Inhibition of Tubulin Guanosine-5′-triphosphatase by Lipid Peroxides: Protective Effects of Vitamin A Derivatives

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ABSTRACT: Lipid peroxidation of cellular proteins has been postulated to be involved in cellular aging. Given the importance of the cytoskeleton in cellular function, it is a prime candidate as a potential target for the deleterious effects of lipid peroxides. In this study, the effects of lipid peroxides on microtubule assembly have been studied in an *in vitro* assay system, as have the protective effects of the vitamin A group (β-carotene, retinal, and retinol). The assay was based on tubulin guanosine-5′-triphosphatase (GTPase) activity, which is associated with all steps of microtubule assembly. Soybean lecithin was utilized as the starting point to generate lipid peroxides. Its selection was based on the high proportion of phospholipids in the cellular membrane. Lipid peroxides were generated by photooxidation of lecithin, dissolved in methanol, in the presence of 0.004% methylene blue at 4°C for 8 h. Lipid peroxides(1.0 mg/mL) inhibited tubulin GTPase by 49%, relative to the control. Vitamin A derivatives (retinol, retinal, and β-carotene) all had the ability to protect against the inhibitory effects of lipid peroxides, presumably owing to their antioxidant activities. This protective effect was more pronounced when utilizing a 30-min, as opposed to a 15-min, reaction time. This suggests a relatively slow rate of reaction between the peroxide and vitamin A group. These studies present a mechanism for the ability of vitamin A to inhibit aging of the cell.
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KEY WORDS: β-carotene, GTPase activity, phospholipid peroxides, retinal, retinol, tubulin.

Lipid peroxides from foods interact with proteins, such as enzymes, in a variety of biological systems, including those involved in lower hepato functions (1). Lipid peroxidation is involved in the pathogenesis of both atherogenesis and myocardial reperfusion injury (2–5). The mechanism of lipid peroxide formation is thought to be due to the peroxidation of unsaturated lipids that is induced by active oxygen radicals, which are generated in a variety of cellular systems (6). The effect of the reaction of lipid peroxides with differing functional proteins on cellular functions is yet to be clarified.

It has been postulated that the chemical modification of proteins upon reaction with lipid peroxides results in the deterioration of cellular function or "aging" of the cell. Which proteins are modified, and which are critical to this process are the subject of intense study. The cytoskeletal proteins are prime candidates as critical targets in this aging process owing to their major role in a variety of cellular processes. In this study, we have investigated the influence of lipid peroxidation on tubulin function, tubulin being the major component of microtubules.

Tubulin is widely distributed in eucaryotic cells, and the specific self-assembly of tubulin results in microtubule formation. The elegant work of Lee *et al.* (7) demonstrated that tubulin can form microtubules *in vitro* in the absence of any microtubule-associated protein. The formed microtubules are involved in many cellular functions, including maintenance of cellular anisometry (8). The interference with tubulin functions as a result of chemical modification *via* lipid peroxidation would be predicted to lead to drastic deterioration of cellular functions.

Tubulin is a dimeric guanosine-5′-triphosphate (GTP)-binding protein that consists of two almost identical molecules, α- and β-tubulin (9–11). It has been postulated that the pathway of microtubule assembly from tubulin proceeds *via* closed cyclic oligomers, followed by microtubule formation (12–14). Tubulin-bound GTP is hydrolyzed to guanosine-5′-diphosphate (GDP) upon formation of microtubules (12). The self-assembly of tubulin into higher structures requires the presence of the divalent metal ion, Mg^{2+} (15,16). The nature of the higher polymer is critically dependent on the concentration of Mg^{2+}; if the concentration of magnesium is kept low, tubulin self-assembly stops at the step of oligomer formation. We have demonstrated previously the presence of tubulin GTPase activity in the presence of low concentrations of magnesium ion, the assay being based on the determination of GDP concentrations by high-performance liquid chromatography (HPLC) (17,18). From these studies it is clear

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that tubulin GTPase is already activated in the step of oligomer formation that precedes microtubule formation (19). This emphasizes that the GTPase of tubulin is active not only in steady state after microtubule assembly but also in all steps of microtubule assembly. In this study, we have investigated the effect of lipid peroxides on *in vitro* microtubule assembly as monitored by their effects on tubulin GTPase activity under conditions that induce microtubule assembly. The effect of the antioxidants in protecting against the effect of lipid peroxidation was also studied by coincubating in the presence of vitamin A (β-carotene, retinal, and retinol). This study is the first report of the effect of antioxidant to restore tubulin GTPase activity as a consequence of exposure to lipid peroxides.

