved with colchicine-binding as evident from the crystal structure or other experimental evidence. Two sets of residues were defined for this purpose, the primary colchicine-binding site (PBS; residues within 4.5 Å of DAMA-colchicine (DC) in the tubulin-DC crystal structure 1SA0 [2]) and the extended colchicine-binding site (EBS; all PBS residues plus residues within 5.0 Å of all PBS residues). Twenty tubulin residues constituted the PBS

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Abbreviations: PBS, primary colchicine-binding site; DC, DAMA-colchicine; EBS, extended colchicine-binding site; PCA, principal component analysis

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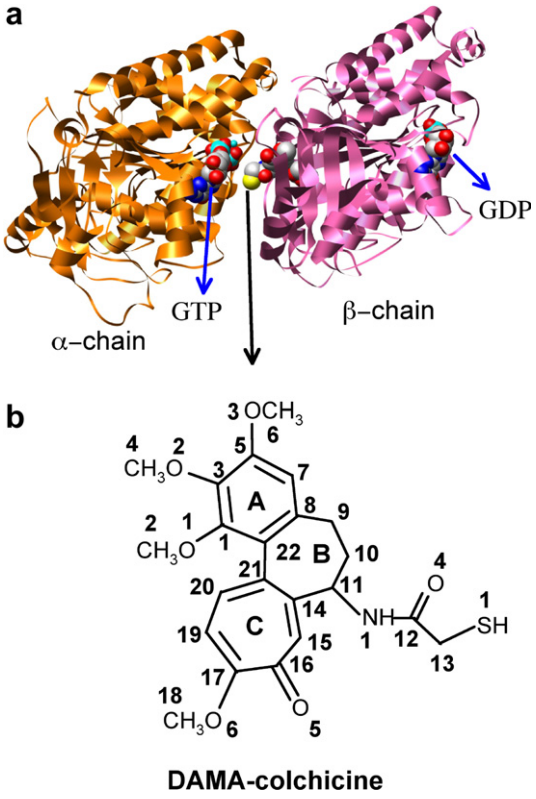

Fig. 1. (a) Structure of animal tubulin hetero-dimer (pdb code: 1SA0) bound to GTP, GDP and DAMA-colchicine (DC) and (b) The chemical structure of DC with atom numbering.

(Table I, Supplementary materials). Unless otherwise stated, the sequence numbering used in this work, including Supplementary materials, is consistent with that used in the pdb file 1SA0 [2], where the sequence numbering was slightly altered from the original sequence numbering to accommodate sequence alignment of α- and the β-chains. The PBS residues in tubulin sequences across eukaryotes were then identified by multiple sequence alignment [17] of 91 α-tubulin and 77 β-tubulin sequences (see Table III Supplementary materials). While four residues (out of 20) in the α-chain constituted the PBS, sequence alignment showed that only one out of these, 180α, exhibited some family-specific conservation (Fig. 1, Supplementary materials). However, the observed conservation was not strictly animal-specific. Therefore, only the remaining β-chain residues (16 residues) were considered to constitute the PBS for subsequent analysis presented here.

2.3. Structural changes compatible with experimental binding energies

The colchicine–tubulin association constant is higher in animals by about three orders of magnitude as compared to other eukaryotes. Using the relationship $\Delta G^{\circ} = -RT\ln K$, this translates to about 4 kcal/mol extra stabilization of the animal tubulin–colchicine complex (at 25 °C). The magnitude of the energy suggests the disruption of specific interactions like H-bonds [18] in non-animal tubulin-colchicine complexes. Tubulin PBS contains a H-bond between the Cys241β side-chain and the colchicine O2 atom (Fig. 1b), disruption of which was shown to result in very little binding of a colchicine analog to rat brain tubulin [19].

3. Principal component analysis in the primary colchicine-binding sequence space

Principal component analysis (PCA) [16] was performed on the PBS set across eukaryotes to ascertain eukaryotic family specific clustering. As shown in Fig. 2a, when projected on the first and second principal component vectors (associated with the highest and the second-highest mean square fluctuations), the four families separate into two subgroups: animals-fungi and plants-protists. Without any distinct group for animals, the PCA result is similar to what has been observed for the phylogenetic analysis of whole tubulin sequences [20]. Several members of the fungi family, viz. *C. albicans*, *S. cerevisiae*, *S. pombe*, *P. carinii*, and *E. nidulans*, were found to overlap with the mostly-animal cluster. At least one of these (*S. pombe*) is known to bind colchicine only weakly [11]. Thus, the identities of amino acid residues comprising the PBS are not sufficient to reflect the animal-specific colchicine-binding trait of tubulin. This led us to look beyond the PBS.

