The colchicine–tubulin interaction: A review

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The plant alkaloid colchicine exerts characteristic antimitotic activity by binding specifically to the dimeric protein tubulin, an important constituent of the mitotic spindle. Apart from its polymerization into microtubules, colchicine binding is one of the primary characteristics of tubulin and the tubulin–colchicine interaction has been studied in detail. Colchicine binding to tubulin results in inhibition of microtubule assembly both in vitro and in vivo. The binding is slow, noncovalent, poorly reversible and occurs with a stoichiometry of 1 mole of colchicine per mole of the tubulin dimer. Both tubulin and colchicine undergo characteristic conformational changes upon interaction, which are often used as probes to monitor the progress of the reaction. The use of structural analogs of colchicine, especially the B ring analogs, has thrown considerable light on the mechanism of colchicine binding. It was observed that the B ring of colchicine, more specifically its C7 substituent, plays an important role in regulating the kinetic and thermodynamic parameters of the binding reaction, although it has no major role in assembly inhibitory or tubulin-binding ability of colchicine. Contact(s) between the B ring substituents, hanging outside the colchicine-binding site on the β subunit of tubulin, and the neighbouring α subunit are thought to be responsible for such regulatory action.

Colchicine, the active principle of Colchicum autumnale, also found in various related plants of the Liliaceae family, has been known since antiquity for its medicinal uses. Purified colchicine is still in use today for the treatment of gout and familial Mediterranean fever.

The presently accepted structure of colchicine (Figure 1) first proposed about 40 years ago, consists of a three-ringed nucleus, of which one is a trimethoxy benzene ring (called ring A), one a methoxy tropone ring (called ring C) and a third, saturated seven-membered ring (B ring), carrying an acetamido substituent at its C7 position. Colchicine disrupts a variety of cellular functions, e.g. mitosis, secretion, cell elongation, cell motility and morphology. It was recognized quite early that colchicine possesses the remarkable ability to modify cell growth by arresting cell division in metaphase—the so-called stathmokinetic effect. Such antimitotic behaviour of colchicine was identified to be due to its very specific and destructive action on the mitotic spindle, and led to extensive use of colchicine in cytology and related fields. Colchicine was, and has remained, one of the main tools for the production of polyploids and amphidiploids in plant breeding.

The 'colchicine-binding protein': Tubulin

The introduction of radioactive colchicine, labelled with tritium on the methoxy group of ring C had spectacular consequences. Early studies indicated that colchicine exerts its antimitotic activity by binding to 'microtubules', the major constituent of the mitotic spindle. Further research demonstrated that [3H]colchicine binding could be traced to a proteinaceous subunit of the microtubule. In fact, [3H]colchicine binding provided the tool necessary for the initial purification and characterization of this 'colchicine-binding protein', subsequently identified as tubulin. Since then, the tubulin–colchicine interaction has been the most intensively studied aspect of microtubule biochemistry and has thrown considerable light on the tubulin–microtubule system and its intracellular functions. Present studies with colchicine and its derivatives indicate that antimitotic activity of colchicine is directly related to its stoichiometric binding with tubulin, which disrupts the tubulin microtubule dynamic equilibrium in a cell.

Any review of colchicine remains incomplete without a discussion on tubulin and a brief résumé of tubulin structure and properties, pertaining to this article is.

Figure 1. Structure of colchicine.
presented here. Tubulin, the subunit protein of microtubules, is a dimer of two similar but nonidentical subunits \( \alpha \) and \( \beta \). The dimer has a Stoke's radius of 4.2 to 4.4 nm, a sedimentation coefficient of 6 S and a molecular weight of about 100 kDa, there being approximately 450 amino acid residues in each subunit. The subunits are held together by non-covalent forces and therefore dissociates reversibly with increasing dilution. The values of \( K_d \) obtained range from 0.2 to 1 \( \mu \)M and depend on the presence of colchicine, excess calcium or GTP hydrolysis\(^{18-22}\).