MATERIALS AND METHODS

Tubulin. Tubulin was prepared from bovine brain by the modified Weisenberg procedure as previously described (20–22). Bovine brain was obtained from a freshly slaughtered animal at the Hanshin Meat Hygienic Laboratory (Nishinomiya, Japan), kept on ice, and used within 30 min of slaughter.

Protein aliquots were stored at –80°C in 10 mM sodium phosphate buffer, pH 7.0, that contained 0.1 mM GTP, 0.5 mM $MgCl_2$, and 1 M sucrose. Prior to each experiment, the bulk of sucrose was removed from the tubulin solution by a Sephadex G-25 batch procedure (22). The resulting protein solution was cleared of aggregates by centrifugation at 20,000 × *g* for 30 min at 4°C. The final equilibration of the protein solution with the desired buffer was by gel chromatography on a Sephadex G-25 column (1.0 × 10 cm; Pharmacia Biotech., Uppsala, Sweden). All steps were performed at 4°C as quickly as possible. Protein concentration was determined in 6 M guanidine hydrochloride at 275 nm with an absorptivity value of 1.09 mL · mg^{-1} · cm^{-1} (23).

Lipid peroxides. Because the cell membrane is composed primarily of phospholipids, soybean lecithin (Lipoids S-100) was used as the starting material for the generation of lipid peroxides. Lipoid S-100 was a gift from Nisshin Oil Mills, Ltd. (Tokyo, Japan). Photosensitized oxidation was carried out in methanol. Methylene blue was added to lecithin–methanol solution as a sensitizer. The final concentration of methylene blue was 0.004%, and that of lecithin was 10 mg/mL. The mixture was peroxidized by irradiation with a 60W tungsten projection lamp, positioned 40 cm above the reaction mixture, for 8 h at 4°C. Upon completion of the reaction, methylene blue was removed by Disposil silica gel columns (Nakalai Tesque, Kyoto, Japan). Methanol was then removed by evaporation. For control samples, no sensitizer was added and no irradiation was performed. Aliquots (50 µL) of the generated lipid peroxides were added to protein solution at a final concentration of 1 mg/mL.

The production of lipid peroxides was verified by reversed-phase HPLC in a Tosoh (Tokyo, Japan) system, composed of a pump (model CCPS), an ultraviolet (UV) absorption detector (model UV-8020), and an integrator (model chromatocorder 21), attached with an electrochemical detector (Irika model Σ 985, Kyoto, Japan), and another integrator (model chromatocorder 21; Tosoh). The column was a packed octadecyl silica gel (ODS) column (4.6 i.d. × 150 mm) from YMC (Kyoto, Japan). The mobile phase solution was a mixture of methanol/water (95:5, vol/vol) that contained 30 mM lithium acetate. Helium gas was bubbled through the solution to eliminate dissolved air. The mobile phase flow rate was 1 mL/min. To detect lipid peroxides, the eluent was monitored at 233 nm by the UV detector and at −300 mV vs. Ag/AgCl by the electrochemical detector (24).

Peroxide value (POV) was determined according to the method of Asakawa and Matsushita (25).

Vitamin A derivatives. Each of the vitamin A derivatives, β-carotene, retinal and retinol, was added as antioxidant to a reaction mixture at a concentration of 0.1 mM. This concentration was selected based on previously reported studies. For example, in studies of the effect of vitamin A on nonenzymatic lipid peroxidation, concentrations in the range from 0.1 to 10.0 mM were used (26). A tubulin assay concentration of 1 mg/mL corresponds to a ratio of 0.1 µmoles of antioxidant/mg protein. The same molar ratio was used by Vile and Winterbourn (27) in studies of the inhibition of lipid peroxidation. These vitamin A derivatives were prepared as described below.

