

HETEROCYCLES, Vol. 79, 2009, pp. 1007 - 1017. © The Japan Institute of Heterocyclic Chemistry  
Received, 4th November, 2008, Accepted, 8th December, 2008, Published online, 12th December, 2008.  
DOI: 10.3987/COM-08-S(D)78

## MAITOTOXIN-PHOTOACTIVE PROBE BINDS TO MEMBRANE PROTEINS IN BLOOD CELLS

**Keiichi Konoki,<sup>a,b,\*</sup> Masaki Hashimoto,<sup>a</sup> Kaori Honda,<sup>a</sup> Kazuo Tachibana,<sup>a</sup>  
Rie Tamate,<sup>b</sup> Futoshi Hasegawa,<sup>b</sup> Tohru Oishi,<sup>b</sup> and Michio Murata<sup>b,\*</sup>**

<sup>a</sup>Department of Chemistry, School of Science, The University of Tokyo, Hongo,  
Bunkyo-ku, Tokyo 113-0033, Japan

<sup>b</sup>Department of Chemistry, Graduate School of Science, Osaka University, 1-1  
Machikaneyama, Toyonaka, Osaka 560-0043, Japan

FAX: (+81)-6-6850-5774, E-mail: konoki@biochem.tohoku.ac.jp (K. K.) and  
murata@ch.wani.osaka-u.ac.jp (M. M.)

**Abstract** – The photoactive and biotinylating ligand was prepared from MTX and maleimide-conjugated Hatanaka reagent with use of Diels-Alder reaction. Blood cells were subjected to affinity labelling experiments using the ligand thus obtained. The labelled band on SDS-PAGE was replaced not by MTX but by brevetoxin B (PbTx2), which suggested the presence of binding proteins in blood cells. Screening of polyether compounds for MTX inhibitory activity using Ca<sup>2+</sup> flux assays in C6 cells disclosed that a synthetic fragment of the hydrophilic portion of MTX inhibited the MTX activity.

## INTRODUCTION

Maitotoxin (MTX, **1**) was first discovered as one of the toxins responsible for ciguatera,<sup>1</sup> seafood poisoning caused by ingestion of coral reef fish, and was later shown to be a metabolite of the dinoflagellate *Gambierdiscus toxicus*.<sup>2</sup> The chemical structure of **1** was determined by spectroscopic methods<sup>3</sup> and by chemical synthesis.<sup>4,5</sup> The striking structural feature is the presence of 32 ether rings encompassing hydroxy and sulfate groups, which comprise a remarkably large heterocyclic molecule. In addition to these unique chemical features, MTX has extremely potent bioactivities exemplified by LD<sub>50</sub> against mice (50 ng/kg, ip.).<sup>3</sup> MTX elicits Ca<sup>2+</sup> influx in virtually all cells and tissues so far tested as reviewed by John Daly and Fabian Gusovsky.<sup>6</sup> This elevation in intracellular calcium concentration leads

Dedication to the great contribution of Dr. John Daly to natural product chemistry.

to secondary events; e.g., phosphoinositide breakdown,<sup>7</sup> arachidonic acid release,<sup>8</sup> muscle contraction,<sup>9</sup> and secretion of dopamine,<sup>10</sup> norepinephrine,<sup>11</sup> and insulin,<sup>12</sup> some of which are elicited by depolarization of the membrane potential.<sup>13</sup> Electrophysiological studies have revealed that channels activated by MTX are non-selective and voltage-independent.<sup>14</sup> On the other hand, some studies indicated that MTX-induced membrane depolarization is essentially independent of  $\text{Ca}^{2+}$  and due mainly to influx of  $\text{Na}^+$ , which suggest the possibility that the direct action of MTX on biomembranes does not always involve  $\text{Ca}^{2+}$  influx, but can be due to the entry of other cations. Many hydrophobic amines, which potentially interact with cation channel proteins to alter their conductivity, have been reported to block the actions of MTX, such as nifedipine, verapamil, SK&F 96365, tetrahexylammonium salt,<sup>15,16</sup> and so on. On the other hand, some non-amine inhibitors of MTX activity, which possess higher probability to interact directly with the MTX target rather than ion channels that are indirectly activated by MTX, are reported so far. One of examples is glycosphingolipid GM1, which has been reported to be a potent blocker of MTX-induced  $\text{Ca}^{2+}$  influx in bovine aortic endothelial cells.<sup>17</sup> Our group has recently demonstrated that synthetic fragments of MTX and structural mimics such as PbTx2 and other ladder-shaped polyethers potentially inhibited the biological activities of MTX.<sup>18</sup> The establishment of the complete chemical structure of MTX allowed us to deduce the three-dimensional shape of the molecule,<sup>4,18</sup> which implies possible interactions with biomembranes. The hydrophobic polycyclic ethers from rings R through F' in MTX (**1**) could penetrate into a plasma membrane, as suggested for other ladder-shaped polyethers,<sup>19,20,21,22</sup> whereas the polyhydroxy groups residing on rings A through Q along with two anionic sulfate esters keeping this hydrophilic half outside the membrane.