The detailed three-dimensional structure of tubulin is not known, as crystals of tubulin suitable for X-ray diffraction have not yet been prepared and tubulin is too large a protein for NMR studies. Insights into the general folding architecture of the tubulin dimer have mainly been obtained from proteolysis\(^{23,24}\), antibody binding\(^{25,26}\) and cross-linking studies\(^{27,28}\), Figure 2 shows a widely-accepted model of the tubulin dimer, based on these results\(^{29,30}\). Both the \( \alpha \) and \( \beta \) subunits are composed of two globular domains, a larger amino (N) terminal domain and a smaller carboxy (C) terminal domain, connected by an exposed loop. Limited digestion of the protein with trypsin and chymotrypsin specifically cleaves \( \alpha \) and \( \beta \) tubulin respectively at a single site only, producing N terminal and C terminal fragments of each subunit\(^{24}\). Cross-linking studies have demonstrated that intra-dimer contacts are between N terminal domain of \( \alpha \) subunit and C terminal domain of the \( \beta \) subunit\(^{31}\). The C-terminal ends of both subunits are remarkably rich in acidic residues, especially in glutamic acid\(^{32,33}\) At physiological pH, these sequences are highly charged and stay in an extended conformation\(^{24}\). Small regions of tubulin, called the C-terminal tails, are exposed to the solvent with little noncovalent interactions with the rest of the protein\(^{23,24}\). They are readily cleaved by the protease subtilisin producing first, dimers with intact \( \alpha \) and cleaved \( \beta \) ends, called hybrid tubulin \((\alpha \beta)\) (refs 23, 31). This is followed by cleavage of \( \alpha \), yielding the doubly cleaved \( \alpha \beta \) or tubulin S (refs 23, 31). The tails of tubulin regulate important functional properties of the protein. Polymerization is progressively enhanced as the tubulin tails are digested; such enhancement is probably due to reduction of charge as the tails are cut off, which facilitates the dimer–dimer interactions necessary for efficient assembly\(^{23,31}\). Colchicine-binding properties of tubulin are also altered by C-terminal cleavage\(^{32}\). The binding sites for several tubulin-binding ligands and proteins, viz. calcium, poly-lysine, the microtubule associated proteins (MAPs), etc. are also located here (shown in Figure 3)\(^{33-35}\).

**Microtubule poisoning by colchicine**

Apart from its polymerization into microtubules, the ability to bind colchicine is one of the primary charac-

![Figure 2](image1.png)

**Figure 2.** A model of the tubulin dimer, showing the larger N domain and smaller C domain of both the \( \alpha \) and \( \beta \) subunits. Trypsin and chymotrypsin cleavage sites are shown on exposed loops connecting the N and C domains of \( \alpha \) and \( \beta \) tubulin respectively. Intradimer contacts between \( \beta C \) and \( \alpha N \) are also shown. The C-terminal tails of both subunits are extended from the dimer surface and are cleavable by subtilisin.

![Figure 3](image2.png)

**Figure 3.** The carboxy terminal sequences of \( \alpha \) and \( \beta \) tubulin, beginning from residue 384 in \( \alpha \) and 374 in \( \beta \), and some of their important features are shown. The lines above the sequences indicate the possible binding sites for MAPs, polylysine, and calcium on both subunits and also, the helical region on \( \beta \) tubulin that is partially unfolded upon colchicine binding. The arrow-labelled Su indicates the major site of subtilisin cleavage.
poison assembly by adding to the growing end of microtubules, effectively ‘capping’ that end and blocking addition of further dimers by rendering that end totally incompetent for supporting assembly – the ‘end poisoning’ model. An alternative model of colchicine action is based on the ‘co-polymerization phenomenon’, in which the tubulin–colchicine complex gets incorporated into the growing microtubule, along with unliganded tubulin monomers. Consequent conformational changes at the microtubule end lowers progressively the affinity of the end for unpolymerized tubulin, resulting in a decrease in the apparent rate of addition of tubulin dimers to the microtubule.