β-Carotene. β-Carotene from Nakalai Tesque Inc. was dissolved in methanol. The concentration was measured spectrophotometrically by using the extinction coefficient, E_{478} = 2,278 $M^{-1} \cdot cm^{-1}$, after diluting with hexane (28). β-Carotene was added to the reaction mixtures to a final concentration of 0.1 mM.

Retinal and retinol. Retinal and retinol, purchased from Nakalai Tesque Inc. and Fluka Chemie AG (Buchs, Switzerland), respectively, were dissolved in methanol. Concentrations were determined by using extinction coefficients of E_{381} = 1,530 $M^{-1} \cdot cm^{-1}$ and E_{325} = 1,832 $M^{-1} \cdot cm^{-1}$ for retinal and retinol, respectively (28). Both compounds were utilized at concentrations of 0.1 mM in the reaction mixtures.

Other reagents. All other chemicals were of reagent grade.

Assay of enzyme activity. The *in vitro* self-assembly of tubulin into microtubules is promoted by high concentrations of glycerol (7). Consequently, glycerol and other compounds (GTP, $MgCl_2$) known to induce assembly were included in all enzymic assays. Assays were performed in 10 mM phosphate buffer, pH 7.0, that contained 0.1 mM GTP, 3.4 M glycerol, and 10 mM $MgCl_2$ with lipid or lipid peroxides at final concentrations of 1.0 mg/mL. The concentration of tubulin was 1.0 mg/mL. As described previously (17,18), a tubulin concentration of 1 mg/mL was used in GTPase assays. Lipid peroxides were added at identical concentrations (1 mg/mL). Although this is considerably higher than concentrations reported *in vivo* (29,30), it was selected so as to increase the rate of peroxidation. The order of addition of chemicals to the assay system was $MgCl_2$, standard buffer, lipid peroxides, vitamin A, and tubulin. The total volume of the assay system was 500 µL in a microtube. The reaction was started by elevation of the assay temperature from 0 to 37°C. About 20 s was needed to elevate the temperature of the reaction mixture to 37°C. This lag time was taken into account in calculation

of the reaction time. Reactions were stopped by the addition of 100 µL of 3 N $HClO_4$ for 10 min after 15 or 30 min of reaction time.

Because the microtubule assembly under the conditions employed reaches a steady state of polymerization at 37°C at around 15 min, 30 min of incubation time is sufficient to examine steady-state GTPase activity. After acid precipitation, denatured protein was removed by centrifugation at 5,000 rpm at 4°C for 15 min. An aliquot of the supernatant was neutralized and buffered by the addition of 1/6 fraction volume of 1 M K_2HPO_4, 0.5 M acetic acid, and of 1/6 fraction volume of 3 M KOH at 0°C. After mixing, samples usually were frozen to ensure precipitation of $KClO_4$ and were stored at –20°C until HPLC analysis (31,32); 25 µL of sample was applied to the HPLC column.

Determination of GDP and GTP by HPLC. GTPase activity of tubulin was measured by determining GDP produced in an HPLC system with a packed ODS column (4.6 × 250 mm) prepared by Nakalai Tesque. For HPLC analysis of nucleotide contents, precipitated $KClO_4$ was removed by centrifugation at 5,000 rpm for 15 min at 2°C. Before injection, aliquots of the supernatants were diluted 1:3 to 1:5 with HPLC mobile phase and cleared by membrane filtration on a Cosmo filter with a pore size of 0.45 µm (Nakalai Tesque). The mobile phase solution was 0.2 M K_2HPO_4, 0.1 M acetic acid, and 4 mM tetrabutylammonium phosphate, pH 6.5. This mobile solution was diluted three- to fourfold with redistilled water and then filtered by membrane filter (pore size 0.45 µm; Millipore Ltd., Bedford, MA). A Tosoh HPLC system was used in all GDP determinations. The flow rate was 1.0 mL/min. Detection wavelength was 253 nm. The extinction coefficient of GTP and GDP at 253 nm was 13,700 $M^{-1} \cdot cm^{-1}$ each. Then, a GTP standard solution of known concentration was prepared (33).