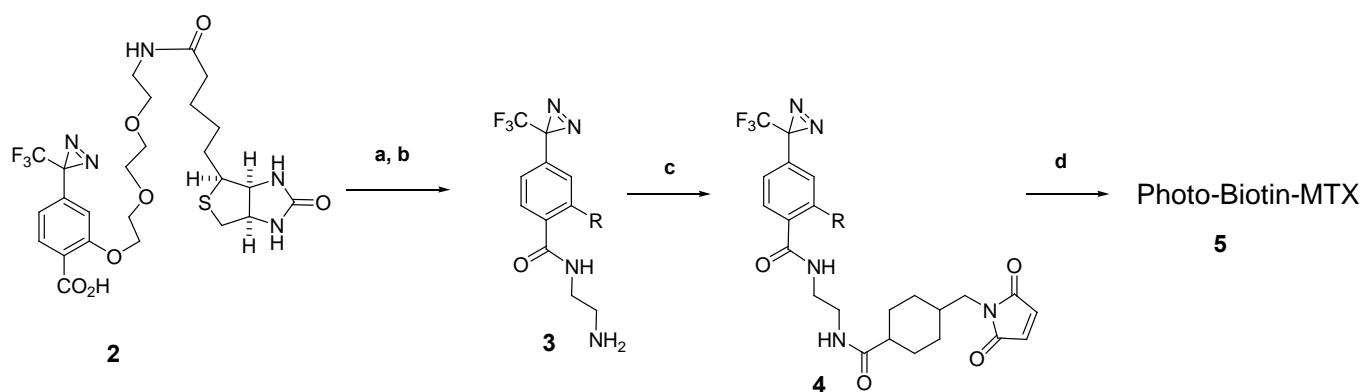
Photoaffinity labeling<sup>23</sup> is one of the most frequently used methods to identify the target proteins of drugs and other bioactive compounds. However, this methodology is usually applied for small molecules or small peptides, thus leaving its applicability to larger non-peptidic compounds like MTX. In this study we prepared a photoactive and biotinylated ligand of MTX and attempted to identify the binding proteins. On the competitive inhibition of MTX-binding, small fragmental structures and structure mimics were used in lieu of MTX.<sup>24</sup>

## RESULTS

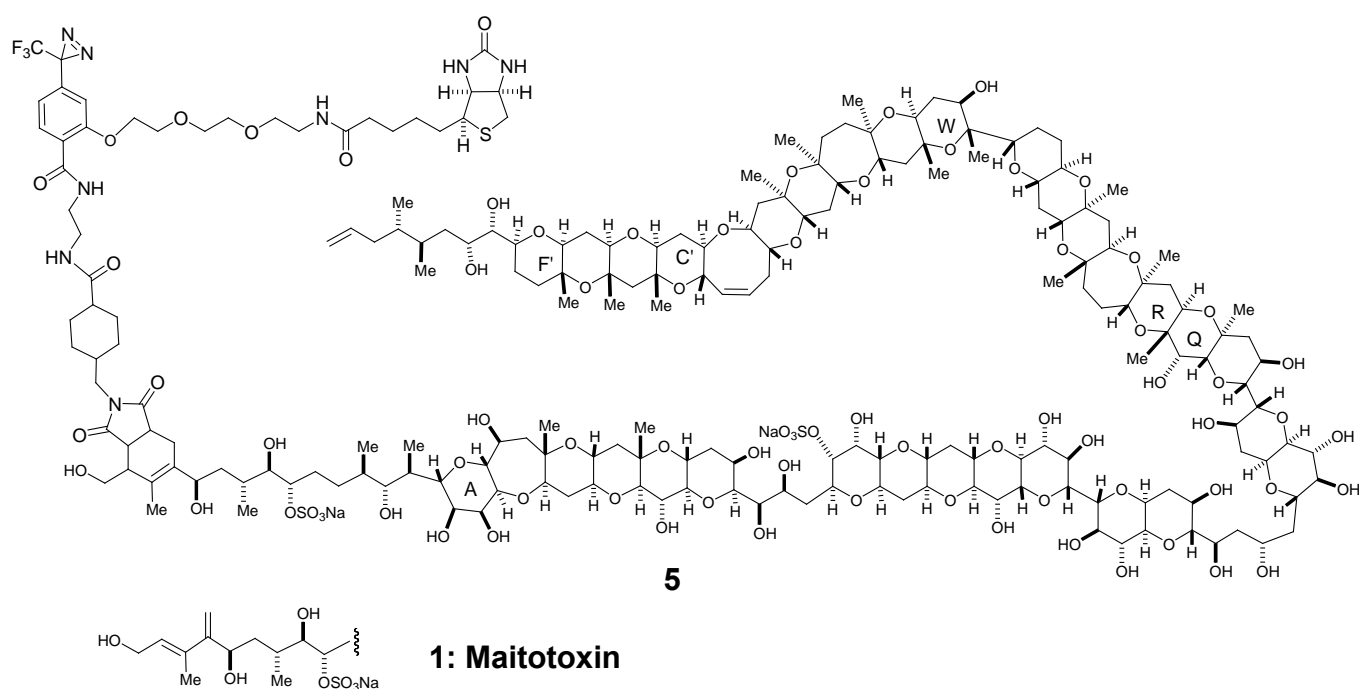
### Preparation of Photoaffinity Probe of MTX.