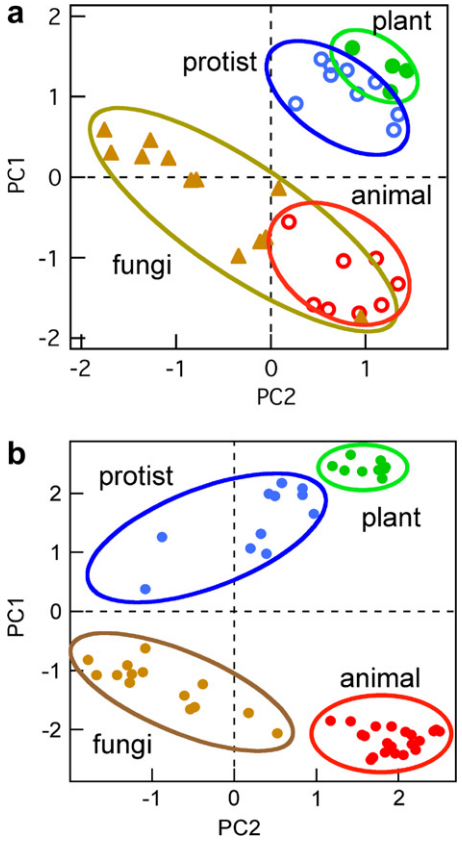

Fig. 2. Projection of: (a) tubulin PBS and (b) EBS sequences onto the first and second principal component sequence vectors. Principal component analysis was performed using a methodology very similar to published earlier [16]. The dimensionality of the sequence space was $\sum N_i$ where N_i corresponds to the total number of residue types at the ith site. A binary code (1 or 0) indicated the presence or absence of a residue type at a given site. The eigen values and eigen vectors (principal component vectors) were obtained by the diagonalization of the variance–covariance matrix $(\sigma_{jk} = \langle(\phi_{ij} - \bar{\phi}_j)(\phi_{ik} - \bar{\phi}_k)\rangle)$, where the indices j and k run over residue positions, and the index i corresponds to individual sequences.

4. Principal component analysis in the extended colchicine-binding sequence space

PCA was performed for the 65 residues (Table II, Supplementary materials) defining the EBS set. Unlike the case with PBS residues (Fig. 2a), tubulin sequences corresponding to the EBS clearly clustered according to their eukaryotic family origin, when projected onto the first two principal component vectors (Fig. 2b). Specifically, the animal tubulin EBS residues were distinct—suggesting that the identity of EBS is sufficient to explain the origin of the animal-specificity of colchicine–tubulin interaction.

Residue variations at 21 sequence positions, found to change along PC1 or PC2, are shown as sequence logo plots [21] in Fig. 3a. Two sequence positions are animal-specific: A250β and P270β. Of these, the identity of 250β position changes to Ser from Ala, and, the identity of 270β position changes to Val/Ile from Pro for non-animal tubulins. We also compared the sequence conservation at binding sites of two other ligands: GTP and GDP (see Fig. 1a), bound to α- and β-tubulin, respectively. As shown in Fig. 3b,c no residue positions show family-specific conservation. In what follows, we consider structural changes and its effect on colchicine-binding upon A250Sβ and P270I/Vβ mutations.