RESULTS

Characterization of lipid peroxides. Lipid peroxides were obtained by photooxidation of lecithin from soybean as described in the Materials and Methods section. Figure 1 shows typical HPLC profiles obtained for soybean lecithin and for lipid peroxides produced by photooxidation. A single sharp peak of phosphatidylcholine was observed on the chromatogram at 9 min (Fig. 1A), illustrating that the lecithin was of high purity. Electrochemical detection (ECD) has been used routinely by a variety of groups to detect and analyze hydroperoxyphospholipids specifically (24,34,35). In this study, the method of Terao (36) was utilized. Based on this approach, the lecithin used in this study was not oxidized. On the contrary, the peak speculated to be the photooxidation product of lecithin, hydroperoxyphosphatidylcholine (PCOOH), eluted at a retention time of 2 min in the HPLC (Fig. 1B). Concomitantly, the peak observed at 9 min due to phosphatidylcholine was decreased. As shown in Figure 2, a single peak at 2 min was also observed by ECD. Based on the characteristics of ECD, this confirms that the peak detected at 2 min was hydroperoxyphosphatidylcholine.

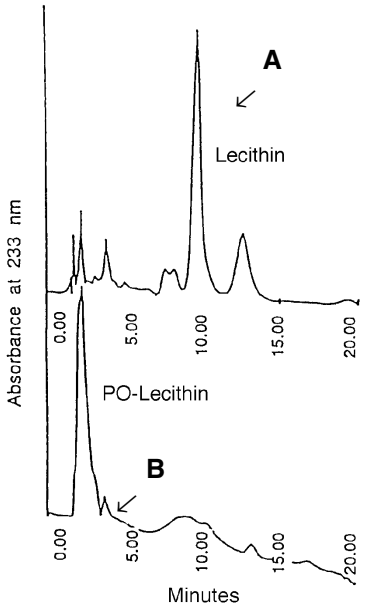

FIG. 1. Generation of lipid peroxides from soybean lecithin. Analysis was performed by high-performance liquid chromatography (HPLC). The column (octadecyl silica gel, 150 mm × 4.6 mm i.d.) was eluted with a mobile phase of methanol/H_2O (95:5 vol/vol with 30 mM lithium acetate). (A) Starting material: soybean lecithin, (B) peroxidation mixture of soybean lecithin after reaction as described in the Materials and Methods section.

No change of POV of control lecithin (lecithin not exposed to sensitizer and irradiation) was observed compared with native lecithin.

Effect of lipid peroxides on GTPase activity of tubulin. The effect of lipid peroxides on the GTPase activity of tubulin was examined upon addition of lipid peroxides at a final concentration of 1.0 mg/mL (Fig. 3). The amount of GDP produced in the control system was 250 ± 11 pmol/0.025 mL at 15 min and 370 ± 12 pmol/0.025 mL at 30 min. The amount of GDP produced between 15 and 30 min was only 32% of that for the first 15 min. On the other hand, when lipid peroxides were added to the system, the amount of GDP produced was 170 ±

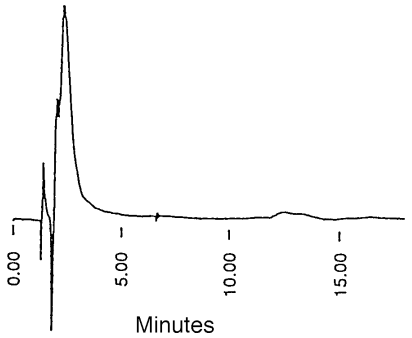

FIG. 2. Analysis of lecithin peroxides by HPLC. Eluted material, as detected in Figure 1B on the basis of absorbance, was detected with an electrochemical detector (model Σ985; Irika, Kyoto, Japan) at –300 mV. Column and elution conditions were as described in Figure 1. See Figure 1 for abbreviation.

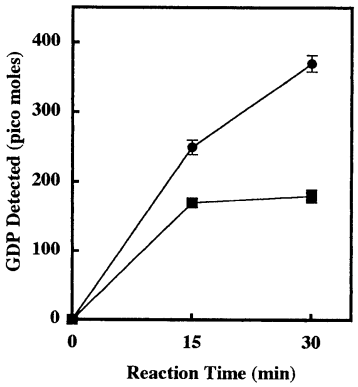

FIG. 3. Inhibition of tubulin guanosine-5′-triphosphatase (GTPase) activity by lecithin peroxides. ●, Control (1 mg/mL tubulin); ■, PO-lecithin (1 mg/mL tubulin, 1 mg/mL lecithin peroxides). All assays were performed at 37°C in 10 mM phosphate buffer, pH 7.0, containing 0.1 mM GTP, 3.4 M glycerol, and 10 mM $MgCl_2$. Values are expressed as means ± SE of seven independent determinations. GDP, guanosine-5′-diphosphate.

