Tohoku J. Exp. Med., 2000, 192, 67-79

# Simple and Rapid Determination of GTPase Activity by Capillary Electrophoresis without Radioisotope

HIROKO KAWATA, KOICHI KURODA, YOKO ENDO,[1] YOSHINORI INOUE and GINJI ENDO

*Department of Preventive Medicine and Environmental Health, Osaka City University Medical School, Osaka 545-8585, and [1]Department of Public Health, Kansai Medical University, Moriguchi 570-8506*

KAWATA, H., KURODA, K., ENDO, Y., INOUE, Y. and ENDO, G. *Simple and Rapid Determination of GTPase Activity by Capillary Electrophoresis without Radioisotope.* Tohoku J. Exp. Med., 2000, **192** (1), 67-79 —— In order to determine guanosine-5′-triphosphatase (GTPase) activity, we developed a simple, rapid and reliable method that utilizes capillary electrophoresis without radioisotope. Tubulin-GTPase was used for simple measurement of GTPase activity utilizing capillary electrophoresis. Tubulin, a component of microtubules, was incubated with guanosine-5′-triphosphate (GTP) in 100 mM 2-(N-morpholino) ethanesulfonic acid (MES) buffer (pH 6.5). Guanosine-5′-diphosphate (GDP) was determined as the hydrolyzed product of GTP. Guanosine-5′-monophosphate, GDP and GTP in the filtrate of the mixture were clearly separated using 10 mM MES buffer (pH 6.5) (migration time, 3.8, 5.5 and 7.2 minutes, respectively) with a fused-silica capillary column. The quantification of GDP was based on the peak area, which increased linearly with the concentration of GDP from 1 to 50 $\mu$M ($r^2 = 0.995$). The peak area and migration time had good reproducibility; the intra-assay coefficient of variation ($n = 6$) was 1.3% for peak area and 0.6% for migration time. As an application of this method, we examined the effect of dimethylarsinic acid, an effective antimitotic agent, on tubulin-GTPase. Dimethylarsinic acid inhibited tubulin-GTPase activity in a dose-dependent manner. The inhibition was not complete and the maximum decrease of the activity was about 50% at 200 $\mu$M dimethylarsinic acid. Thus, since this method is clean, simple and rapid, its application to the study of various GTPase proteins is expected to be useful.

———— capillary electrophoresis; GTPase; tubulin; GDP; non-radioisotope

© 2000 Tohoku University Medical Press

Guanosine-5′-triphosphatase (GTPase) hydrolyzes guanosine-5′-triphosphate (GTP) to guanosine-5′-diphosphate (GDP) and releases Pi. GTPase activity is

---

Received June 27, 2000; revision accepted for publication September 13, 2000.

Address for reprints: Hiroko Kawata, Department of Preventive Medicine and Environment Health, Osaka City University Medical School, 1-4-3 Asahi-machi, Abeno-ku, Osaka 545-8585, Japan.

e-mail: hirozou@med.osaka-cu.ac.jp

reflected by Pi- or GDP-production, and is usually analyzed using radioisotope [$^{32}$P]-labeled GTP (David-Pfeuty et al. 1978; Mejillano et al. 1996). Recently, a non-radioisotope assay for GTPase activity has been developed. Okada et al. (1985) and Shimada et al. (1995) reported methods of detecting GDP by high performance liquid chromatography (HPLC) with non-labeled GTP. However, a simpler preparation, smaller sample volume and shorter analysis time are needed to study GTPase proteins.

Tubulin is a component of microtubules and one of the extensively studied GTPase proteins. GTP binds the E-site of tubulin to enhance tubulin assembly. Hydrolysis of GTP to GDP results in a change of tubulin conformation and induction of tubulin disassembly. Tubulin-GTPase activity is important in controlling microtubule dynamics, an essential function for many biological processes, including cell motility, cell division, morphogenesis and axonal transport (Alberts et al. 1994). It is important to investigate the inhibition of tubulin-GTPase activity. Tubulin-assembly and -GTPase assay are performed in MES buffer (David-Pfeuty et al. 1978). Many antimitotic agents, such as colchicine, paclitaxel and vinblastine, inhibit normal tubulin assembly and modify tubulin-GTPase activity (Correia 1991). These effects are related to non-mutagenic carcinogenicity and apoptosis. Arsenic is a non-mutagenic carcinogen and its carcinogenesis has been studied in vivo and in vitro (IARC 1987). Dimethylarsinic acid (DMA), a major metabolite of inorganic arsenic in mammals, is an effective antimitotic agent, which arrests the cell cycle in metaphase (Endo et al. 1992), and disrupts spindle formation in mitotic cells (Ochi et al. 1999). The effects of DMA on tubulin-assembly and GTPase, however, remain to be elucidated.