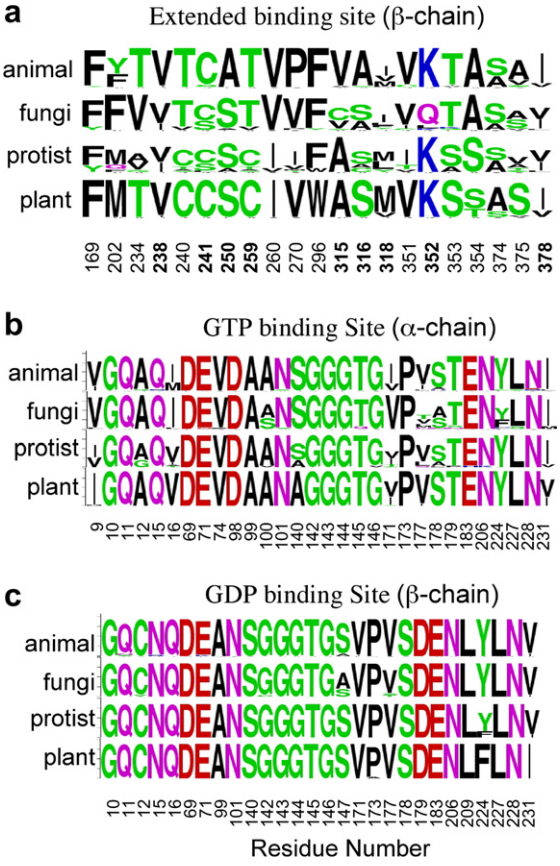

Fig. 3. (a) Sequence logo plots [21] depicting sequence variations at: (a) residue positions in EBS that change along PC1 and PC2 (Fig. 2b), (b) GTP-binding residues in the α-tubulin, and (c) GDP-binding residues in β-tubulin.

5. Structural consequences of A250βS mutation

The A250Sβ mutation in non-animal tubulins can alter a specific colchicine–tubulin interaction present in the crystal structure, the C241β-colchicine H-bond, in two ways ($\chi 1_{250\beta}$ = trans or gauche-). In the colchicine-free state, S250β in the trans isomeric state can form a H-bond with the S^{γ} atom of C241β (Fig. 4a). This H-bond and the C241β-colchicine H-bond are mutually exclusive, implying that A250Sβ mutation has the potential to counter any energetic advantage of C241β-colchicine H-bond in the colchicine-bound state. In the gauche-isomeric state, S250β can form a ST-turn H-bond [22] with the backbone amide hydrogen atom of L252β (Fig. 4b). Upon colchicine-binding, if the ST-turn H-bond is retained, it will disrupt favorable L242β–colchicine interactions (Fig. 4b). On the other hand, if the H-bond gets disrupted, the colchicine-bound state will become energetically less favorable than the colchicine-free state by a H-bond that will counter any favorable energy from the C241β–colchicine H-bond.

6. Structural consequences of P270βV/I mutation

The other unique residue in animal colchicine, P270β, is a little removed from the colchicine molecule in the crystal structure. Proline is rarely found in regular secondary structural elements [23], both in α-helix and β-sheet. This is due to the restricted nature of its allowed backbone dihedral angle ϕ (∼−63°) and the lack of backbone amide hydrogen necessary to participate in H-bonding networks. However, Pro is consistently present in the center of a β-strand ($\phi = -92°$; $\psi = -158°$ in 1SA0) at position 270β in animal tubulins. In all non-animal eukaryotes the position is occupied by either Val or Ile, both ranked as top β-strand formers.

In the crystal structure (1SA0), the β-strand (267–272β) containing P270β is flanked by two (374–381β and 200–205β) other β-strands connected by inter-strand H-bond network (Fig. 4c). The network gets disrupted due to the lack of a backbone amide hydrogen atom of P270β, which could have been H-bonded to the backbone carbonyl of C203β. As shown in Fig. 4d, the lack of this potential backbone–backbone H-bond is somewhat compensated for by the presence of a C^{δ}–H···O H-bond between C^{δ}–H (P270β side-chain) and O═C (C203β backbone), similar to what has been observed for Pro in α-helices [24]. Upon a P270βV/I mutation, the C^{δ}–H···O═C H-bond is likely to be replaced by a NH···O═C H-bond, bringing the backbones of 270β and 203β closer, thereby also bringing the two β-strands closer. In addition, with a non-Pro (V/I) residue at position 270β, the constraint on the backbone dihedral angle ϕ will be relaxed, leading to the possibility of forming one extra inter-strand H-bond (between $205\beta^{NH}$ and $270\beta^{C=O}$). This extra H-bond may cause significant rearrangement of the backbone, especially in the context of β-strand H-bonding network around 270β.