Colchicine-induced depolymerization of preformed microtubules has also been observed. The depolymerization also occurs in an end-wise fashion, where the binding of tubulin to colchicine lowers the free tubulin concentration to below the critical concentration required for polymerization, thus shifting the tubulin–microtubule equilibrium towards the dimer. It has been reported that the microtubule-associated proteins significantly reduce the rate of colchicine-induced depolymerization.

Both colchicine and tubulin undergo conformational adjustments upon interaction

The molecular mechanism of colchicine binding to tubulin has received considerable attention. Although colchicine is not altered chemically upon binding, it undergoes some conformational changes during the binding process, as indicated by marked changes in its spectroscopic properties. Colchicine does not fluoresce when free in aqueous or organic solvents. However, binding to tubulin causes a dramatic increase in colchicine fluorescence, with emission maximum near 435 nm and a quantum yield of about 0.03 (refs 51, 52). The development of its fluorescence upon binding to tubulin has provided a simple technique for detailed kinetic and thermodynamic analyses of the reaction. The fluorescence has been ascribed to the troponle moiety (C ring) of colchicine and is probably due to a $\pi - \pi^*$ transition, with fluorescence lifetime of 1.14 ± 0.02 ns, which is characteristic of the singlet state. The CD spectrum of colchicine is also altered upon binding to tubulin (loss of negative CD band at 340 nm).

The basis of promotion of colchicine fluorescence upon binding to tubulin is not well understood. It has been shown that the promotion of colchicine fluorescence upon binding to tubulin can be mimicked by immobilizing the troponle ring, e.g. on increasing solvent viscosity, upon freezing or upon inclusion into micelles. This suggests that the induction of colchicine fluorescence is primarily due to its immobilization on binding to tubulin. It is also established that solvent polarity plays a role in determining the ability of colchicine to fluoresce in absence of tubulin although the relative contribution of the two factors, viz. viscosity and polarity, when the drug is bound to tubulin is yet to be determined. The change in CD spectrum probably signifies a flattening of the molecule upon binding to tubulin, with a marked decrease in the dihedral angle between the A and C rings from about 55° to less than 20° (ref. 54). This more planar conformation permits extended conjugation and hence the observed enhancement of fluorescence.

Tubulin also undergoes a conformational change upon binding colchicine as indicated by small changes in its absorption and CD spectra. A time-dependent quenching of tubulin fluorescence on binding colchicine is also observed. The quenching is proportional to the occupancy of the colchicine-binding site and in the saturated complex, almost 35–40% of intrinsic fluorescence of the unliganded protein is quenched. This fluorescence quenching is believed to be due to Forster's energy transfer between the bound colchicine and one or more tryptophans neighboring the colchicine site on tubulin. That colchicine induces a local unfolding in tubulin has also been concluded from proteolytic studies. Colchicine binding causes changes in tubulin sulfhydryl group reactivity; intra-β-subunit covalent cross-linking of two reactive cysteines, Cys 239 and Cys 354, by N,N’-ethylenebis(iodoacetamide) (EBI) is prevented, indicating a conformational shift. Changes in immunological properties of tubulin upon binding colchicine are also reported. Colchicine binding also enhances the intrinsic GTPase activity of tubulin and lowers the $K_d$ of the tubulin dimer.

Based on kinetic data, a two-step model of the binding reaction has been proposed:

$$T + C \rightarrow [TC] \rightarrow [TC]^*,$$

which involves an initial, rapid, equilibrium step between colchicine (C) and tubulin (T), to form a low affinity, non-fluorescent complex [TC]. The second step is slow and poorly reversible, yielding the high affinity, fluorescent complex [TC]*. The slow step is where conformational changes of both tubulin and colchicine occur.