Capillary electrophoresis is a popular and powerful technique that can provide good separation of ionic and ionizable compounds such as amino acids, peptides, DNA fragments, proteins, drugs or even inorganic ions (Lehmann et al. 1997). The mechanisms responsible for separation by capillary electrophoresis are different from those in liquid chromatography or HPLC. Capillary electrophoresis separates ionic species based on their charge and ion radius. The merits of capillary electrophoresis are convenience of sample preparation, environmental friendliness, good reproducibility of peak area and migration time, and small sample volumes. Many assays using separation of many mono-, di-, and tri-phosphate nucleotides by capillary electrophoresis have been reported (Tsuda et al. 1983; O'Neill et al. 1994; Uhrova et al. 1996; Geldart and Brown 1997, 1998). Considering these methods, we applied capillary electrophoresis in an assay of GTPase with tubulin, and tried to use this technique to separate and determine guanosine-5′-monophosphate (GMP), GDP and GTP in the tubulin-GTPase assay-mixture. However, good results were not obtained. We therefore developed new conditions for the separation and determination. To clarify whether this method could be used to measure the inhibition of tubulin-GTPase activity

by DMA, we treated tubulin with DMA and detected GDP production.

## Materials and Methods

### *Reagents*

Tubulin (Lot 87H4024), GMP (>99%) and GDP (98%) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). GTP (biochemical grade) was purchased from Wako Pure Chemical (Osaka). Tubulin was purified from bovine brain by assembly-disassembly cycles and contained approximately 15% microtubule associated proteins. DMA-sodium salt (purity>99.99%) was obtained from Tri-Chemical Lab. (Yamanashi). Other reagents were purchased for superior quality from Wako Pure Chemical.

### *Sample preparation*

Tubulin was prepared according to the instruction of the supplier. Briefly, about 7.5 mg of tubulin was dissolved in 1 ml of 100 mM 2-(N-morpholino) ethanesulfonic acid (MES)-NaOH (pH 6.8), 1 mM ethylene glycol-bis ($\beta$-aminoethyl ether)-N, N, N', N'-tetraacetic acid (EGTA), 0.1 mM GTP, 0.1 mM ethylenediamine tetraacetic acid (EDTA), 0.5 mM $MgCl_2$, 1 mM dithiothreitol, 1 $\mu$g/ml leupeptin, 1 $\mu$g/ml aprotinin, and 0.3 mM sucrose, and shaken gently for 5 minutes at 37°C. The suspended tubulin was sonicated for 5 minutes at 0°C, and centrifuged at 27 000×$g$ for 40 minutes at 4°C. Then, the supernatant was collected and kept at −70°C until electrophoresis. The frozen supernatant contained only tubulin-dimer. The protein concentration of tubulin was determined by the method of Bradford (1976) using an assay kit (Bio-Rad Laboratories, Hercules, CA, USA). Stock standard solutions of GMP, GDP and GTP were 10 to 100 $\mu$M in 20 mM MES (pH 6.5). Their working standard solutions were 5 to 50 $\mu$M in 10 mM MES/10% ethanol since ethanol was added to stop the tubulin-GTPase reaction. For selection of a running buffer, 100 $\mu$M GMP, GDP and GTP in 10 mM MES were used as standard solution.

### *Tubulin-GTPase assay*

Tubulin-GTPase assays were performed in 100 mM MES buffer, containing 1 mM EGTA, 0.1 mM EDTA, 0.5 mM $MgCl_2$, and 2.5 M glycerol (pH 6.5). The concentration of tubulin protein was 0.8 mg/ml. The activation of GTPase was started by incubation at 37°C after the addition of GTP up at to 1 mM. The reaction was stopped by addition of an equivalent amount of ice-cold ethanol (99.5%) (Shimada et al. 1995). The reaction mixture was centrifuged at 27 000×$g$ for 10 minutes at 4°C and the supernatant was centrifuged at 4 500×$g$ for 30 to 60 minutes at 4°C with an Ultrafree-MC 10 000 NMWL Filter Unit (Millipore Co., Bedford, MA, USA). The GDP in the filtrate was kept at −20°C until analysis.

In order to examine the effects of DMA on tubulin-GTPase, tubulin was treated with DMA (10 to 200 $\mu$M). The DMA solution was adjusted to a pH of

6.5. and premixed on ice with GTP. Tubulin-GTPase was activated by addition of a GTP-DMA mixture at 37°C for 30 minutes. The final concentration of GTP was 0.8 mM.

*Capillary electrophoresis*

The measurements were performed using a Hewlett-Packard 3DCapillary Electrophoresis System with a diode array detector (Hewlett-Packard, Waldbronn, Germany). The capillary column, which consisted of fused silica, was 75 $\mu$m inner-diameter $\times$ 48.5 cm total length (40 cm effective length). Sample solution and running buffer were filtrated through a 0.22 $\mu$m polyvinylidene fluoride membrane (Millipore Co.). Prior to injection, the capillary was preconditioned for 1 minute by flushing with running buffer, 20 mM borate (pH 9.0), 20 mM phosphate (pH 7.0) or 10 mM MES (pH 6.5). The filtrated-sample solution was diluted with water to 2–5 volumes and 20 nl was injected automatically at a pressure of $5 \times 10^3$ Pa for 4.0 seconds. The applied voltage was set at 30 kV. The capillary was maintained at 20°C. Three guanosine nucleotides were detected at 254 nm with a band-width of 10 nm. Concentrations of GDP were calculated from peak area at 254 nm. The analysis was performed at least three times and the mean was used.

