Interaction Mechanism between Microtubule-Associated Proteins and Microtubules. A Proton Nuclear Magnetic Resonance Analysis on the Binding of Synthetic Peptide to Tubulin†

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ABSTRACT: An amino acid sequence essential for microtubule-associated proteins (MAPs) to bind to microtubules is presented [Aizawa et al. (1989) *J. Biol. Chem. 264*, 5885–5890]. A synthetic peptide of 23 amino acid residues which corresponded to the sequence [tubulin binding peptide (TBP)] was active in binding to tubulin and inducing its assembly. The TBP–tubulin interaction mechanism was analyzed by proton nuclear magnetic resonance spectroscopy as a simplified model for MAP–microtubule interactions. Intraresidue transferred nuclear Overhauser effects (TRNOEs) of TBP in TBP–tubulin mixtures were analyzed, and strong binding of two Val and two Lys residues of TBP to tubulin was detected. Among the sharply peaked signals from tubulin aromatic rsidues, those due to Tyr ring protons broadened upon mixing with TBP, suggesting the involvement of Tyr residue(s) in the binding with TBP. Irradiation of the tubulin Tyr protons resulted in an intermolecular TRNOE at TBP methyl proton resonances. Evidently, hydrophobic interactions between Val and Tyr residues are important for the binding of TBP to tubulin. Hydrophobic interactions have not been taken into account previously in the widely accepted electrostatic model for the binding of MAPs to microtubules.

Microtubules are present in a wide variety of cells and play diverse roles in cellular events. They consist of tubulin, a major component, and several accessory proteins generically termed microtubule-associated proteins (MAPs).[1] Because tubulin is a conserved protein among both species and tissues, the diverse functions of microtubules are attributed the diversity of MAPs (Vallee et al., 1984). Four classes of MAPs [MAP1, MAP2, τ, and MAP-U (200-kDa MAPs)] have been characterized (Wiche, 1989), and even though they have distinct characteristics from each other, they all possess an ability to stimulate tubulin polymerization and to bind microtubules.

Each MAP molecule consists of two parts, a microtubule binding domain and a projection domain (Wiche, 1989; Hirokawa et al., 1988). The isolation of microtubule binding chymotryptic fragments of τ and bovine MAP-U was previously reported (Aizawa et al., 1987, 1988), and a common amino acid sequence, termed assembly-promoting (AP) sequence, was identified in the two fragments (Aizawa et al., 1989). A synthetic peptide corresponding to the AP sequence was active in binding to the microtubules, and in inducing microtubule elongation. This AP sequence coincides with one of the triple-repeat motives of mouse τ (Lee et al., 1988). The mouse MAP2 sequence (Lewis et al., 1988) also contains a homologous portion. These studies suggest that the AP sequence is evolutionally conserved among MAPs and that it forms a tubulin binding site. Thus, it is implied that even though the four MAP classes are molecularly diverse, they can still bind to tubulin in a common mechanism.

Nuclear magnetic resonance (NMR) spectroscopy is a useful tool in obtaining structural information so as to analyze the binding mechanism of a macromolecule and a ligand. When part of the macromolecule is in close proximity to a bound ligand, a nuclear Overhauser effect (NOE) can be observed in the ligand if the macromolecule protons are irradiated (Balaram et al., 1972; James & Cohn, 1974; James, 1976). Transferred NOE (TRNOE) measurements give information on the conformation of the bound ligand (Albrand et al., 1979; Clore & Gronenborn, 1982, 1983; Behling et al., 1988). The TRNOE method has been applied to investigations on nucleotide–enzyme interaction (Cayley et al., 1979; Levy et al., 1983; Gronenborn et al., 1984a; Banerjee et al., 1985; Machida et al., 1985; Ferrin & Mildvan, 1985; Leanz & Hammes, 1986; Koide et al., 1989), peptide–phospholipid bilayer interaction (Wakamatsu et al., 1986, 1987; Milon et al., 1990), codon–anticodon interaction (Gronenborn et al., 1984b; Clore et al., 1984), and peptide–enzyme interaction (Clore et al., 1986; Meyer et al., 1988).

Unlike the ligand–macromolecule interactions, studies of interactions between large macromolecules are difficult due to the loss of resolution. To overcome this obstacle, synthetic peptides were used in antibody–antigen interaction studies (Anglister et al., 1988, 1989; Levy et al., 1989). A peptide has already been synthesized by the authors (Aizawa et al., 1989) which corresponds to an "active site" of MAPs [tubulin binding peptide (TBP)]; therefore, the tubulin–TBP interaction was analyzed by the TRNOE measurements in the paper in order to research the mechanisms of MAP–microtubule in-

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[1] Abbreviations: MAP, microtubule-associated protein; AP sequence, assembly-promoting sequence; TBP, tubulin binding peptide; NMR, nuclear magnetic resonance; NOE, nuclear Overhauser effect; TRNOE, transferred NOE; NOESY, two-dimensional NOE spectroscopy; ROESY, rotational frame NOE spectroscopy; 2D-HOHAHA, two-dimensional homonuclear Hartmann–Hahn spectroscopy; FID, free induction decay.

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teractions. Results indicating the participation of hydrophobic interaction modes were obtained.