Properties of the tubulin–colchicine interaction

Binding of colchicine to tubulin is slow, requiring almost 60–90 min at 37°C to reach completion, noncovalent and poorly reversible. The thermodynamic and kinetic parameters of the binding reaction have also been determined by many workers. Reports on rate constants have ranged from $10^5$ to $2 \times 10^9$ M$^{-1}$ h$^{-1}$ while off-rate constant values between 0.009 and 0.039 h$^{-1}$ have been obtained. Much of this variability is
probably due to differences in reaction conditions, reaction temperature and source of tubulin. The rate of binding of colchicine to tubulin is enhanced by certain anions like tartarate or sulphate\(^\text{30}\). The activation energy of the forward reaction is about 20 kcal/mol (refs 70, 71). Such high values of activation energy have been attributed to the conformational changes of both tubulin and colchicine that accompany their binding reaction. The enthalpy values (\(\Delta H\)) reported were 7–16 kcal/mol and the entropy values (\(\Delta S\)), 47–80 entropy units. The reaction is entropy driven.

Colchicine–tubulin interaction is strongly influenced by pH. The pH profile of colchicine-binding activity of tubulin shows a maximum at pH 6.8, decreasing on either side of this optimum pH (ref. 32). The binding activity is labile, and is often used as a probe for aging of the tubulin. However, it appears to be more resistant to inactivation than polymerization activity of tubulin\(^\text{72}\). A variety of agents are known to stabilize the colchicine-binding site, e.g. sucrose\(^\text{72-74}\), glycerol\(^\text{75}\), MAPs\(^\text{36}\), polylysine\(^\text{38}\) and antimiotic drugs like vinblastine and colchicine itself\(^\text{73,77}\). Many of these stabilizing agents bind to the C-terminus of tubulin, suggesting that the C-terminus must be involved in the lability. It has been shown that both pH dependence of colchicine binding and time dependent decay of the site are very much reduced in tubulin S, obtained by C-terminal cleavage\(^\text{32}\). Also the affinity for colchicine is lowered due to increase in off-rate constant of the complex\(^\text{12}\). These changes are probably regulated by the \(\alpha\) C-terminus as hybrid tubulin had properties similar to tubulin\(^\text{32}\).

Using Scatchard analysis, most workers have reported that colchicine binds to native tubulin with a stoichiometry of approximately 1 mole per mole of the dimer\(^\text{51}\). Some reports, however, indicate two classes of colchicine-binding sites on tubulin; one being of high affinity and the other of very low affinity\(^\text{78-81}\). The presence, origin and implications of these two classes of colchicine-binding sites on tubulin are still not confirmed.

### Role of colchicine analogs to understand the mechanism of drug–protein interaction

In order to understand clearly the mechanism and various aspects of the tubulin–colchicine interaction, such as (1) specificity and necessity of different rings of colchicine in its binding to tubulin, (2) slow association and high activation energy, (3) reversibility and (4) thermodynamic properties of the binding reaction, several structural analogs of colchicine, that bind to the same site on tubulin, have been synthesized and their binding to tubulin studied in some detail.

Structure–activity studies indicate that the A and C rings of colchicine comprise the minimum structural features of the molecule required for its high affinity binding to tubulin. Insertion of a bulky group in the A ring of colchicine, as in colchicoside, causes complete loss of binding\(^\text{32}\). On the other hand, several changes in the C ring, such as different substitutions at the C10 position (e.g., thiocholichine) or a replacement of the seven-membered ring with a six-membered phenyl ester (e.g., allocolchicine) are tolerated\(^\text{83-87}\). However, replacement of the tropolone ring with a phenyl ring, as in colchino, causes total loss of binding ability\(^\text{51}\). Also, correct positioning of the carbonyl group in the C ring appears to be important as isocolchicine, which has altered positioning of the carbonyl and methoxy groups in ring C, binds to tubulin very feebly\(^\text{51}\). Single ring analogs of colchicine (A ring and C ring) also bind to tubulin specifically and reversibly although the standard free energy change of individual ring binding is lower than that of colchicine\(^\text{88}\).