## Results

*Selection of running buffer components on electrophoresis*

In order to separate the three nucleotides, 20 mM borate buffer (pH 9.0), 20 mM phosphate buffer (pH 7.0) and 10 mM MES buffer (pH 6.5) were examined as running buffer. The components and the pH of the running buffer had a significant effect on the migration time and the separation of the three nucleotides.

When 20 mM borate buffer (pH 9.0) was used, the migration times of GMP, GDP and GTP were 7.4, 8.0 and 7.5 minutes, respectively. As shown in Fig. 1, the three nucleotides could not be separated completely and an unknown peak (at 9 minutes) was observed, although the reproducibility of migration time of each nucleotide was so good that the coefficient of variation (CV) was less than 0.2%. Next, we examined phosphate buffer since it usually provides for good separation of compounds. With 20 mM phosphate buffer (pH 7.0), the migration times of GMP, GDP and GTP were 5.6, 7.0 and 8.1 minutes, respectively (Fig. 2A). The reproducibility of migration time was not good (CV value was greater than 6%) and the peak shape of GTP was broad and tailing (Fig. 2B).

Finally, because MES (pH 6.5) was a major component of the tubulin-GTPase reaction buffer, and the component and pH of the running buffer would therefore not be affected by the sample solution, we examined it as running buffer. In contrast to the other two buffers, MES buffer provided good separation and reproducibility. The migration times of GMP, GDP and GTP were 3.2, 4.8 and 7.0 minutes, respectively, and the CV value was 0.4% ($n=3$).

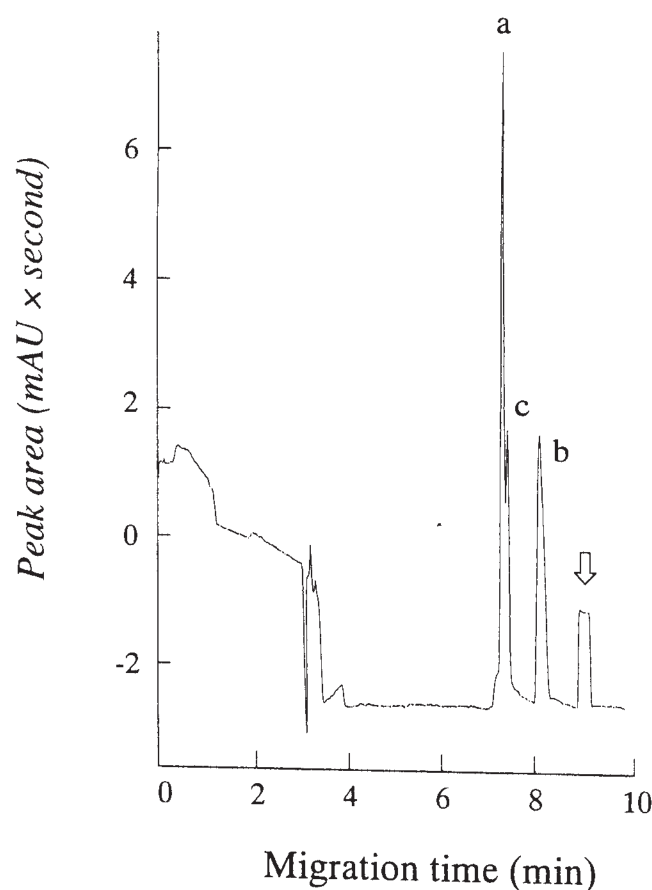


Fig. 1. Separation of GMP, GDP and GTP in a running buffer of 20 mM borate buffer (pH 9.0). Each guanosine nucleotide (100 $\mu$M) was added in 20 mM MES sample buffer (pH 6.5). a, GMP; b, GDP; c, GTP. Arrow, unknown peak.

Since 100 mM MES buffer used as the sample buffer for reaction of tubulin-GTPase, the effect of 10–100 mM MES on the electrophoresis in the three buffers was surveyed. When 50–100 mM MES was applied for electrophoresis in 20 mM borate- or phosphate-buffer, the current was unstable, the peak shape of MES was broad, and the base line of the electrophoregram was disrupted. In contrast, using 10 mM MES as running buffer, the current was stable and the peak shape of MES was sharp.

These results suggested that the MES buffer was the best for determining the concentration of the nucleotides produced by tubulin-GTPase.