Experimental Procedures

The TBP sequence used was H-Lys-Asn-Val-Arg-Ser-Lys-Val-Gly-Ser-Thr-Glu-Asn-Ile-Lys-His-Gln-Pro-Gly-Gly-Gly-Arg-Ala-Lys-OH, supplied by Applied Biosystems Japan, and was purified as described in Aizawa et al. (1989). The microtubule protein was obtained similar to Shelanski et al. (1973) with some modifications incorporated (Kotani et al., 1984). The tubulin was purified by the method of Weingarten et al. (1975).

For the ^1H NMR measurements in ^2H_2O, TBP in buffer [20 mM phosphate buffer (pH 7.0) containing 100 mM KCl, 0.1 mM $MgCl_2$, and 0.1 mM GTP] was evaporated and then dissolved in the original volume of ^2H_2O (99.85% ^2H). The solution was evaporated a second time and dissolved in the original volume of ^2H_2O (100% ^2H). For the ^1H NMR measurements in H_2O, TBP in the buffer was evaporated and dissolved in the original volume of 10% ^2H_2O, and the pH value of the solution was adjusted to pH 3.1 with HCl. To prepare a tubulin solution for NMR measurements, a deuterated buffer (1.5 mL) was added to the tubulin solution, and the solution was concentrated to 0.3 mL using a Centricon 30 microconcentrator. This procedure was repeated 5 times.

A 400-MHz proton NMR spectrum (8K data points at a spectral width of 6.0/6.5 kHz) was recorded on a Bruker AM-400 spectrometer at a probe temperature of 25 °C (unless otherwise stated). Chemical shifts of proton resonances were measured relative to that of the proton resonance of internal 2,2-dimethyl-2-silapentane-5-sulfonate. In the H_2O solution, the solvent signal was suppressed by 0.8 s of presaturation.

For the two-dimensional experiments, 512 free induction decays (FID's) of 2K data points were collected by using a time proportional phase increment (Ernst et al., 1987), and spectra of 1K × 2K were obtained with zero-filling, after which a two-dimensional Fourier transformation with squared sine-bell window functions for both dimensions was performed. The mixing time for the two-dimensional homonuclear Hartmann–Hahn spectroscopy (2D-HOHAHA) measurement (Davis & Bax, 1985; Bax & Davis, 1985b; Ernst, 1988) was 54 mn, and that for the rotating-frame NOE spectroscopy (ROESY) measurement (Bothner-By et al., 1984; Bax & Davis, 1985a) and two-dimensional NOE spectroscopy (NOESY) measurement (Ernst et al., 1987) was 200 ms.

The MLEV-17 pulse sequence (Bax & Davis, 1985) was used for the spin-echo experiment with a spin-echo time of 10 ms. Spin diffusion (Bother-By, 1979; Jardetzky & Roberts, 1981) was measured with irradiations at 4.78 ppm for 1.0 s, using a decoupler power of 2k dB below 0.2 W. TRNOE action spectra (Clore & Gronenborn, 1983) of γ-methyl proton signals of TBP Val residues were obtained with selective irradiation (38 dB below 0.2 W for 0.5 s) between 7.05 and 6.50 ppm at intervals of 0.05 ppm.

Results

Assignments of the TBP Proton Resonance. To obtain the sequence-specific resonance assignment (Wüthrich, 1983), TBP ^1H NMR spectra were observed for a H_2O solution at pH 3.1. The resonances of the side chain protons were assigned with reference to the intrinsic chemical shifts of amino acid residues (Bundi & Wüthrich, 1979). The spin system of each amino acid residue was identified by a 2D-HOHAHA measurement, and then each spin system was connected by a ROESY measurement. Examples of these spectra are presented in Figures 1 and 2 (showing the assignments of four residues), and the assignments of all signals at pH 3.1 are summarized in Table I. The resonances of protons, except for the amido ones in ^2H_2O at pH 6.8, were assigned as shown in Table II, by comparing the 2D-HOHAHA spectra at pH 6.8 with those at pH 3.1. Chemical shifts both of the Glu-11 and Lys-23 carboxyl protons and of the His-15 aromatic protons were different at pH 3.1 and 6.8 due to the differences in their protonation state. Chemical shifts of the other protons were the same for these two pH conditions. The proton resonances of Arg-4 and Arg-2 residues could not be discriminated. No measurable higher order structures in free TBP were found.

NMR Spectra of the Tubulin–TBP Mixture. In solution, tubulin molecules exist in an equilibrium between polymerized and unpolymerized forms. In the present study, a solution

Table I: Assignments of TBP Proton NMR Signals in H_2O (pH 3.1, Meter Reading)