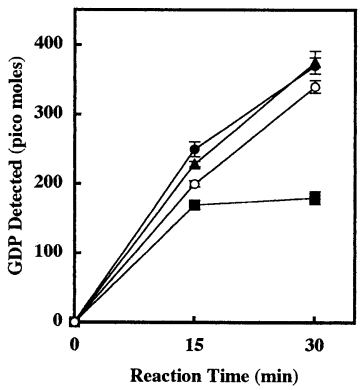

FIG. 4. Protective effect of β-carotene against the inhibitory effect of lecithin peroxides or tubulin GTPase activity. ●, Control (1 mg/mL tubulin); ■, PO-lecithin (1 mg/mL tubulin, 1 mg/mL lecithin peroxides); ▲, β-carotene (1 mg/mL tubulin, 0.1 mM β-carotene); ○, PO-lecithin + β-carotene (1 mg/mL tubulin, 1 mg/mL lecithin peroxides, 0.1 mM β-carotene). Assay conditions were as described in Figure 3. Values are expressed as means ± SE of four independent determinations for β-carotene and PO-lecithin + β-carotene. For abbreviations see Figure 3.

6 pmol/0.025 mL at 15 min and 180 ± 9 pmol/0.025 mL at 30 min. The increase of GDP in the second 15 min was only 5.9% of that for the first. Comparing the reaction system in the presence of lipid peroxides with the control system, GTPase activity was decreased by 32% for the first 15 min and by 51% for the 30-min reaction times. Therefore, the GTPase activity of tubulin was decreased nearly half by the addition of lipid peroxides. From this it is clear that even low concentrations of lipid peroxides are sufficient to inhibit tubulin GTPase activity. In fact, the amounts of GDP produced in the presence of lipid peroxides at the concentration of 0.5 mg/mL were 208 ± 10 pmol/0.025 mL at 15 min and 226 ± 5 pmol/0.025 mL at 30 min. The inhibition of tubulin GTPase by lipid peroxides was dependent on the concentration of lipid peroxides. The inhibition by lipid peroxides was significantly greater for the second 15 min in comparison to the first. This presumably reflects the rate of reaction of lipid peroxides with tubulin; it is not complete within the initial 15-min reaction phase. These results indicate that lipid peroxides have the ability to inhibit the GTPase activity of tubulin and, consequently, interfere with tubulin functions. Such data are consistent with the postulate that lipid peroxides could interfere with cellular function owing to their ability to react with tubulin.

Protection against inhibitory effects of lipid peroxides on tubulin GTPase by vitamin A and its derivatives. Effect of added β-Carotene. Figure 4 shows the effect of β-carotene (0.1 mM) on the ability of lipid peroxides to inhibit tubulin GTPase activity. When β-carotene was added to the reaction system, GTPase activity of tubulin (375 ± 16 pmol/0.025 mL) was equal to that of the control system (370 pmol/0.025 mL). In the presence of lipid peroxides, GTPase activity after 15 min was lowered 20%, compared to the control system, when β-carotene was added. This value was similar to that obtained in the presence of lipid peroxides and in the absence of β-carotene. However, after 30 min in the presence of β-carotene, GTPase activity was only decreased 8% relative to the control system, in comparison to 51% in its absence. Clearly, the effect of β-carotene is much more strongly manifested at 30 min than at 15 min, suggesting that β-carotene reacts slowly with lipid peroxides in this reaction system. These results indicate that β-carotene (37), a well-known antioxidant, has a strong capacity to protect tubulin GTPase against inhibition by lipid peroxides.

Effect of added retinal. The influence of retinal on the ability of lipid peroxides to inhibit tubulin GTPase is illustrated in Figure 5. Whereas the lipid peroxides inhibited tubulin GTPase activity by 29 and 67% at 15 and 30 min, respectively, in the presence of retinal, the percentage inhibition was significanty reduced to 12 and 23% for the corresponding time periods. These results support the conclusion that retinal behaves in a similar manner as β-carotene in protecting tubulin enzymic activity against the chemical modifying activities of lipid peroxides, presumably owing to its antioxidant activities.