*Effect of stop solution for the tubulin-GTPase reaction*

Ethanol or 10% perchloric acid is generally used to stop the GTPase reaction. An equal volume of perchloric acid or ethanol was added to the sample solution, and the mixture was subjected capillary electrophoresis. When perchloric acid was added, the base line of the electrophoregram and peak shapes of the nucleotides were disrupted by the acidity of perchloric acid, which strongly affected the pH of the running buffer (data not shown). The addition of ethanol, how-

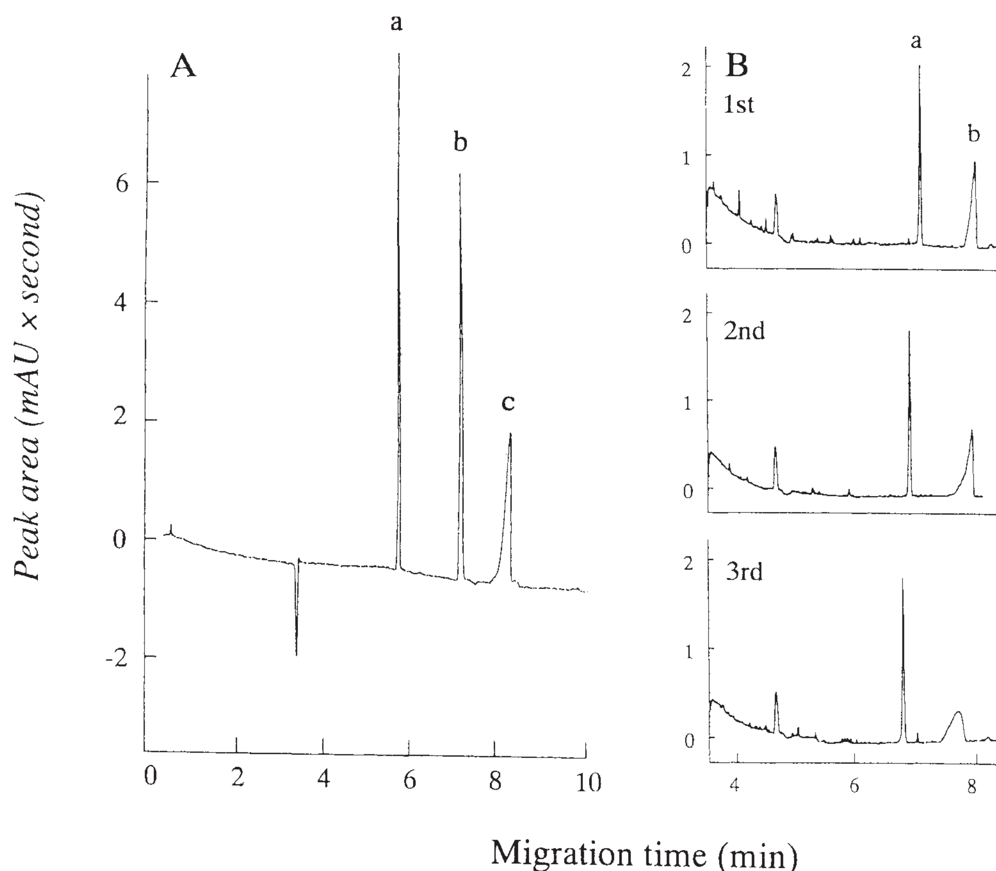


Fig. 2. Electrophoregrams of a mixture of guanosine nucleotides in a running buffer of 20 mM phosphate buffer (pH 7.0). A: Separation of GMP, GDP and GTP. Each guanosine nucleotide (100 $\mu$M) was added in 20 mM MES sample buffer (pH 6.5). a, GMP; b, GDP; c, GTP.
B: Alteration of the migration time and the peak shape in repeated analyses ($n=3$). Each guanosine nucleotide (50 $\mu$M) was resolved in 20 mM MES buffer (pH 6.5). *a*, GDP; *b*, GTP.

ever, did not disrupt the base line of the electrophoregram because it did not change the pH of the running buffer. The organic solvent had some effects as follows: the peak shapes of the nucleotides were sharper and the separation of the nucleotides was much better than with only MES buffer. The migration time of the nucleotides was slightly delayed but the reproducibility of the migration time was not affected. When the sample containing ethanol was diluted 5-fold with water, that is, the ethanol concentration was changed to 10–50% (v/v) of the solution, there was no effect on the migration time of GDP (CV was 1.6% [$n=4$]). These effects were slight and did not affect the determination. Therefore, we used ethanol as the stop solution for the tubulin-GTPase reaction in this study.