Colchicine analogs modified at or even depleted of the B ring (structures shown in Figure 4) retain antimiotic and assembly inhibitory activity and bind as well to tubulin at the colchicine site. This apparently rules out any major role of the B ring in antimiotic activity or tubulin-binding property of colchicine\(^\text{67,71,89}\). Nevertheless, the presence of the B ring alone, or more specifically its C7 substituent significantly influences the binding mechanism, kinetics, association and dissociation rates, activation energy, reversibility, temperature dependence, thermodynamic properties of the binding reaction and also quantum yields of their complexes with tubulin\(^\text{67,71,83,90-94}\). These have been discussed below.

Colchicine analogs with progressively smaller or no substituent in the B ring bind to tubulin remarkably faster than colchicine. Colchicine and its analog, 2-methoxy-5-(2′,3′,4′-trimethoxyphenyl)tropane (AC; having no B ring) are two extreme drugs; while colchicine binding to tubulin is slow, requiring almost
90 min to attain completion at 37°C, AC binds tubulin even at 4°C and the binding is almost instantaneous at 37°C (refs 67, 89). The association rate constant of AC binding to tubulin has been calculated to be 187.2 × 10^6 M^-1 h^-1, while the dissociation rate constant is 216 h^-1 (ref. 71). The activation energy of the AC–tubulin interaction is significantly lower than that for the tubulin–colchicine reaction, only about 12–13 kcal/mol\(^7\). The rapid binding and low activation energy of AC binding to tubulin might be attributed to its structural simplicity compared to colchicine (absence of the B ring) and free rotation through the phenyl-tropone bond. The on rate and off rate of desacetylcolchicine (DAAC; having the B ring but no C7 substituent) binding with tubulin is 17 times slower than that of AC although the activation energies of both are similar\(^93\,94\). This is not unexpected as the B ring of colchicine immobilizes its A and C rings and hence hinders their free rotation. A significant drop in association rates and a significant enhancement in the activation energies occur when an NH\(_2\) group is present at the C7 position of the B ring (NH\(_2\)-DAAC; deacetylcolchicine\(^93\,94\). In fact, the activation energy of the tubulin–deacetylcolchicine interaction was found to be about 5 kcal/mol greater than that of the colchicine–tubulin association. However, substitution of further bulky groups as in the aminocolchicinoids (e.g. NHMe-DAAC; demecolcine/colcemid and NMe\(_2\)-DAAC; N-methyldecolcine) does not affect either the association rate or the activation energy\(^94\). These results suggest that not only the presence of the B ring itself, but also the nature of its C7 substituent affects significantly the reaction kinetics and the activation energy of binding. Also, electronic rather than steric properties of the B ring side chain appear to be of greater importance in the activation enthalpy of the aminocolchicinoids binding to tubulin.

Another important property of the colchicine–tubulin interaction that has been found to be related to the B ring is the reversibility of the reaction. Thus, while colchicine–tubulin interaction is essentially irreversible, AC–tubulin interaction is > 90% reversible\(^67\,69\). Compounds such as DAAC, NH\(_2\)-DAAC, NHMe-DAAC and NMe\(_2\)-DAAC are partially reversible, their dissociation rates being similar to each other and about 18–30 fold less than that of AC\(^93\,94\). Results of reversibility clearly indicate that it is the carbonyl group of the B ring substituent present, rather than the size of the substituent or the B ring itself that is responsible for the irreversibility of the colchicine–tubulin interaction. The dissociation rate of the colchicine analog, colchicine fluorescein, where the >C=O of the side chain was substituted by >C=S, was found to be similar to DAAC and the other aminocolchicinoids and much greater than colchicine\(^94\), thus supporting the above-mentioned hypothesis.