In addition to the backbone rearrangement, a P270βV/I mutation will also introduce changes in the side-chain packing interaction around 270β to accommodate a V/I residue at that position. Residues that are in proximity of 270β (202β, 234β, 238β, 378β) are shown in Fig. 4c. Of these, 238β directly contacts C241β, a crucial residue for a side-chain mediated

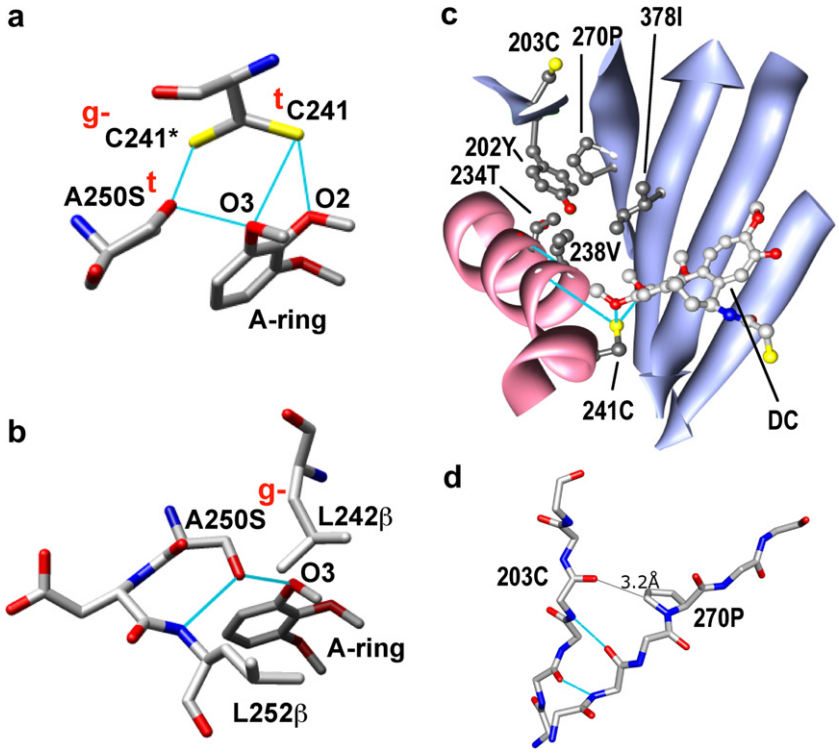

Fig. 4. (a) Effect of A250βS (t conformer) substitution in the PBS. C241β side-chain is shown in trans (crystal structure) as well as in gauche-conformation. (b) Formation of a Type-I ST-turn [22] upon A250βS (gauche-conformer) substitution in the PBS; in the gauche-conformation, S250β side-chain hinders close contact of L242β side-chain and colchicine. (c) Disposition of Pro 270β and neighboring residues, including C241β and DC, in the crystal structure (1SA0). (d) C^{δ}–H···O═C hydrogen bond (C^{δ}···O═C = 3.2 Å; H^{δ}···O═C = 2.7 Å; C^{δ}–H^{δ}···O = 111°; H^{δ}···O═C = 138°) between C203β backbone and P270β side-chain.

H-bond with colchicine. The other three residues (202β, 234β, 378β) make secondary contact with C241β via 238β. Therefore, a P270βV/I mutation might also disrupt the colchicine-241β H-bond interaction by modulating side-chain packing around C241β.

7. Summary and perspective

The recent 3.58 Å X-ray structure of colchicine-bound animal tubulin [2] provided the first comprehensive picture of the colchicine–tubulin interaction. In addition to pinpointing specific and non-specific interactions, the structure can lead to the prediction of common themes in colchicine–tubulin binding. For example, a recent study [25] used the crystal structure as a template to derive a common pharmacophore for a diverse set of colchicine site inhibitors using molecular docking and molecular dynamics studies. The animal-specificity of tubulin affinity of colchine can also be understood from a similar approach, albeit in a reverse sense. Instead of studying the interaction of tubulin with a diverse set of molecules that bind the colchicine-binding site, the interaction of colchicine with a diverse set of eukaryotic tubulins can provide clues about the origin of the animal-specific colchicine-binding of tubulin, as was attempted recently [26] to identify the molecular origin of biphasic kinetics of colchicine-binding to animal tubulin, originating from tubulin isoforms. In this work we have demonstrated that two residues in β-tubulin, at sequence positions 250 and 270, whose identities show animal-specific conservation (A250β and P270β in animals; S250β and V/I270β in plants, fungi and protists), are critical for the observed animal-specific colchicine-binding to tubulins across eukaryotes.

The idea that endogenous molecules might bind the colchicine-binding site has been suggested before. The family-specific conservation of colchicine-binding site in tubulins, pointed out in this paper, further suggests that the endogenous molecules may actually be family-specific (Roy, S. personal communication). Our results also call for the elucidation of non-animal tubulin structures for capturing subtle but important changes in the core tubulin structure, especially in the vicinity of the colchicine-binding site, as a result of sequence changes reported here, especially P270βV/I.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2007.09.047.

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