*Verification of GDP production using capillary electrophoresis*

A typical electrophoregram of a standard mixture of 20 $\mu$M nucleotides is shown in Fig. 3. The migration times of GMP, GDP and GTP were 3.8, 5.5 and 7.2 minutes, respectively. The figure shows good separation of the three

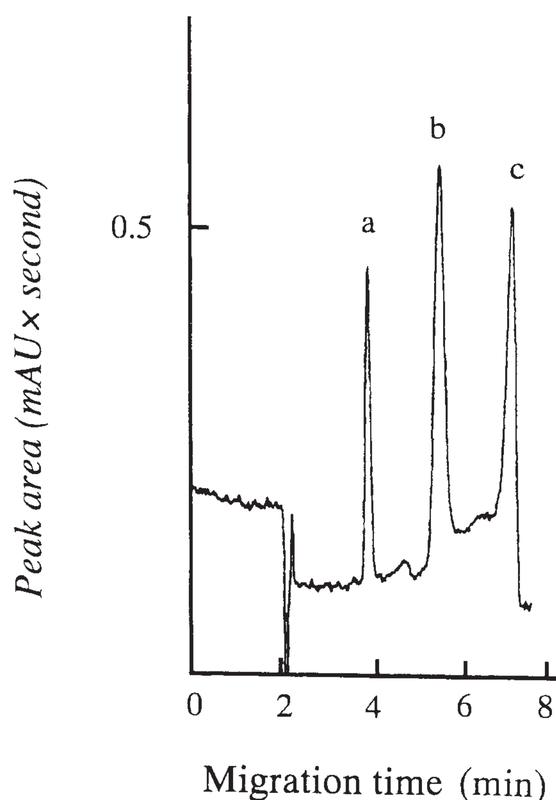


Fig. 3. Electrophoregram of a mixture of GMP, GDP and GTP in a running buffer of 10 mM MES buffer (pH 6.5). Each guanosine nucleotide (20 $\mu$M) was added in 10 mM MES sample buffer containing 10% ethanol (v/v). *a*, GMP; *b*, GDP; *c*, GTP.

guanosine nucleotides within 10 minutes. One analysis took less than 15 minutes, including column washing, sample application, electrophoresis and detection. The CV value of the migration time of 10 $\mu$M GDP was 0.6% ($n=6$). The spectrum and migration time were used for detection and identification of GDP. The calibration curve for GDP based on peak area (mAU×second) at 254 nm was linear from 1 to 50 $\mu$M ($r^2=0.995$). The detection limit of GDP was 1 $\mu$M (20 fmol, 8.86 pg), and this sensitivity was good enough for our study. The peak area of GDP had very good reproducibility, as follows: the intra-assay CV value ($n=6$) of the peak area for 10 $\mu$M GDP was 1.3% and the inter-assay CV ($n=3$) was 1.5%.

*Determination of GDP in tubulin-GTPase reaction mixture using capillary electrophoresis*

Tubulin-GTPase assays were performed in 100 mM MES buffer (pH 6.5). Tubulin incubated at 37°C with GTP for 0–60 minutes. GDP was determined as tubulin-GTPase product, and it increased with increasing reaction time (Figs. 4 and 5). The results showed that GDP was produced by tubulin-GTPase. Thus, this method is useful for the measurement of GTPase activity without radioisotope.

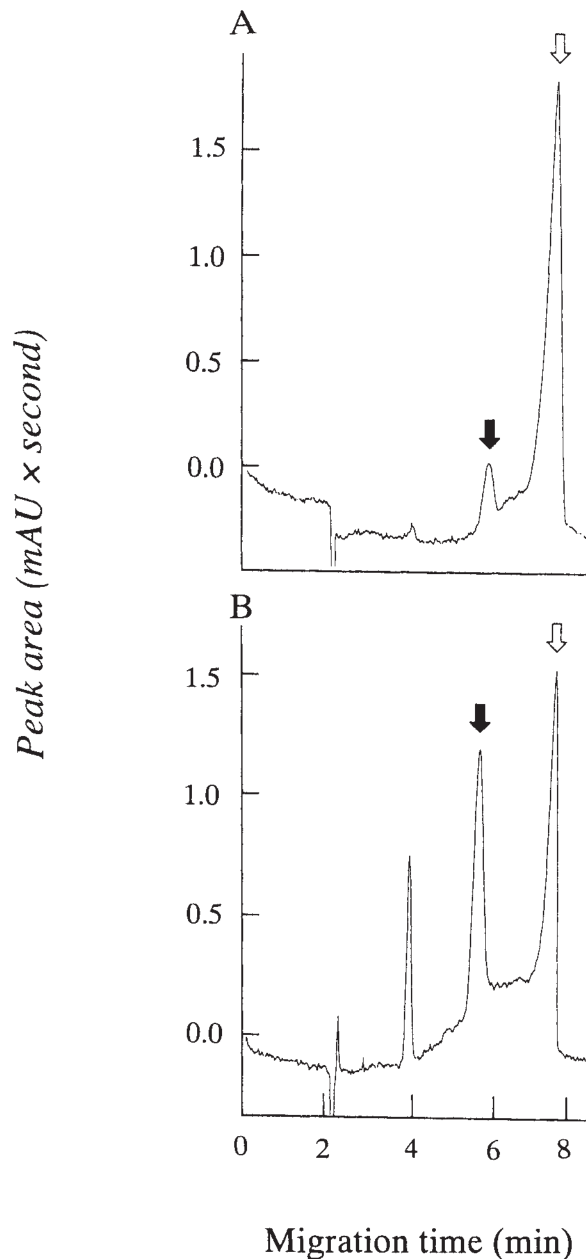


Fig. 4. The increase of GDP produced by tubulin-GTPase in 100 mM MES buffer (pH 6.5) with increasing reaction time. A: the reaction mixture immediately after GTP addition. B: GDP was assayed at 30 minutes (min) after GTP was added to the reaction mixture. The concentration of tubulin protein was 0.8 mg/ml. The activation of GTPase was started by incubation at 37°C after the addition of GTP at up to 1 mM. Running buffer, 10 mM MES buffer (pH 6.5). Black arrow, GDP; white arrow, GTP.