Photoactive-biotinylating agent (**2**, Hatanaka Reagent) was kindly gifted by Prof. Y. Hatanaka.<sup>23</sup> As shown in the Scheme 1, the reagent was treated with mono-*N*-(*t*-Boc)-ethylenediamine in the presence of EDC and HOBt followed by deprotection by TFA to provide monoamine amide **3**. The coupling with maleimide-containing activated carboxylic acid proceeded to give rise to photoactive-biotinylating dienophile **4**. The crucial Diels-Alder reaction with the diene group of MTX was carried out under high

pressure of 1.0 GPa for 3 days. Production of the Diene-Alder adduct **5** was confirmed by HPLC and mass spectrometry as shown in Figure 1. Although an attempt to measure NMR was unsuccessful due to a small sample amount and large molecular weight, the mass number obtained in ESIMS completely agreed with that of intended product **5**. The biological activity of the product was examined by  $\text{Ca}^{2+}$  assays using erythrocytes where the product showed significantly  $\text{Ca}^{2+}$  influx into blood cells (data not shown).



Scheme 1. (a) Mono-*N*-(*t*-Boc)-ethylenediamine, EDC, HOBt, 1:1 THF-MeOH, rt, 3 h; (b) 1:1 TFA- $\text{CH}_2\text{Cl}_2$ , rt, 30 min; (c) Succinimidyl-4-(*N*-maleimidomethyl) cyclohexane-1-carboxylate,  $\text{Et}_3\text{N}$ , DMF, rt, 4 h (35% in 3 steps); (d) MTX, 10:46:44 MeOH- $\text{H}_2\text{O}$ -MECN, 1 x 1.0 GPa, rt, 3 days



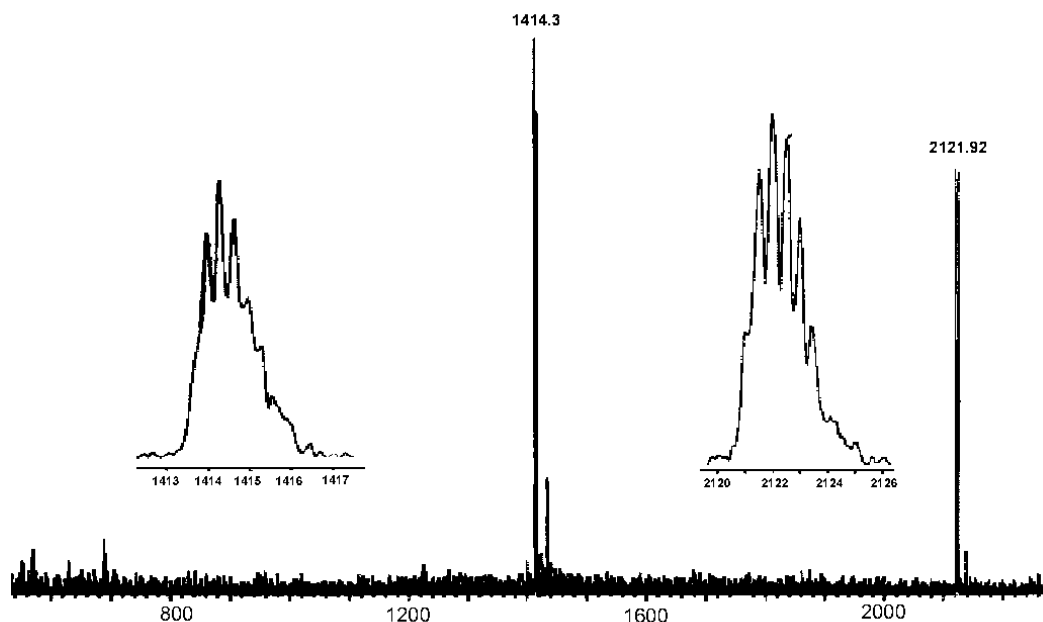
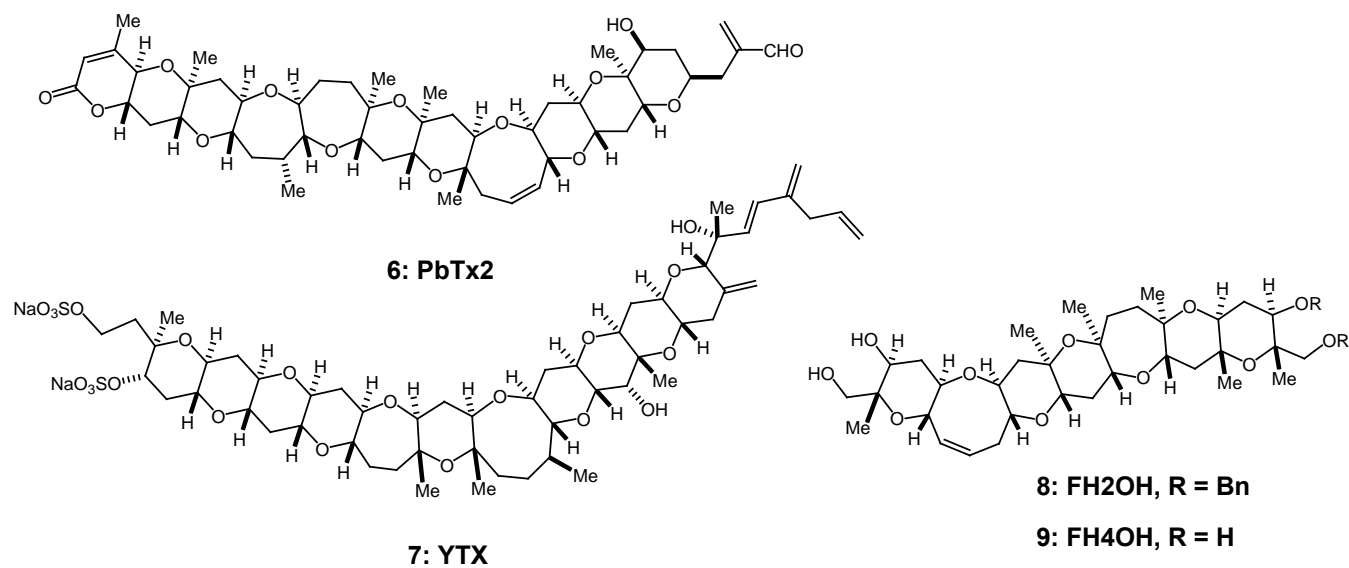