Effect of added retinol. Similar experiments as described for β-carotene and retinal are illustrated in Figure 6 for retinol. Surprisingly, a small but reproducible increase in GTPase activity relative to the control was observed upon addition of retinol. The quantity of hydrolyzed GTP increased from 250 to 275 pmol/0.025 mL at 15 min and from 370 to 395 pmol/0.025 mL in the presence of retinol, relative to the control.

The mode of action for this direct stimulating effect of retinol on tubulin GTPase activity is not clear but may reflect the direct interaction of the ligand with tubulin. Further study is required to identify the mechanism behind this effect. In addition to the direct effect of retinol on tubulin function, as just described, retinol also retained the ability to protect against the inhibitory effect of lipid peroxides. The observed

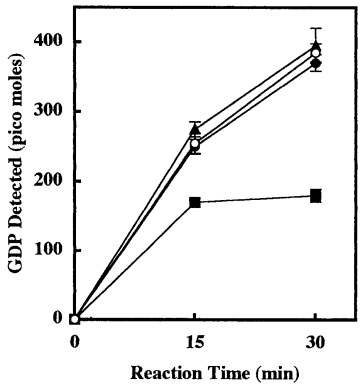

FIG. 5. Protective effect of retinal against the inhibitory effect of lecithin peroxides or tubulin GTPase activity. ●, Control (1 mg/mL tubulin); ■, PO-lecithin (1 mg/mL tubulin, 1 mg/mL lecithin peroxides); ▲, retinal (1 mg/mL tubulin, 0.1 mM retinal); ○, PO-lecithin + retinal (1 mg/mL tubulin, 1 mg/mL lecithin peroxides, 0.1 mM retinal). Assay conditions were as described in Figure 3. Values are expressed as means + SE of four independent determinations for retinal and PO-lecithin + retinal. For abbreviations see Figure 3.

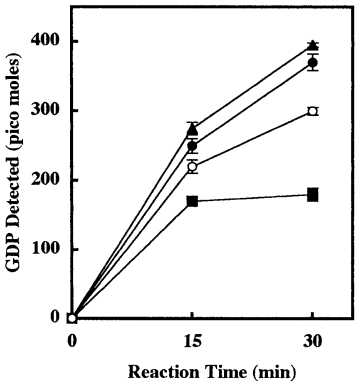

FIG. 6. Protective effect of retinol against the inhibitory effect of lecithin peroxides or tubulin GTPase activity. ●, Control (1 mg/mL tubulin); ■, PO-lecithin (1 mg/mL tubulin, 1 mg/mL lecithin peroxides); ▲, Retinol (1 mg/mL tubulin, 0.1 mM retinol); ○, PO-lecithin + retinol (1 mg/mL tubulin, 1 mg/mL lecithin peroxides, 0.1 mM retinol). Assay conditions were as described in Figure 3. Values are expressed as means ± SE of four independent determinations for retinol and PO-lecithin + retinol. For abbreviations see Figure 3.

activities were 2–3% above that of the control reaction mixtures. Retinol was significantly more potent than retinal in its protective action, which emphasizes its high effectiveness as an antioxidant.

DISCUSSION

Because biomembranes are composed largely of phospholipids, they must clearly be considered when investigating the effect of lipid peroxidation *in vivo*. Indeed, it has been reported that unsaturated fatty acids of membrane phospholipids are particularly susceptible to lipid peroxidation, leading to the loss of membrane integrity (37–39). The peroxidation of membrane phospholipids is supported by the observation that phospholipid-hydroperoxides were detected in vital samples (40). It is well documented that proteins are the major target for lipid peroxides, and it seems likely that lipid peroxides produced at the inner side of cell membranes interact with cytoplasmic proteins and lead to deterioration of cellular function.

In studies on the red blood cell membrane, peroxidation of lipid was observed to proceed from the inner side of the cell membrane (40), and peroxidized phospholipid accumulated in liver, leading to an aging phenomenon (41). It was deduced that the secondary products of lipid peroxides inhibited intracellular functional proteins.