The B ring substituent influences significantly the thermodynamic parameters of the reaction as well. Both AC–tubulin and DAAC–tubulin interactions are characterized by a small positive entropy change and negative enthalpy of binding\(^71\,94\). The reactions are enthalpy-driven ones unlike colchicine–tubulin interaction which is entropy-driven\(^91\,95\). Progressive introduction of amine and alkyl amine substituents into the B ring results in a concomitant increase in entropy of the binding reaction\(^94\). Thus, the bare B ring itself has no significant effect on the thermodynamics of drug–tubulin interaction. Rather, it is the B ring substituent that converts an enthalpy-driven reaction (as in AC, DAAC) to an entropy-driven one as in colchicine.

The solvation properties of colchicine and its congeners are very much influenced by the side chain at the C7 position. Whereas colchicine is highly soluble in water, its analogs mentioned here are weakly soluble. The B ring substituent of colchicine and its analogs is also crucial for the subsequent fluorescent properties of their complexes with tubulin. The promotion of colchicine fluorescence upon binding tubulin has already been mentioned. Like colchicine, AC and DAAC exhibit marked fluorescence upon binding and have a quantum yield of approximately 0.02 (ref. 91). Other colchicine analogs where the B ring substituent is an acetylated amine have quantum yields comparable to colchicine and the fluorescence was not particularly sensitive to the bulk of the substituent, once the complex was formed. In contrast, the addition of free amine or alkyl amine (the aminocolchicinoids) substituent at the C7 position yields analogs that fluoresce very poorly or not at all when bound to tubulin\(^96\). This low fluorescence of the aminocolchicinoids bound to tubulin has been explained by exciplex formation between the lone electron pair of the nitrogen and the \(\pi\) system of the C ring which leads to fluorescence quenching of the latter\(^96\).

The colchicine binding site on tubulin

Many workers have tried to localize the colchicine-binding site on tubulin. Efforts to define the colchicine-binding site have generally involved induction of covalent interactions between tubulin and different affinity and photoaffinity labelled derivatives of colchicine. The results obtained were apparently contradictory to each other. Initial studies with bromoacetylcolchicine (label on side chain of B ring) indicated binding to \(\alpha\) tubulin\(^7\). Proteolytic studies with the colchicine-tubulin complex yielded colchicine bound peptides, having molecular weight of 16–18 kDa, belonging to \(\alpha\) tubulin\(^97\). Colchicine localization to the \(\alpha\) subunit has also been shown using photoaffinity label on colchicine with a long spacer arm\(^98\); however a shorter spacer arm led to colchicine binding to both \(\alpha\) and \(\beta\) tubulin\(^91\). Predominant labelling of \(\beta\) tubulin has been obtained with A ring derivatives, viz. chloroacetates of 2- or 3-demethyl-
thiocolchicine\textsuperscript{61}. Direct photoaffinity labelling with [\textsuperscript{3}H]colchicine has shown that the labelling occurs preferentially on \(\beta\) tubulin, with some labelling of the \(\alpha\) subunit at longer times, suggesting that the site is located near the \(\alpha/\beta\) interface\textsuperscript{100}. Analysis of these results shows that when the reactive group is in the side chain of the B ring of colchicine, the \(\alpha\) subunit is always labelled. In contrast, when the affinity group is on the A ring of colchicine, the labelled peptide belongs to \(\beta\). This suggests that the A ring is probably a part of the \(\beta\) subunit while the B ring substituent is close to \(\alpha\). Lujan and Roach suggested colchicine binding to \(\beta\) subunit on the basis of drug-induced inhibition of specific cross-linking of sulfhydryl groups on \(\beta\)\textsuperscript{101}. In yet another approach, several groups have shown that many mutations associated with colchicine resistance occur on \(\beta\) tubulin\textsuperscript{102,103}. Also, different isotypes of \(\beta\) tubulin in the A\(\beta\) dimer lead to different kinetics of colchicine binding\textsuperscript{104}. That colchicine binding occurs on the \(\beta\) subunit has also been shown directly in some recent reports. Photolabelling of tubulin monomers induced by lactoperoxidase with [\textsuperscript{3}H]colchicine showed that the bulk of radioactivity becomes attached to \(\beta\) tubulin\textsuperscript{105}. Colchicine-induced unfolding of tubulin is believed to occur in the carboxy terminal region of \(\beta\) tubulin, around Arg 390 (ref. 60). Recently it has been shown that Cys 354 of \(\beta\) tubulin is directly involved in colchicine binding\textsuperscript{106,107}.