*Application of this method (Alteration of tubulin-GTPase activity by treatment with DMA)*

This analytical procedure was used to measure the alteration of GTPase activity of DMA-treated tubulin. As shown in Fig. 6, DMA significantly reduced the GDP production catalyzed by tubulin, i.e., it inhibited tubulin-GTPase activity, in a dose-dependent manner. The maximum decrease was about 50%,

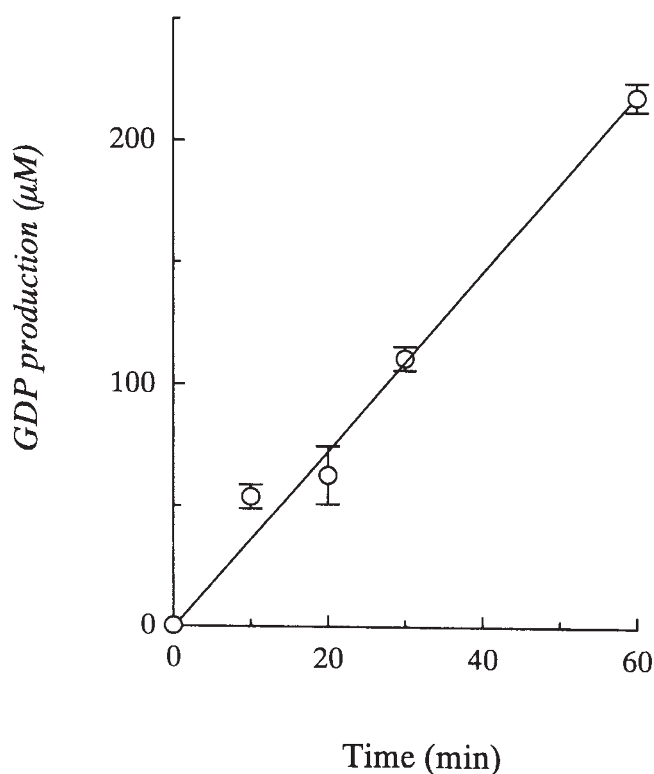


Fig. 5. Time course of GDP production catalyzed by tubulin. Tubulin-GTPase reaction was performed in 100 mM MES buffer (pH 6.5). Values represent the mean ± S.D. for three experiments. The quantification was performed using the peak area (mAU × second) at 254 nm. Other conditions are the same as described in the legend for Fig. 4.

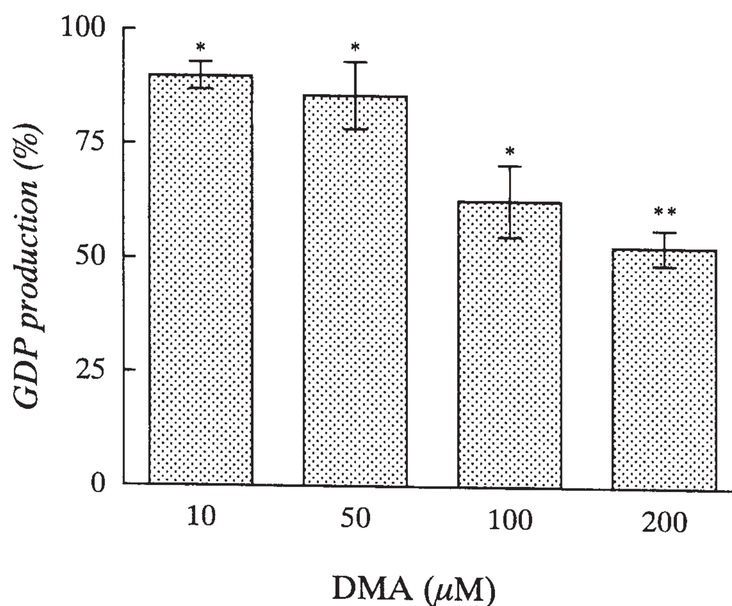


Fig. 6. Inhibition of tubulin-GTPase activity by DMA. Tubulin-GTPase activity is represented as a percentage of the GDP production by tubulin-GTPase in the absence of DMA. The final concentration of GTP was 0.8 mM. Values represent the mean ± S.D. for six experiments. Other conditions are the same as described in the legend for Fig. 4. $^{*}p<0.05$ and $^{**}p<0.01$ by Student's $t$-test.

with decreases of 10.0±3.0, 14.3±7.4, 37.3±7.8 and 47.2±3.8% (mean±S.D., $n$=6) at 10, 50, 100 and 200 $\mu$M DMA, respectively.