Tubulin is a protein that is found in large concentrations in the brain (10% of soluble protein). It forms microtubules by self-assembly and participates in various cellular functions. Tubulin is consequently a prime candidate as a target for lipid peroxide action.

Until this time, there have been no reports on the interaction between cytoskeletal proteins and lipid peroxides in evaluating the effects of lipid peroxidation *in vivo*. The aim of this study was to evaluate this interaction and to clarify the behavior of lipid peroxides on the cytoskeleton protein, tubulin, taking note of functions of tubulin in a biological system.

With the present experiment, we confirmed that the GTPase activity of tubulin was inhibited when a small amount of lipid peroxides was added to a reaction mixture that contained tubulin (Fig. 3). From this we deduced that lipid peroxides, formed *in vivo*, have the potential to modify tubulin and inhibit microtubule formation. These experiments stress that a portion of the observed effect of lipid peroxides on cellular function could be a consequence of effects on microtubule function.

The higher molar ratios used in this study were selected to increase the reaction rate and to enable us to effectively monitor the reaction rate in an *in vitro* assay in an appropriate time frame. *In vivo*, however, it would be postulated that lower peroxide concentrations but longer exposure times result in similar extents of lipid peroxidation and consequent effects on tubulin function.

It seems likely that biological compounds with antioxidant properties have the capacity to protect cells and tissues against the deleterious effects of reactive oxygen species and other free radicals. Jialal *et al.* (42) reported that β-carotene inhibited low-density lipoprotein oxidation and might have an important role in the prevention of atherosclerosis. In addition, β-carotene is an efficient quencher of singlet oxygen and significantly decreases heart disease and vascular disease because it can function as a radical-trapping antioxidant.

Ciaccio *et al.* (43) reported that an increase of vitamin A within cell membranes results in an increased resistance of membrane lipids to peroxidation, both endogenously produced and induced *in vitro*. These results are consistent with the hypothesis that vitamin A might act as a physiological antioxidant in cell membranes where it is localized.

To probe the effects of antioxidants in the microtubule system, vitamin A (β-carotene, retinal, and retinol) was added to the reaction system, and then the GTPase activity of tubulin was measured. When β-carotene or retinol was added to the reaction mixture, tubulin GTPase activity was almost identical to that of the control.

These results indicate that both β-carotene and retinol have the capacity to protect against the inhibitory effects of lipid peroxides on tubulin GTPase activity, and consequently, on microtubule assembly.

In contrast to β-carotene or retinol, the protective effect of retinal was significantly lower in 30-min reactions ($P < 0.05$), although a significant difference was not observed for the 15-min reactions. When retinal was added to the tubulin reaction mixture in the presence of lipid peroxides, only a small restoration of GTPase activity of tubulin was observed, compared with those reactions when β-carotene or retinol was added. These results suggest that, besides the role of antioxidant, there may be an additional mechanism of action that contributes to the effect of vitamin A analogs in protecting against the inhibitory effects of lipid peroxides on microtubule assembly. This is supported by the observation that tubulin GTPase activity in the presence of retinol was enhanced, relative to the control, which suggests that retinol may bind directly to tubulin. These results emphasize the potential beneficial effects of vitamin A antioxidants in protecting against aging by inhibiting the deleterious effects of lipid peroxides on the various cellular functions of microtubules.

In this study, the protective effect of the vitamin A group was much more significant over a 30-min time period, as opposed to the initial 15-min time frame. In fact, GTPase activity measured after 15 min was similar to that of the control, whereas there was a large difference after a reaction of 30 min (Figs. 4–6). This was interpreted to indicate that the rate of reaction of the vitamin A group with the lipid peroxides is relatively slow in comparison with the rate of peroxidation of tubulin.

The results in this study are consistent with many reports that the vitamin A group has the ability to prevent peroxidation in a living cell (42–46).

In summary, this study demonstrated that tubulin self-association into microtubules may be inhibited by lipid peroxides, presumably owing to their ability to chemically modify tubulin. Because microtubules are intrinsic to cellular function, these data pinpoint tubulin as a potential target for the well-characterized damaging effects of lipid peroxides, which in turn could contribute to aging of the cell. More detailed studies of the mechanism of the effect of lipid peroxides on tubulin function and its relationship to the aging phenomenon are under way.

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