amino acid	NH	αH	βH	γH and other H
Lys-1		4.04	1.90	1.43, δH 1.70, εH 3.0
Asn-2	8.91	ND[a]	2.82, 2.76	
Val-3	8.38	4.16	2.10	γH 0.93
Arg-4	8.45	4.36	1.83, 1.77	γH 1.65, δH 3.19
Ser-5	8.32	4.42	3.85	
Lys-6	8.46	4.36	1.76	γH 1.43, δH 1.61, εH 3.0
Val-7	8.18	4.10	2.06	γH 0.94
Gly-8	8.51	4.00		
Ser-9	8.25	4.54	3.93, 3.88	
Thr-10	8.28	4.37	4.28	γH 1.20
Glu-11	8.31	4.35	2.09, 1.97	γH 2.44
Asn-12	8.47	ND	2.81, 2.74	
Ile-13	8.02	4.11	1.83	γH 1.41, 1.16; γH_3 0.80; δH 0.83
Lys-14	8.33	4.25	1.73	γH 1.45, δH 1.72, εH 3.0
His-15	8.49	4.69	3.23, 3.16	H2 8.63, H4 7.29
Gln-16	8.51	4.62	2.10, 1.91	γH 2.37
Pro-17		4.43	3.79, 3.67	γH 2.06, 1.97; δH 2.30
Gly-18	8.58	4.00		
Gly-19	8.34	3.99		
Gly-20	8.35	3.96		
Arg-21	8.17	4.34	1.83, 1.77	γH H 1.66, δH 3.19
Ala-22	8.37	4.29	1.99	
Lys-23	8.22	4.24	1.87	γH 1.43, δH 1.76, εH 3.0

[a] Not determined.

Table II: Assignments of TBP Proton NMR Signals in ^2H_2O (pH 6.8)

amino acid	αH	βH	γH and other H
Lys-1	4.01	1.90	γH 1.44, δH 1.71, εH 2.99
Asn-2	4.93	2.83, 2.77	
Val-3	4.16	2.10	γH 0.94
Arg-4	4.37	1.88, 1.78	γH 1.66, δH 3.20
Ser-5	4.42	3.85	
Lys-6	4.37	1.7	γH 1.44, δH 1.68, εH 3.00
Val-7	4.10	2.06	γH 0.95
Gly-8	4.00		
Ser-9	4.54	3.97, 3.89	
Thr-10	4.37	4.30	γH 1.20
Glu-11	4.27	2.03, 1.94	γH 2.24
Asn-12	4.69	2.82, 2.74	
Ile-13	4.11	1.85	γH 1.43, 1.17; γH_3 0.83; δH 0.85
Lys-14	4.27	1.73	γH 1.37, δH 1.68, εH 2.98
His-15	4.66	3.18, 3.12	H2 8.32, H4 7.18
Gln-16	4.63	2.08, 1.91	γH 2.37
Pro-17	4.44	3.77, 3.69	γH 2.05, 1.98; δH 2.32
Gly-17	4.00		
Gly-19	4.00		
Gly-20	3.95		
Arg-21	4.37	1.88, 1.78	γH 1.66, δH 3.20
Ala-22	4.31	1.40	
Lys-23	4.12	1.84	γH 1.41, δH 1.69, εH 3.00

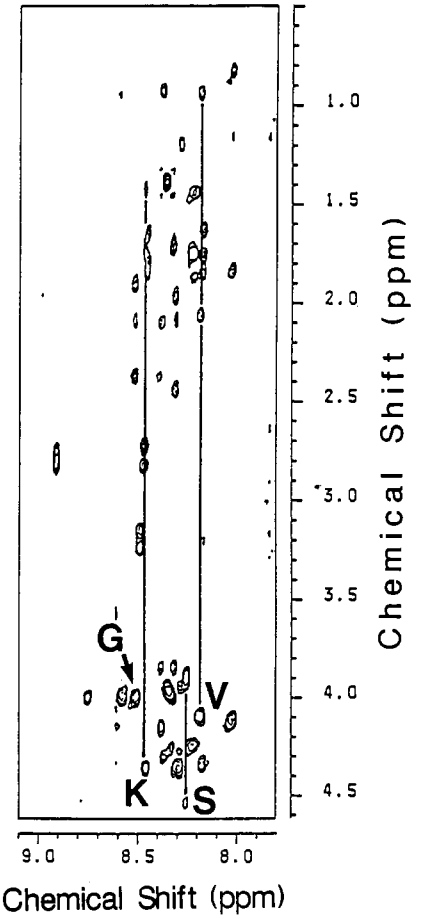

FIGURE 1: Spin systems of Gly, Lys, Val, and Ser of TBP (0.06 mM) as revealed by 2D-HOHAHA experiments. Cross-peaks derived from each residue were connected by solid lines. Gly has a single cross-peak because the two methylene proton signals could not be separated. The spectral width was 6500 Hz, and 512 × 2K FID's were accumulated 64 times. A $\pi/4$-shifted squared sine-bell window function was used for the F2 dimension, and a $\pi/8$-shifted squared sine-bell window function was used for the F1 dimension. A spectrum of 1K × 2K was obtained by zero-filling before Fourier transformation.