## Discussion

In capillary electrophoresis, selection of the running buffer is very important not only for separation of compounds but also for reproducibility of peak area and migration time, because the electroosmotic flow depends on the concentration, components and pH of the buffer solution and on the condition of the column wall (Lehmann et al. 1997). Many nucleotides dissolved in water (Uhrova et al. 1996; Geldart and Brown 1997), in 0.9% NaCl at pH 6.5-7.5 (Tsuda et al. 1983) or in Tris-phosphate buffer at pH 5.28 (O'Neill et al. 1994) can be clearly separated using borate- and/or phosphate-buffer as running buffer. The tubulin-GTPase reaction is performed in a high-concentration MES buffer (100 mM) at pH 6.5. We first compared 20 mM borate- (pH 9.0), 20 mM phosphate- (pH 7.0) and 10 mM MES-buffer (pH 6.5) as running buffer to separate GMP, GDP and GTP in 20 mM MES sample buffer. MES buffer produced good separation in a short time and with better reproducibility than borate- and phosphate-buffer. It was considered that using MES buffer, a stable electroosmotic flow and buffer capacity could be obtained to give the best conditions for separation of the three nucleotides in the present study. The reason for the good results with MES are as follows. The components and pH of the sample buffer and running buffer were the same, and there was no effect of the sample buffer on these features of running buffer. The migration-order and -time of nucleotide elution found by Uhrova et al. (1996) with a phosphate/borate buffer in the same pH range was different from these found by Geldart and Brown (1997), using sodium carbonate/bicarbonate buffer. The rate of electroosmotic flow is dependent on the pH of the running buffer, and an alkaline running buffer provides a fast and stable electroosmotic flow. The pH of the buffer and pKa of solutes are important factors for separation of solutes. Since the pKa2 of GMP, GDP and GTP is at pH 6-7 (Hirokawa et al. 1985), MES buffer provided good separation of the nucleotides. It took more than 20 minutes for separation of the nucleotides in sodium carbonate/bicarbonate buffer at pH 10 (Geldart and Brown 1997), or more than 40 minutes in Tris-phosphate buffer at pH 5.2 (O'Neill et al. 1994). Sodium borate is the most commonly used alkaline buffer for nucleotides. However, we found that borate buffer could not yield complete separation of the three nucleotides. The order of migration (GMP, GTP and GDP) was unusual, and an unknown peak was observed. Furthermore, in a neutral phosphate buffer which was tested as the second choice, the analysis was not expected to be reliable because of poor reproducibility. It has been reported that such a phenomenon is the result of incomplete ionization of the triphosphate, indicating that interaction occurs between solutes and either the capillary wall or buffer component (Uhrova et al. 1996). The differences of component and pH between sample- and running-buffer affected the electroosmotic flow and the buffer

capacity, resulting in effects on the separation and the reproducibility of the peak area and the migration time. Also, it has been reported that addition of organic solvents, such as methanol, ethanol, and propanol, to a neutral or acidic running buffer improves the shapes and separation of peaks (Sarmini and Kenndler 1997). According, we found that a rapid and reliable analysis could be achieved by using MES as the running buffer and adding ethanol to the sample solution. Tubulin-GTPase activity was therefore determined with capillary electrophoresis in 10 mM MES buffer as running buffer.

A simple and rapid method for the non-radioactive determination of GDP produced by GTPase was then developed. HPLC has been used routinely to quantify nucleotides and to determine GTPase activity (Musacchio and Schen 1983; Okada et al. 1985; Shimada et al. 1995). HPLC and capillary electrophoresis have many advantages for nucleotide analysis: they are reproducible, sensitive and selective, and are easily automated. Moreover, the development of analytical chemistry requires investigation of methods with different theoretical bases. Our method has the following advantages compared to the HPLC methods: our analysis time is short (less than 15 minutes) because the separation of the three guanosine nucleotides is rapid, and the time-consuming steps of column-equilibration and -washing are not needed. It takes more than 30 minutes for separation of the nucleotides by HPLC (Musacchio and Schen 1983; Okada et al. 1985), that is, the total analytical time is nearly an hour for one analysis. Next, analyses can be made repeatedly with less than 20 $\mu$l of sample solution because of using capillary electrophoresis (the volume needed for one analysis is 20 nl). HPLC requires a relatively large volume of sample, often between 10–100 $\mu$l, for one analysis (Musacchio and Schen 1983; Okada et al. 1985; Shimada et al. 1995). Finally, the bare capillary column is robust enough to use almost permanently, and various chemicals can be separated with the same capillary column by changing the buffer conditions.