Figure 1. Mass spectrum of Diels-Alder adduct **5**. The Diels-Alder product after HPLC purification was subjected to ESI-TOF mass spectrometer. The ion peaks at 1414.3 and 2121.92 correspond to  $[M-\text{Na}_2-\text{H}]^{3-}$  and  $[M-\text{Na}_2]^{2-}$ , respectively. Their isotopic patterns (inset figures) for those at  $m/z$  1414 and 2121 depict that these ions are divalent and trivalent, respectively.



### Inhibition of MTX Activity by Synthetic Fragments and Yessotoxin

Since preliminary binding experiments using tritium-labeled MTX disclosed that MTX did not block or even enhanced the binding of the tritium ligand, we sought a better inhibitor for identification of an MTX target. We examined synthetic fragments of MTX (**8** and **9** corresponding W-C' rings) together with natural products brevetoxin B (PbTx2, **6**) and yessotoxin (YTX, **7**). MTX markedly stimulated  $\text{Ca}^{2+}$  influx in rat glioma C6 cells as reported previously;<sup>18</sup> MTX (1 nM) induced approximately 10-fold  $\text{Ca}^{2+}$  influx, which was dose-dependently inhibited by PbTx2 (Figure 2). Synthetic fragments **8** and **9** of hydrophobic portions of MTX<sup>24</sup> were subjected to the inhibition assays. Dihydroxy and dibenzyloxy derivative **8** showed the inhibitory activity while its tetrahydroxy derivative **9** and YTX were virtually devoid of the activity. For erythrocytes, MTX (10 nM)-induced hemolysis was blocked down to 20% by fragment **8** (10  $\mu\text{M}$ ) as we reported.<sup>24</sup> These findings together with our previous data<sup>18,24</sup> imply that the inhibitory activity of MTX-induced  $\text{Ca}^{2+}$  influx by polyether compounds is decreased by hydrophilic groups and increased by hydrophobic substitution.

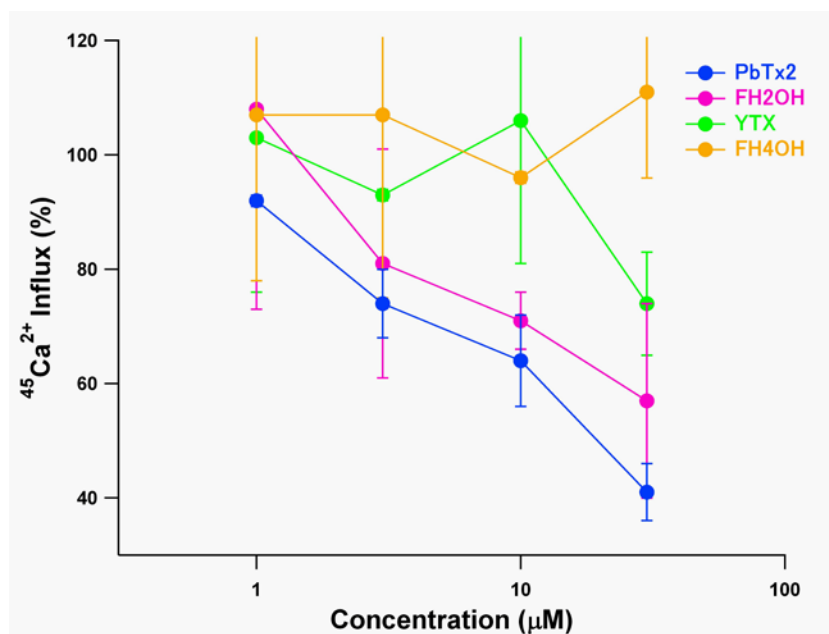


Figure 2. Blockade of MTX (1 nM)-induced  $\text{Ca}^{2+}$  influx in C6 cells by natural and synthetic polyether compounds. Error bars were obtained from three experiments in duplicate. For the conditions of inhibition assays, see Experimental section.