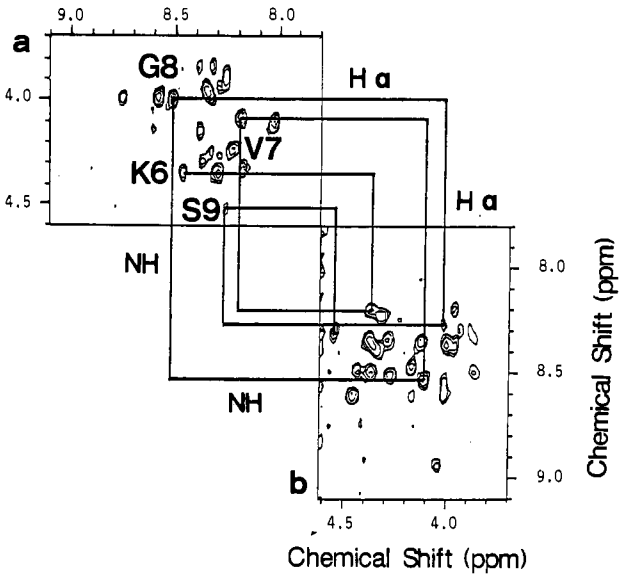

FIGURE 2: Connection of spin systems shown in Figure 1 by the 2D-HOHAHA spectrum (a) and the ROESY spectrum (b). (a) shows NH–Hα cross-peaks of the same residues, and (b) shows cross-peaks between Hα and the next (C-terminal side) NH. Starting from Lys, the order of Lys-Val-Gly-Ser is revealed. By comparison of this order with a known primary structure, these signals were respectively assigned to Lys-6, Val-7, Gly-8, and Ser-9. The 2D-HOHAHA spectrum was recorded and manipulated as in Figure 1. The ROESY spectrum was recorded and manipulated similar to the 2D-HOHAHA, except for the accumulation times (48 times).

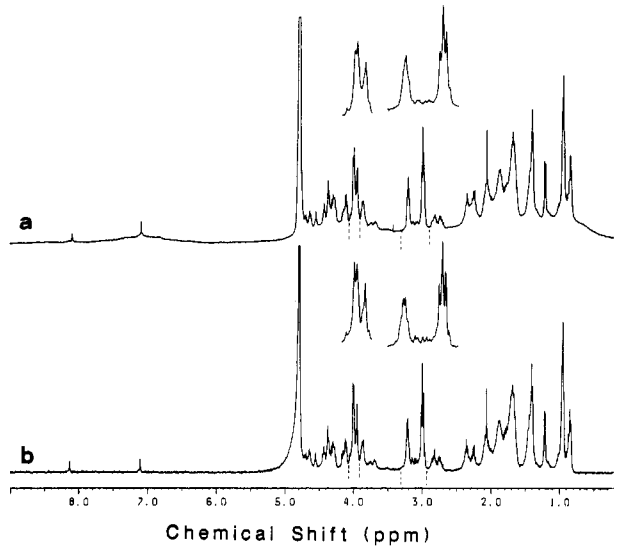

FIGURE 3: ^{1}H NMR spectra of TBP with tubulin (a) and without tubulin (b). TBP concentration was 0.1 mM for (a) and 0.6 mM for (b). Tubulin concentration was 1.0 mg/mL. Broadening of Gly peaks (around 4.0 ppm) and Lys peaks (around 3.0 ppm) is evident when the expanded spectra are compared. A total of 400 FID's for (a) and 16 for (b) were accumulated. Broadening of 1 Hz was applied. Spectral width was 6000 Hz.

condition was chosen which left most of the tubulin molecules protomeric (i.e., in an unpolymerized form).[2] After TBP is mixed with tubulin, some of the TBP binds to tubulin as seen by the broadening of serveral TBP-derived peaks (Figure 3a). The K_d value of TBP–tubulin binding (0.18 mM; Aizawa et al., 1989) suggests that the chemical exchange of TBP between the free state and the tubulin-bound state is fast. Positive proof for this was obtained by performing temperature-dependent measurements (Figure 4). TBP-derived peaks in the mixture are sharper for higher temperature (Figure 4a) and broader for lower temperature (Figure 4c), indicating that the exchange is fast on the NMR chemical shift scale (Kohda et al., 1987).

Tubulin is a labile protein at room temperature, and ^2H_2O affects its assembly kinetics (Dustin, 1984). In these reports, Woody et al. (1983) carried out detailed experiments for NMR measurements of tubulin and provided evidence that tubulin retains its original property during the course of NMR measurements. The stability and the assembly competency of tubulin was also tested in this study using dark-field light microscopy, i.e., after each series of NMR measurements, the sample was observed to confirm that it was free from amorphous aggregates derived from denatured tubulin. Taxol was then added to the sample, and it was again observed to check the microtubule formation.

Detection of TBP Amino Acid Residues Involved in the Interaction with Tubulin. Amino acid residues which are

[2] At conditions favorable for tubulin polymerization, the dynamic properties of tubulin and microtubules (e.g., tubulin–tubulin interaction, GTP hydrolysis accompanied by tubulin polymerization, and treadmilling and dynamic instability of microtubules) can complicate the results; thus, a phosphate buffer was used which is unfavorable for microtubule assembly. Low concentrations of GTP, $MgCl_2$, tubulin, and peptide, and a temperature of 25 °G, were also used because these conditions are also unfavorable for assembly. Previous studies have indicated that MAPs specifically bind to protomeric tubulin, or proteolytic fragments of tubulin, as well as to microtubules (Serrano et al., 1984a; Littauer et al., 1985); thus, it is considered that a specific tubulin–TBP interaction can be analyzed with the presented method.