Tubulin has been extensively studied for its role in cell cycle progression. Tubulin dimer, which is composed of $\alpha$-and $\beta$-tubulin, has GTPase activity. GTP binding to the E-site is necessary for the assembly to occur, while after assembly the E-site on microtubules contains GDP. Therefore, hydrolysis of the E-site GTP apparently takes place during assembly, that is, the GTPase is associated with tubulin assembly. David-Pfeuty et al. (1978) reported that at the assembly equilibrium, the rate of GTPase should be directly proportional to the concentration of microtubule ends. Many antimitotic agents bind to tubulin and thereby alter its structure, resulting in inhibition of normal tubulin-assembly and -GTPase activity (Correia 1991). As shown in Fig. 6, tubulin-GTPase was clearly inhibited by DMA. It is not clear whether DMA inhibits the GTPase of tubulin itself or the tubulin-assembly-associated GTPase. However, because the inhibition of tubulin-GTPase by DMA was weak (the maximum inhibition was about 50%) and DMA is an antimitotic agent, DMA may inhibit the GTPase associated

with tubulin assembly rather than the GTPase of tubulin itself.

In conclusion, since this method is clean, simple and rapid, its application to the study of various GTPases is expected to be useful.

**Acknowledgments**

This investigation was supported by grants (11670383) from the Ministry of Education, Science, Sports and Culture of Japan.

**References**

1) Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K. & Watson, J.D. (1994) The cytoskelton. In: *Molecular Biology of the Cell*, 3rd ed., edited by R. Adams & M. Robertson, Garland Publishing Inc., New York, pp. 787-862.
2) Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Chem.*, **31**, 248-254.
3) Correia, J.J. (1991) Effects of antimitotic agents on tubulin-nucleotide interactions. *Pharmacol. Ther.*, **52**, 127-147.
4) David-Pfeuty, T., Laporte, J. & Pantaloni, D. (1978) GTPase activity at ends of microtubules. *Nature*, **272**, 282-284.
5) Endo, G., Kuroda, K., Okamoto, A. & Horiguchi, S. (1992) Dimethylarsenic acid induces tetraploids in Chinese hamster cells. *Bull. Environ. Contam. Toxicol.*, **48**, 131-137.
6) Geldart, S.E. & Brown, P.R. (1997) Opitimization for the separation of ribonucleotides by capillary electrophoresis at high pH. *J. Chromatogr. A*, **792**, 67-73.
7) Geldart, S.E. & Brown, P.R. (1998) Analysis of nucleotides by capillary electrophoresis. *J. Chromatogr. A*, **828**, 317-336.
8) Hirokawa T., Kobayashi S. & Kiso Y. (1985) Isotachophoretic determination of mobility and pKa by means of computer simulation. *J. Chromatogr.*, **318**, 195-210.
9) IARC (1987) Arsenic and arsenic compounds (Group 1). In: *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Human*, Suppl. 7, IARC, Lyon, pp. 100-106
10) Lehmann, R., Voelter, W. & Liebich, H.M. (1997) Capillary electrophoresis in clinical chemistry. *J. Chromatogr. B Biomed. Sci. Appl.*, **697**, 3-35.
11) Mejillano, M.R., Shivanna, B.D. & Himes, R.H. (1996) Studies on the nocodazole-induced GTPase activity of tubulin. *Arch. Biochem. Biophys.*, **336**, 130-138.
12) Musacchio, J.M. & Schen, C. (1983) Failure of opiates to increase the hydrolysis of GTP in neuroblastoma-glioma 108-15 cells. *Life Sciences*, **33**, 879-887.
13) Ochi, T., Nakajima, F. & Nasui, M. (1999) Distribution of $\gamma$-tubulin in multipolar spindles and multinucleated cells induced by dimethylarsinic acid, a methylated derivative of inorganic arsenics, in Chinese hamster V79 cells. *Toxicology*, **136**, 79-88.
14) Okada, D., Tsukida, K. & Ikai, A. (1985) Functional properties of cattle rhodopsin in soluble complex with phospholipids and deoxycholate. *Photochem. Photobiol.*, **42**, 405-411.
15) O'Neill, K., Shao, X., Zhao, Z., Malik, A. & Lee, M.L. (1994) Capillary electrophoresis of nucleotides on Ucon-coated fused silica colums. *Anal. Biochem.*, **222**, 185-189.
16) Sarmini, K. & Kenndler, E. (1997) Influence of organic solvents on the separation selectivity in capillary electrophoresis. *J. Chromatogr. A*, **792**, 3-11.
17) Shimada, T., Ito, H. & Kasai, H. (1995) Development of a new assay method for GTPase activity using HPLC. *Seikagaku*, **67**, 475-477. (in Japanese)
18) Tsuda, T., Nakagawa, G., Sato, M. & Yagi, K. (1983) Separation of nucleotides by

high-voltage capillary electrophoresis. *J. Appl. Biochem.*, **5**, 330–336.

19) Uhrova, M., Deyl, Z. & Suchanek, M. (1996) Separation of common nucleotides, mono-, di- and triphosphates, by capillary electrophoresis. *J. Chromatogr. B Biomed. Sci. Appl.*, **681**, 99–105.