### Photoaffinity Labeling Experiments.

We have previously disclosed that MTX elicited  $\text{Ca}^{2+}$  influx in erythrocyte ghosts, whereas showing no activity to phospholipid liposomes.<sup>18</sup> The finding indicated the presence of MTX-binding protein(s) on the erythrocyte membrane, which allowed us to use erythrocyte ghosts as the membrane protein library.

The inhibition experiments described above demonstrated PbTx2 to be the best inhibitor among those tested. We thus used PbTx2 to evaluate specific photolabeling by the probe **5**. After the erythrocyte ghosts were incubated with **5** either in the presence or absence of PbTx2 for 2.5 h, the mixture was irradiated at 365 nm for 60 min. The electrophoresis in Figure 3 revealed that one band at around 23 kDa was significantly faded in the presence of PbTx2; similar experiments were carried out with MTX but the competitive displacement of labeling was not observed. However, since the possible target protein at 23 kDa was covalently conjugated with the large photoactive probe of 4 kDa (**5**) possessing two sulfate groups, it was very difficult for us to estimate the molecular weight of the original protein. Due to a short supply of the labeling probe and the poor reproducibility of the binding replacement by PbTx2 or other inhibitors, we could not so far identify the protein.

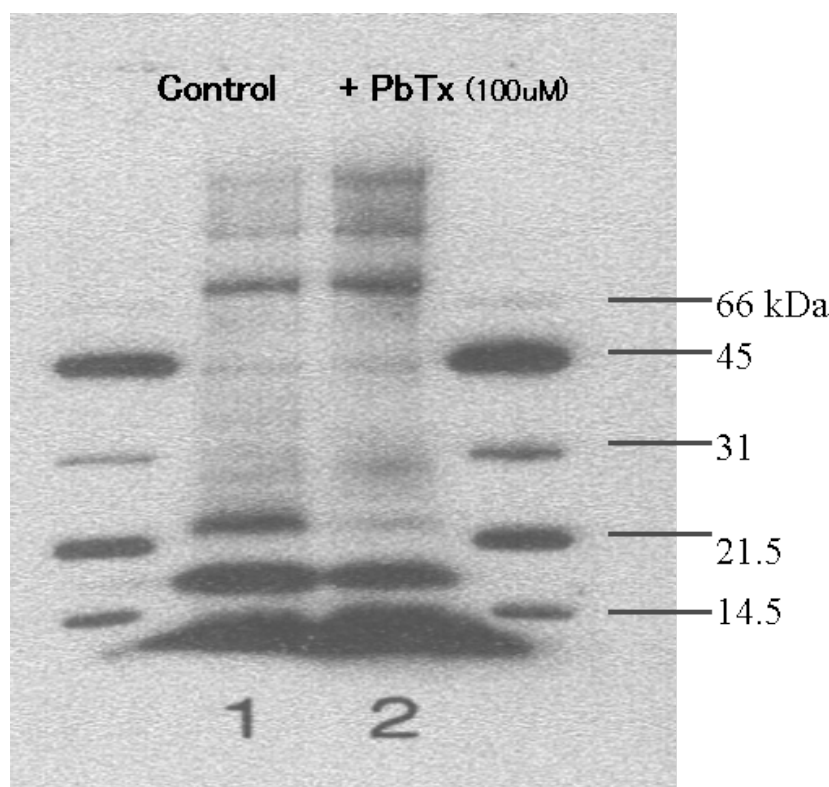


Figure 3. SDS-PAGE pattern of membrane proteins from erythrocytes with biotin-sensitive detection. Lane 1: Erythrocyte membrane proteins labeled with **5**; Lane 2: the same membrane proteins labeled with **5** in the presence of 100  $\mu$ M PbTx2 (**6**). For the sample preparation and electrophoresis conditions, see Experimental section.

## DISCUSSION

The target molecule of MTX has not yet been characterized in spite of great efforts by scientists in various fields. As seen in previous unsuccessful attempts by our group,<sup>25</sup> the difficulties in biochemical experiments with MTX are often caused by adhesion of MTX to the surface of the cells and containers. The complicated structure of MTX also prevented us from applying conventional chemical methods that

were originally designed for drugs or smaller molecules. These fruitless efforts using MTX itself prompted us to seek for an alternative agent with a smaller molecular weight.<sup>16</sup> The chemical structure of MTX implied us that PbTx2 might be an appropriate substitute for MTX because of their close resemblance in the hydrophobic polyether structures. In addition to PbTx2, synthetic fragment **8** with hydrophobic benzyloxy sidechains revealed the comparable inhibitory activity (Figure 2) despite of its fewer ether rings than those of PbTx2. As reported previously,<sup>25</sup> we examined inhibition of MTX binding to rat brain synaptosomes by PbTx2 using <sup>3</sup>H-labeled hydrogenated MTX, which was prepared in John Daly's laboratory.<sup>25</sup> The binding of tritiated-MTX to synaptosomes was often enhanced by unlabeled MTX, which was particularly unusual in binding replacement tests. This may be accountable by the mode of binding of MTX to biomembranes; one possible explanation is formation of dimers or oligomers of MTX upon binding to the target, where a mixed assembly of labeled and unlabeled MTX enhanced the total binding. When the same experiment was carried out with PbTx2 as an inhibitor, binding replacement was not observed.<sup>18</sup> This unexpected results can be explained by the manner of MTX binding to biomembranes. MTX was not completely washed out from rat brain synaptosomes even after repeated perfusions,<sup>16</sup> which suggests that its amphiphilicity stabilizes the molecule and results in abundant nonspecific binding of <sup>3</sup>H-labeled hydrogenated MTX. Thus, photolabeling experiments were carried out in this study to confirm the binding replacement of MTX by PbTx2. We successfully prepared the ligand from MTX and observed the promising band, for which binding of MTX-derivative was blocked by PbTx2. Further examinations to identify the protein have been hampered due to difficulties in preparing the ligand from MTX and in effectively labeling the protein. Rapid progress in proteomics technology will soon enable us to identify the target protein in an extremely small quantity.