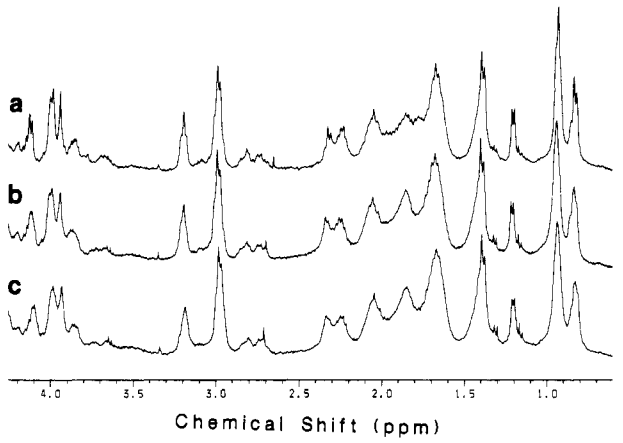

FIGURE 4: Temperature dependence of the ^{1}H NMR spectra of TBP with tubulin. A total of 1000 FID's were accumulated under the same conditions as in Figure 3a, except for the temperature: 40 °C (a); 25 °C (b); 18 °C (c). Broadening of 0.5 Hz was applied before Fourier transformation.

involved in an interaction will show intraresidue TRNOEs among side chain protons, as previously shown by the binding of yeast α-mating factor to membranes (Wakamatsu et al., 1986). After TBP was mixed with tubulin, strong intraresidue TRNOE cross-peaks emerged for proton resonances of Val-3 and Val-7 side chains (Figure 5a) and weak ones for those of Ile-13, Lys-1, Lys-14, Arg(s), and Pro-17. When alone, TBP showed no significant cross-peaks on an NOESY spectrum (Figure 5b); thus, the conformation rigidation of these residues occurred upon mixing with tubulin, suggesting that they are in close molecular contact with tubulin. Other residues, which did not show intraresidue TRNOEs, are concluded to be flexible and do not participate in the interaction. The involvement of Lys residues in the interaction is consistent with the electrostatic model for MAP–microtubule interaction, whereas the involvement of Val and Ile residues was not predicted. This suggests that the hydrophobic interactions are also important in the binding of TBP to tubulin.

Detection of Tubulin Amino Acid Residues Involved in the Interactions with TBP. In order to confirm the significant finding of the participation of hydrophobic interactions in MAP–microtubule binding, the counter residue(s) of tubulin was(were) determined. Although NOESY measurement is an excellent method to detect intermolecular TRNOE, a high concentration of tubulin is needed. Since the tubulin concentration should be kept low, a TRNOE action spectrum (Clore & Gronenborn, 1983) was used.

Residues which are involved in the interaction with aliphatic residues are normally hydrophobic (aromatic or aliphatic) and situated on the surface of a molecule. Mobile tubulin aromatic protons were detected by spin diffusion and spin-echo techniques (Figure 6), because there are few TBP-derived signals in the aromatic region of the tubulin–TBP spectra. A peak at 6.8 ppm, located among several other sharp peaks caused by aromatic ring protons of mobile residues, broadened after mixing with TBP (see arrows in Figure 6a,c). A TRNOE action spectrum of methyl proton signals of the TBP Val residues was obtained by irradiating the tubulin aromatic protons for 0.5 s (Figure 7), and a single peak was observed at the same chemical shift (6.8 ppm) as the broadened peak in Figure 6. A similar TRNOE action spectra profile was obtained even though a shorter irradiation time was used (0.1 s, data not shown), indicating that this is a direct NOE and not mediated by other protons. The observed intermolecular NOE is as large as the Val Intraresidue NOE. This significant NOE can only be explained by the close contact between the Val methyl group and the aromatic group. Judging from the chemical shift, the 6.8 ppm peak derived from the exposed mobile proton of tubulin is most likely due to the aromatic ring protons of the Tyr residue (Bundi & Wüthrich, 1979).

Discussion

MAPs usually have a large basic domain, so that acidic macromolecules such as DNA and RNA can bind to MAPs and inactivate them, inhibiting microtubule assembly. Tubulin, on the other hand, is an acidic protein, and basic macromolecules such as histone, polylysine, etc. bind to it to induce its aggregation and/or polymerization (Dustin, 1984). As a result, it was considered that MAPs electrostatically bind to microtubules. This type of interaction may be suspect because