A model of the tubulin–colchicine complex

Having established that colchicine and its analogs interact primarily with the \(\beta\) subunit, a pertinent question is where on the \(\beta\) subunit does the binding take place and what contribution, if any, does the \(\alpha\) subunit make to the binding process? A schematic model of the tubulin–colchicine complex (Figure 5), in conformity with most experimental observations, answers the question. It consists of the following characteristics. The colchicine site is on \(\beta\) tubulin, near the \(\alpha/\beta\) interface. It consists of two chemically different and independent subsites which serve as attachment points for colchicine, one for ring A and the other for ring C of the drug. It is believed that colchicine binds to tubulin, first through its tropolone ring by means of hydrogen bonding or ring stacking\textsuperscript{59,65}. This induces a structural change in tubulin that facilitates binding of the A ring through hydrophobic interactions with the protein. The B ring substituent resides outside the colchicine-binding site in the equilibrium complex. Recently it has been proposed that the B ring substituent faces \(\alpha\) tubulin and probably makes some kind of contact(s) with the protein, possibly with the \(\alpha\) subunit\textsuperscript{93,94,105,108,109}.

These contact(s) between the B ring substituent, protruding from \(\beta\) tubulin, and the protein are responsible for regulating the unusual properties of the tubulin–colchicine reaction, viz. slow association rate, high activation energy, poor reversibility and high entropy. Thus an analog with no side chain (e.g. DAAC) or no B ring (AC) and hence incapable of making contact(s) with the \(\alpha\) subunit, binds tubulin with properties dramatically different from colchicine. The \(\alpha\) subunit probably imposes constraints on the tubulin–colchicine interaction, making the entry and exit of the drug difficult. This contact(s) would cause a reorganization of water structure around the protein and the drug towards greater disorder of the water molecules compared to the isolated and individually hydrated species, thus providing a simple explanation for the observed high values of entropy accompanying the binding reaction. It also accounts for the \(\alpha\) subunit being labelled when the affinity label is in the side chain of colchicine, while \(\beta\) tubulin is labelled preferentially when the A ring carries the chemically reactive group.

Additional support for this model is provided by a recent study which describes colchicine binding to tubulin monomers\textsuperscript{110}. In the dissociated state of tubulin, the association rate of the tubulin–colchicine interaction increases 3–4 fold, the activation energy is lowered to 13 kcal/mol and the reversibility of the reaction is considerably increased. Moreover, the tubulin–colchicine reaction which is normally entropy-driven becomes enthalpy-driven when tubulin is in the monomeric form. The situation is thus analogous to DAAC or AC binding to tubulin dimers and confirms that the B ring substituent makes a contact with \(\alpha\) tubulin, that is responsible for the peculiar properties that characterize colchicine binding to tubulin. These properties are thus markedly different in cases where such contacts are not
possible, e.g. when colchicine binds to tubulin monomers or when the substituent is absent (AC or DAAC). The possibility of such regulatory contact(s) between the B ring substituent and the β subunit (that bears the colchicine site) is eliminated for in this case, the binding parameters would be independent of whether tubulin is in its dimeric or monomeric states.

The nature of these contacts is not clear although a possible involvement of the amino and carbonyl groups of the B ring side chain in forming hydrogen bonds with the surroundings, might be visualized. Which part of α tubulin participates in such contacts is also not clear. It is strongly believed that the B ring side chain makes contact with the C-terminus of α tubulin; that is why subtilisin cleavage of the α tail modifies the colchicine−tubulin interaction. Definite evidence in support of this hypothesis is, however, awaited.

RESEARCH ACCOUNT


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