The present results confirmed that the synthetic fragments prominently inhibit MTX-induced Ca<sup>2+</sup> influx, and further support the idea that the hydrophobic portion of MTX plays an important role in recognizing the target molecule. If the hydrophobic portion of MTX binds to the transmembrane  $\alpha$ -helix domain of its target proteins in a manner similar to PbTx2 binding to sodium channels,<sup>18</sup> it is plausible that a relatively high concentration of polyether compounds could mask the MTX-binding site despite their low affinities to the site.

The present experimental results together with previous findings allow us to propose a hypothetical mechanism for the biological activities of MTX:<sup>18</sup> MTX binds to a plasma membrane with its hydrophobic tail (R-F' rings), whereas its hydrophilic portion staying outside. MTX interacts to a transmembrane  $\alpha$ -helix domain of the target proteins with its hydrophobic portion to modify the protein function to allow ions to pass across membrane, which causes large Ca<sup>2+</sup> influx. Further investigations using synthetic fragments are underway in the laboratories to gain a better understanding of the action mechanism of this unique marine toxin.

## EXPERIMENTAL

### Materials

MTX was isolated from the French Polynesian strain of the dinoflagellate *Gambierdiscus toxicus* collected off Gambier Island.<sup>2</sup> The toxin was dissolved in 50 % aqueous methanol (290 nM) and kept at -30 °C until use. Rat glioma C6 cells, RPMI 1640, a penicillin-streptomycin solution, fetal bovine serum, and trypsin (tissue culture grade) were purchased from Dainippon Pharmaceutical (Osaka, Japan). <sup>45</sup>CaCl<sub>2</sub> and [<sup>3</sup>H]-leucine were from New England Nuclear (Boston, USA).

### Preparation of Photoactive-Biotinylating MTX

*Synthesis of dienophile.* Hatanaka Reagent<sup>23</sup> (20.0 mg, 0.033 mmol) in 1:1 THF-MeOH was reacted with EDC (7.0 mg, 1.1 eq), HOBt (5.4 mg, 1.2 eq) and mono-*N*-(*t*-Boc)-ethylenediamine (12.8 mg, 2.4 eq) at rt for 3 h. The mixture was evaporated, and partitioned between AcOEt (15 mL) and H<sub>2</sub>O (5.0 mL). The organic layer was washed with brine and dried over MgSO<sub>4</sub>. After evaporated, the crude mixture primarily containing *t*-Boc protected amine was directly used for the following reaction. The crude mixture dissolved in 250 μL of CH<sub>2</sub>Cl<sub>2</sub> was added dropwise to 250 μL of trifluoroacetic acid. After 30 min, the mixture was evaporated and the concentrate was dissolved in 500 μL of DMF. The solution was treated with Et<sub>3</sub>N (4.6 μL, 0.033 mmol) and succinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (12.1 mg, 0.0362 mmol, Thermo Fisher Scientific, Rockford, USA) at rt for 4 h. The mixture was concentrated and subjected to ODS column chromatography (1.0 g, YMC ODS-AM, YMC, Kyoto, Japan). The fraction eluted with 1:1 MeCN-H<sub>2</sub>O was concentrated to give the dienophile (14.3 mg, 45% in 3 steps). A part of the product was further purified with HPLC (YMC ODS AM323, φ 10 x 250 mm, 1.0 mL/min, 50% MeCN, 400 nm) for the following Diels-Alder Reaction.

*Diels-Alder Reaction.* Maitotoxin (50 μg, 15 nmol) dissolved in 36 μL of 83% aqueous MeOH was added to a specified reaction container for high-pressure reaction. The dienophile (639 μg, 740 nmol) dissolved in 250 μL of 50% aqueous MeCN was added to the container. The mixture was covered with hexane to fill the rest of the space in the container, which was then tightly capped. The mixture was gradually pressurized up to 1.0 x 1.0 GPa and maintained at 25 °C for 3 days. The whole mixture was transferred to a vial and the lower layer (not the hexane layer) was transferred to another vial. The mixture was dried under stream of N<sub>2</sub>. To remove the excess dienophile, the concentrate was treated with H<sub>2</sub>O (100 μL) and washed with AcOEt (100 μL x 2), CHCl<sub>3</sub> (100 μL), and then hexane (100 μL x 2). The aqueous layer was concentrated and subjected to HPLC analysis (Develosil TMS-5, φ 4.6 x 150 mm, 35% MeCN/10 mM NH<sub>4</sub>OAc, 1.0 mL/min, 230 nm). The fractions for Diels-Alder adduct were estimated by comparison of HPLC profile with that for the mock reaction without MTX. ESI-TOF measurements

using Q-ToF 2 (Micromass, UK) to identify the fraction containing Diels-Alder reaction adduct was first carried out at Jasco International Corp (Tokyo, Japan).