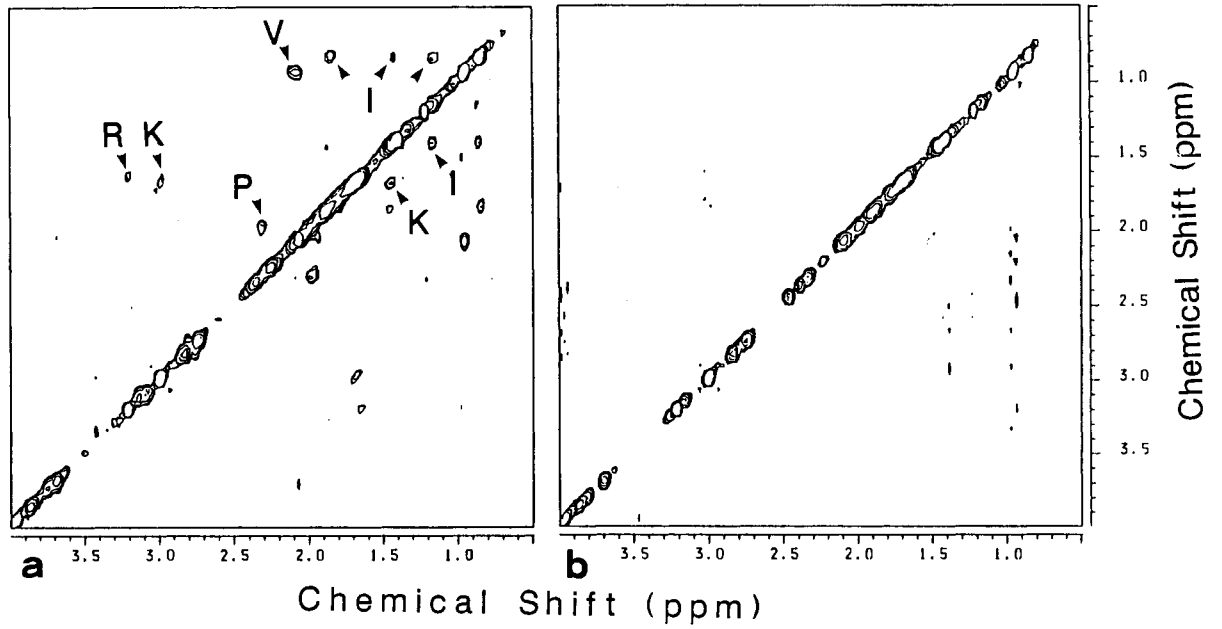

FIGURE 5: NOESY spectra of TBP with tubulin (a) and without tubulin (b). The concentration of TBP and tubulin is the same as in Figure 3a,b. The spectral width was 6500 Hz. FID's (512 × 2K) were accumulated 96 times for (a) and 48 times for (b). A $\pi/4$-shifted squared sine-bell window function was used for the F2 dimension, and a $\pi/8$-shifted squared sine-bell window function was used for the F1 dimension. A spectrum of 1K × 2K was obtained by zero-filling before Fourier transformation.

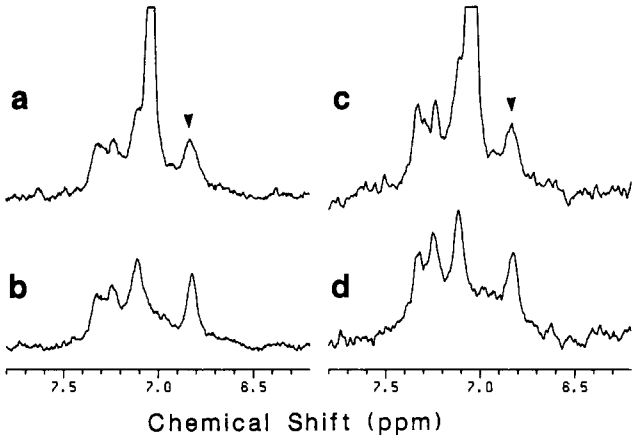

FIGURE 6: Spin-echo (a and b) and spin diffusion (c and d) spectra of tubulin with TBP (a and c) and without TBP (b and d). A total of 4000 FID's were accumulated for each spectrum. Broadening of 5 Hz was applied for each FID before Fourier transformation. A portion of the spectrum which shows peaks due to the aromatic protons (6.1–7.4 ppm) is shown.

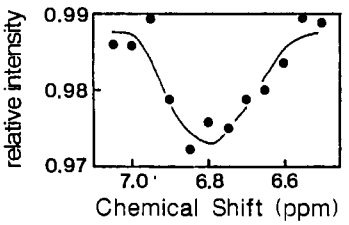

FIGURE 7: TRNOE action spectrum of methyl proton signals of TBP valine residues in the presence of tubulin. Aromatic protons of tubulin were irradiated. The relative intensity of the methyl proton signals of TBP Val residues was measured for each irradiation experiment.

of the potentiality of a variety of nonspecific substances to induce tubulin polymerization (Timasheff, 1979), and though a more detailed physicochemical analysis is required for the understanding of this binding mechanism, interaction studies could not be furthered because tubulin and MAP molecules are too large to be measured by conventional methods for physicochemical analyses.

Previously, an "active site" of MAPs in the restricted region of 23 amino acid residues was found (Aizawa et al., 1988), making it possible to apply physicochemical techniques to analyze the MAP–microtubule interaction mechanism. In the present paper, TBP–tubulin interactions were used as a "model system" to study MAP–microtubule interactions. Although the binding could not be measured by using the NOESY difference spectroscopy method (Anglister et al., 1989), because of the low tubulin concentration, several novel findings were obtained by combining NOESY and TRNOE action spectrum measurements. The most important result is the detection of hydrophobic interactions between TBP and tubulin. Using the assumption that MAPs and tubulin interact electrostatically, Serrano et al. (1984a,b) proposed a charge neutralization mechanism that involved charge neutralization of an acidic tubulin molecule by a basic MAP region to induce tubulin polymerization, yet hydrophobic interactions have not yet been considered.

The electrostatic interaction between TBP and tubulin was in fact detected; i.e., among the six basic residues of TBP, three residues (two Lys and one Arg) are found to interact with tubulin. However, the tightest binding was observed for the TBP residues Val-3 and Val-7. The counterresidue of tubulin was suspected to be Tyr; therefore, MAPs do not interact with tubulin by simple charge neutralization but by a specific mechanism that includes both electrostatic and hydrophobic interactions. Lys and Arg residues also have, in addition to a positive charge, a hydrophobic part in their side chains, and the hydrophobic environment constructed by these side chains possibly serves to determine the electrostatic binding site between MAPs and tubulin and to strengthen the binding.

Using previous studies, Tyr residue(s) candidate for interactions with TBP can be elucidated. Limited proteolysis experiments indicated that tubulin binds to MAPs through its C-terminal 5-kDa domain (Serrano et al., 1984a, 1985), and recently it as also found that the C-terminal end portion amino acid residues are not needed for MAP binding (Fridovich-Keil et al., 1987; Maccioni et al., 1988; Vera et al., 1988, 1989; Kats et al., 1988; Matsuzaki et al., 1988). On the basis of these findings, several regions of the tubulin molecule have been proposed as a binding site for MAPs. A single region of β-tubulin [amino acid residues 422–434 in Maccioni et al. (1988) and Vera et al. (1988)] is considered the most promising candidate to interact with TBP because TBP binds to tubulin dimmer at a molar ratio of 1 (Aizawa et al., 1989). Subsequently, Tyr-422 and/or Tyr-425 are possibly the key residues for MAP–tubulin interactions.

Polycations induce tubulin aggregation and/or polymerization, and also, proteolytic elimination of the C-terminal region of tubulin is apparently sufficient for the polymerization without MAPs (Serrano et al., 1984b, 1985; Sackett et al., 1985). These reports, however, also state that the presented assembly systems resulted in the formation of many nonmicrotubular polymers such as sheets, spirals, and double-walled microtubules. These results are interpreted as follows: The C-terminal acidic region of tubulin is a structural hindrance for assembly, so the elimination, as well as nonspecific binding of polycations to this region, promotes interactions between tubulin molecules. This results in the formation of polymers, some of which look like microtubules. However, a specific conformational change, induced by specific MAP binding, is still necessary for tubulin to efficiently assemble into a normal microtubule. This interpretation agrees with references showing that the positive charge by itself is inadequate to induce normal microtubule formation (Kuznetsov et al., 1978; Dustin, 1984).

To further research MAP–tubulin interaction mechanisms, NMR studies are in progress which include conformational analysis of the bound TBP, analysis of the interaction of tubulin with point-mutated TBP, and analysis of TBP–microtubule interactions.

Registry No. TBP, 121310-57-4; L-Val, 72-18-4; L-Tyr, 60-18-4.

REFERENCES

Aizawa, H., Murofushi, H., Kotani, S., Hisanaga, S., Hirokawa, N., & Sakai, H. (1987) *J. Biol. Chem. 262*, 3782–3787.

Aizawa, H., Kawasaki, H., Murofushi, H., Kotani, S., Suzuki, K., & Sakai, H. (1988) *J. Biol. Chem. 263*, 7703–7707.

Aizawa, H., Kawasaki, H., Murofushi, H., Kotani, S., Suzuki, K., & Sakai, H. (1989) *J. Biol. Chem. 264*, 5885–5890.

Albrand, J. P., Birdsall, B., Feeny, J., Roberts, G. C. K., & Burgen, A. S. V. (1979) *Int. J. Biol. Macromol. 1*, 37–41.

Anglister, J., Jacob, C. O., Assulin, O., Ast, G., Pinker, R., & Arnon, R. (1988) *Biochemistry 27*, 717–724.

Anglister, J., Levy, R., & Scherf, T. (1989) *Biochemistry 28*, 3360–3365.

Balaram, P., Bothner-By, A. A., & Dadok, J. (1972) *J. Am. Chem. Soc. 94*, 4015–4017.

Banerjee, A., Levy, H. R., Levy, G. C., & Chan, W. W.-C. (1985) *Biochemistry 24*, 1593–1598.

Bax, A., & Davis, D. G. (1985a) *J. Magn. Reson. 63*, 207–213.