### **Photoaffinity Labeling**

Two micro liters of human red blood cells were added to 96  $\mu\text{L}$  of a buffer consisting of 150 mM NaCl, 5 mM KCl, 2 mM  $\text{CaCl}_2$ , 5 mM D-glucose, and 10 mM Hepes/Tris (pH 7.4). The suspension was mixed at 0 °C for 2.5 h with 1.0  $\mu\text{L}$  of the photoactive biotinylated maitotoxin either in the presence or absence of 1.0  $\mu\text{L}$  of intact maitotoxin or PbTx2. The mixture was irradiated with a black light lamp (365 nm) for 1 h and then heated at 95 °C for 10 min.

### **SDS-PAGE**

The Sample Buffer consists of 1.0 mL of 0.5 M Tris/HCl (pH 6.8), 2.0 mL of 10% SDS, 0.6 mL of 10% DTT, 1.0 mL of glycerol, 0.4 mL of mili-Q grade water and 3 drops of 1% aqueous Bromophenol Blue. After photolabeling, the mixture was mixed with the Sample Buffer and heated at 95 °C for 10 min. The denatured sample was loaded to a polyacrylamide gel (Ready Gel J 5-20%, Biorad, Hercules, USA) as well as biotinylated protein markers (Biorad, Hercules, USA). The acryl amide gel was electroblotted to a nitrocellulose membrane. The membrane was blocked with 5% skim milk/0.1% Tween 20/PBS (T-PBS), washed 5 times with 0.1% T-PBS for 2, 2, 15, 5 and 5 min and reacted with horse raddish peroxidase-conjugated streptavidin for 1 h. ECL western blotting reagent (GE, Chalfont St. Giles, UK) was used for detection. The chemiluminescence was recorded with Hyperfilm ECL (GE, Chalfont St. Giles, UK).

### **Cell culture**

Rat glioma C6 cells were cultured at 37 °C in a humidified atmosphere of 5%  $\text{CO}_2$ /95% air with a  $\text{CO}_2$  incubator. The culture media consisted of RPMI 1640 medium supplemented with 10% fetal bovine serum, 50 units/mL penicillin, and 50  $\mu\text{g}/\text{mL}$  streptomycin. C6 cells were grown for 2 days (the cell density usually reached about  $2.4 \times 10^6$  cells/mL) and inoculated onto new media to a cell density of  $1.2 \times 10^6$  cells/mL.

### **$^{45}\text{Ca}^{2+}$ influx assays with inhibitors**

After passage, rat glioma cells were grown for 2 days, harvested by treatment of trypsin, and diluted to  $6.7 \times 10^5$  cells/mL. The detached cells were transferred to 12 well plates and [ $^3\text{H}$ ]-leucine (0.1  $\mu\text{Ci}/\text{mL}$ ) was added. After overnight incubation, the medium was replaced with 238  $\mu\text{L}$  of buffer A containing 150 mM NaCl, 5 mM KCl, 2 mM  $\text{CaCl}_2$ , 5 mM glucose, and 50 mM Hepes (pH 7.4 adjusted by Tris), along

with an inhibitor tested. After 12 min of preincubation, 50  $\mu\text{L}$  of buffer A containing  $^{45}\text{CaCl}_2$  (1.5  $\mu\text{Ci}/\text{mL}$ ), and 12  $\mu\text{L}$  of MTX solution in 50% aqueous methanol were added to the media. The cells were incubated for 12 min and then washed three times with buffer A followed by solubilization with 250  $\mu\text{L}$  of 1% sodium dodecyl sulfate (SDS) in 0.5 M NaOH at 37°C for 20 min. The solution was transferred to a scintillation vial (20 mL), neutralized with 250  $\mu\text{L}$  of 0.5 M HCl, and mixed with 5 mL of scintillation cocktail. The radioactivity was measured with a scintillation counter set for simultaneous counting of  $^3\text{H}$  and  $^{45}\text{Ca}$ . Differences in the growth of the cells among microplate wells were normalized by the  $^3\text{H}$ -count for incorporated [ $^3\text{H}$ ]-leucine.

## ACKNOWLEDGEMENTS

This work was supported by Grant-In-Aids for Scientific Research (A) (No. 15201048) and (S) (No. 18101010), for Priority Area (A) (No. 16073211) from MEXT, Japan, and by a grant from the CREST, Japan Science and Technology Corporation. We are grateful to late Dr. John W. Daly for discussion and collaboration, and to Prof. Takeshi Yasumoto for encouragements of maitotoxin research. We also thank Prof. Masaharu Nakamura, Kyoto University, for Diels-Alder reaction, and Dr. Nobuaki Matsumori in our group for discussion.