Bax, A., & Davis, D. G. (1985b) *J. Magn. Reson. 65*, 355–360.
Behling, R. W., Yamane, T., Navon, G., & Jelinski, L. W. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 6721–6725.
Bothner-By, A. A., Stephens, R. L., Lee, J.-M., Warren, C. D., & Jeanloz, R. W. (1984) *J. Am. Chem. Soc. 106*, 811–813.
Bundi, A., & Wüthrich, K. (1979) *Biopolymers 18*, 285–298.
Cayley, P. J., Albrand, J. P., Freeney, J., Roberts, G. C. K., Piper, E. A., & Burgen, A. S. V. (1979) *Biochemistry 18*, 3886–3895.
Clore, G. M., & Gronenborn, A. M. (1982) *J. Magn. Reson. 48*, 402–417.
Clore, G. M., & Gronenborn, A. M. (1983) *J. Magn. Reson. 53*, 423–442.
Clore, G. M., Gronenborn, A. M., & McLaughlin, L. W. (1984) *J. Mol. Biol. 174*, 163–173.
Clore, G. M., Gronenborn, A. M., Carlson, G., & Meyer, E. F. (1986) *J. Mol. Biol. 190*, 259–267.
Davis, D. G., & Bax, A. (1985) *J. Am. Chem. Soc. 107*, 2820–2821.
Ennulat, D. J., Liem, R. K. H., Hashim, G. A., & Shelanski, M. L. (1989) *J. Biol. Chem. 264*, 5327–5330.
Ernst, R. R. (1988) XIII International Conference on Magnetic Resonance in Biological Systems, Madison, WI.
Ernst, R. R., Bodenhausen, G., & Wokaun, A. (1987) *Principles of Nuclear Magnetic Resonance in One and Two Dimensions*, Clarendon Press, Oxford.
Ferrin, L. J., & Mildvan, A. S. (1985) *Biochemistry 24*, 6904–6913.
Fridovich-Keil, J. L., Bond, J. S., & Solomon, F. C. (1987) *Mol. Cell. Biol. 7*, 3792–3798.
Gronenborn, A. M., Clore, G. M., Hobbs, L., & Jeffery, J. (1984a) *Eur. J. Biochem. 145*, 365–371.
Gronenborn, A. M., Clore, G. M., McLaughlin, L. W., Graeser, E., Lorber, B., & Giege, R. (1984b) *Eur. J. Biochem. 145*, 359–364.
Hirokawa, N., Shiomura, Y., & Okabe, S. (1988) *J. Cell Biol. 107*, 1449–14559.
James, T. L. (1976) *Biochemistry 15*, 4724–4730.
James, T. L., & Cohn, M. (1974) *J. Biol. Chem. 249*, 2599–2604.
Kats, W. S., & Solomon, F. (1988) *Mol. Cell. Biol. 8*, 2730–2736.
Kohda, D., Kawai, G., Yokoyama, S., Kawakami, M., Mizushima, S., & Miyazawa, T. (1987) *Biochemistry 26*, 6531–6538.
Koide, H., Yokoyama, S., Matsuzawa, H., Miyazawa, T., & Ohta, T. (1989) *J. Biol. Chem. 264*, 8676–8679.
Kotani, S., Murofushi, H., Nishida, E., & Sakai, H. (1984) *J. Biochem. 96*, 959–969.
Kuznetsov, S. A., Gelfand, V. I., Rodionov, V. I., Rosenblat, V. A., & Gulyaeva, J. G. (1978) *FEBS Lett. 95*, 3443–3446.
Leanz, G. F., & Hammes, G. G. (1986) *Biochemistry 25*, 5617–5624.
Lee, G., Cowan, N., & Kirschner, M. (1988) *Science 239*, 285–288.
Levy, H. R., Ejchart, A., & Levy, G. C. (1983) *Biochemistry 22*, 2792–2796.
Levy, R., Assulin, O., Scherf, T., Levitt, M., & Anglister, J. (1989) *Biochemistry 28*, 7168–7175.
Lewis, S. A., Wang, S., & Cowan, N. J. (1988) *Science 242*, 936–939.
Maccioni, R. B., Serrano, L., Avila, J., & Cann, J. R. (1986) *Eur. J. Biochem. 156*, 375–381.
Maccioni, R. B., Rivas, C. I., & Vera, J. C. (1988) *EMBO J. 7*, 1957–1963.
Machida, M., Yokoyama, S., Matsuzawa, H., Miyazawa, T., & Ohta, T. (1985) *J. Biol. Chem. 260*, 16143–16147.
Matsuzaki, F., Matsumoto, S., & Yahara, I. (1988) *J. Cell Biol. 107*, 1427–1435.
Meyer, E. F., Clore, G. M., Glonenborn, A. M., & Hansen, H. A. S. (1988) *Biochemistry 27*, 725–730.
Milon, A., Miyazawa, T., & Higashijima, T. (1990) *Biochemistry 29*, 65–75.
Sackett, D., Bhattacharyya, B., & Wolff, J. (1985) *J. Biol. Chem. 260*, 43–45.
Serrano, L., Avila, J., & Maccioni, R. B. (1984a) *Biochemistry 23*, 4675–4681.
Serrano, L., De La Torre, J., Maccioni, R. B., & Avila, J. (1984b) *Proc. Natl. Acad. Sci. U.S.A. 81*, 5989–5993.
Serrano, L., Montejo De Garcini, E., Hernández, M. A., & Avila, J. (1985) *Eur. J. Biochem. 153*, 595–600.
Shelanski, M. L., Gaskin, F., & Cantor, C. R. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70*, 765–768.
Timasheff, S. N. (1979) *Trends Biochem. Sci. (Pers. Ed.) 4*, 61–65.
Vallee, R. B., Bloom, G. S., & Luca, F. C. (1984) in *Molecular Biology of the Cytoskeleton* (Borisy, G. G., Cleveland, D. W., & Murphy, D. B., Eds.) pp 111–130, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
Vera, J. C., Rivas, C. I., & Maccioni, R. B. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 6763–6767.
Vera, J. C., Rivas, C. I., & Maccioni, R. B. (1989) *Biochemistry 28*, 333–339.
Wakamatsu, K., Okada, A., Suzuki, M., Higashijima, T., Masui, Y., Sakakibara, S., & Miyazawa, T. (1986) *Eur. J. Biochem. 154*, 607–615.
Wakamatsu, K., Okada, A., Miyazawa, T., Masui, Y., Sakakibara, S., & Higashijima, T. (1987) *Eur. J. Biochem. 163*, 331–338.
Weingarten, M. D., Lockwood, A. H., Hwo, S. Y., & Kirschner, M. W. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 1858–1862.
Wiche, G. (1989) *Biochem. J. 259*, 1–12.
Wüthrich, K. (1983) *Biopolymers 22*, 131–138.