## REFERENCES

1. T. Yasumoto, R. Bagnis, and J. P. Vernoux, *Bull. Jpn. Soc. Fish.*, 1976, **42**, 359.
2. A. Yokoyama, M. Murata, Y. Oshima, T. Iwashita, and T. Yasumoto, *J. Biochem.*, 1988, **104**, 184.
3. M. Murata, H. Naoki, S. Matsunaga, M. Satake, and T. Yasumoto, *J. Am. Chem. Soc.*, 1994, **116**, [7098](#).
4. M. Sasaki, N. Matsumori, T. Maruyama, T. Nonomura, M. Murata, K. Tachibana, and T. Yasumoto, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 1672.
5. W. Zheng, J. A. DeMattei, J.-P. Wu, J. J.-W. Duan, L. R. Cook, H. Oinuma, and Y. Kishi, *J. Am. Chem. Soc.*, 1996, **118**, 7946.
6. F. Gusovsky and J. W. Daly, *Biochem. Pharmacol.*, 1990, **39**, 1633.
7. F. Gusovsky, T. Yasumoto, and J. W. Daly, *FEBS Lett.*, 1989, **243**, 307.
8. O. H. Choi, W. L. Padgett, Y. Nishizawa, F. Gusovsky, T. Yasumoto, and J. W. Daly, *Mol. Pharmacol.*, 1989, **37**, 222.
9. Y. Ohizumi, A. Kajiwara, and T. Yasumoto, *J. Pharmacol. Exp. Ther.*, 1983, **227**, 199.
10. M. Tagliatalata, S. Amoroso, T. Yasumoto, G. D. Renzo, and L. Annunziato, *Brain Res.*, 1986, **381**, [356](#).
11. M. Takahashi, M. Tatsumi, Y. Ohizumi, and T. Yasumoto, *J. Biol. Chem.*, 1983, **258**, 10944.

12. D. Soergel, F. Gusovsky, T. Yasumoto, and J. W. Daly, *J. Pharmacol. Exp. Ther.*, 1990, **255**, 1360.
13. D. Xi., F. M. Van Dolah, and J. S. Ramsdell, *J. Biol. Chem.*, 1992, **267**, 25025.
14. L. I. Escobar, C. Salvador, M. Martinez, and L. Vaca, *J. Neurobiol.*, 1998, **6**, 59.
15. J. W. Daly, J. Lueder, W. L. Padgett, S. Yangmee, and F. Gusovsky, [\*Biochem. Pharmacol.\*, 1995, \*\*50\*\*, 1187.](#)
16. K. Konoki, M. Hashimoto, M. Murata, K. Tachibana, and T. Yasumoto, *J. Natur. Toxins*, 1996, **5**, 209.
17. J. P. Bressler, L. Belloni-Olivi, and S. Forman, [\*Life Sci.\*, 1993, \*\*54\*\*, 49.](#)
18. K. Konoki, M. Hashimoto, T. Nonomura, M. Sasaki, M. Murata, and K. Tachibana, *J. Neurochem.*, 1998, **70**, 409.
19. V. L. Trainer, D. G. Baden, and W. A. Catterall, *J. Biol. Chem.*, 1994, **269**, 19904.
20. R. E. Gawley, K. S. Rein, G. Jeglitsch, D. J. Adams, E. A. Theodorakis, J. Tiebes, K. C. Nicolaou, and D. G. Baden, [\*Chem. Biol.\*, 1995, \*\*2\*\*, 533.](#)
21. S. Matile, N. Berova, and K. Nakanishi, [\*Chem. Biol.\*, 1996, \*\*3\*\*, 379.](#)
22. S. Matile and K. Nakanishi, [\*Angew. Chem., Int. Ed. Engl.\*, 1996, \*\*35\*\*, 757.](#)
23. T. Tomohiro, M. Hashimoto, and Y. Hatanaka, [\*Chem. Rec.\*, 2005, \*\*5\*\*, 385;](#) Y. Hatanaka, M. Hashimoto, and Y. Kanaoka, [\*J. Am. Chem. Soc.\*, 1998, \*\*120\*\*, 453;](#) Y. Hatanaka, M. Hashimoto, and Y. Kanaoka, [\*Bioorg. Med. Chem.\*, 1994, \*\*2\*\*, 1367;](#) Y. Hatanaka, M. Hashimoto, H. Kurihara, H. Nakayama, and Y. Kanaoka, [\*J. Org. Chem.\*, 1994, \*\*59\*\*, 383.](#)
24. T. Oishi, F. Hasegawa, K. Torikai, K. Konoki, N. Matsumori, and M. Murata, [\*Org. Lett.\*, 2008, \*\*10\*\*, 3599.](#)
25. M. Murata, F. Gusovsky, T. Yasumoto, and J. W. Daly, [\*Eur. J. Pharmacol.\*, 1992, \*\*227\*\*, 43;](#) M. Murata, M. Sasaki, A. Yokoyama, T. Iwashita, F. Gusovsky, J. W. Daly, and T. Yasumoto, *Bull. Soc. Path. Exot.*, 1992, **85**, 470.
26. M. Estacion, H. B. Nguyen, and J. J. Gargus, *Am. J. Physiol.*, 1994, **270**, C1145.