

Production and Characterization of Antibodies against Microcystins

FUN S. CHU,^{1*} XUAN HUANG,¹ R. D. WEI,¹ AND W. W. CARMICHAEL²

Food Research Institute and Department of Food Microbiology and Toxicology, University of Wisconsin-Madison, Madison, Wisconsin 53706,¹ and Department of Biological Sciences, Wright State University, Dayton, Ohio 45435²

Received 3 April 1989/Accepted 11 May 1989

Antibodies against a microcystin (MCYST) leucine-arginine variant (MCYST-LR) were demonstrated 4 weeks after immunization of rabbits with either MCYST-LR-polylysine- or MCYST-LR-ethylenediamine-modified bovine serum albumin. A radioimmunoassay (RIA), a direct competitive enzyme-linked immunosorbent assay (ELISA), and an indirect competitive ELISA were developed for characterization of the antibodies. Indirect ELISA and RIA revealed that MCYST-LR-ethylenediamine-bovine serum albumin was a better immunogen. Competitive RIA and direct ELISA revealed that the antibodies had good cross-reactivities with an MCYST-arginine-arginine variant (MCYST-RR), MCYST-LR, an MCYST-tyrosine-arginine variant (MCYST-YR), and nodularin (NODLN); but they had lower reactivities with variants MCYST-leucine-tyrosine (MCYST-LY) and MCYST-leucine-alanine (MCYST-LA). The antibodies did not cross-react with ozonolyzed MCYST-LR. The concentrations causing 50% inhibition of binding of reduced MCYST-LR to the antibodies by MCYST-RR, MCYST-LR, MCYST-YR, NODLN, MCYST-LA, and MCYST-LY in the RIA were 43, 105, 112, 503, 671, and 1,920 ng/ml, respectively. The concentrations causing 50% inhibition of binding of MCYST-LR-horseshoe peroxidase to the antibodies by MCYST-RR, MCYST-LR, MCYST-YR, NODLN, MCYST-LY, and MCYST-LA in the ELISA were 1.75, 2.2, 3.4, 4.6, 50, and 114 ng/ml, respectively.

Recent investigations of animal and human water-based diseases caused by certain freshwater cyanobacteria, including *Anabaena flos-aquae*, *Microcystis aeruginosa*, *Oscillatoria agardhii*, and *Nodularia spumigena*, have led to the isolation of a series of penta- or heptapeptide toxins (Fig. 1) referred to as microcystins (MCYSTs) and nodularin (NODLN) (1, 4, 5, 17; W. W. Carmichael, V. R. Beasley, D. Bunner, J. Eloff, I. Falconer, P. Gorham, K.-I. Harada, M.-J. Yu, T. Krishnamurthy, R. E. Moore, K. L. Rinehart, M. T. Runnegar, O. M. Skulberg, and M. Watanabe, *Letter, Toxicol* **26**:921-923). These toxins cause intermittent but repeated poisonings of wild and domestic animals in many parts of the world, as well as liver damage, gastroenteritis, diarrhea, and dermatitis in humans (12). These toxins are low-molecular-weight monocyclic peptides (824 to 1,044 daltons) with intraperitoneal 50% lethal doses (in mice) of approximately 60 to 200 µg/kg. The current method for analysis and purification of these toxins involves organic solvent extraction and cleanup, followed by high-performance liquid chromatography (13, 14, 16). The mouse bioassay is most frequently used to detect the toxins in cyanobacterial water blooms (3, 4). With the recent development of immunoassays for a number of low-molecular-weight toxins, including mycotoxins (6-11) and marine toxins (2, 15; Y. Hokama, M. N. Kobayashi, L. N. Nakagawa, A. Y. Asahina, and J. T. Miyahara, *Abstr. 7th Int. Symp. Mycotoxins and Phycotoxins*, p. 10, 1988; T. Uda, *Abstr. 7th Int. Symp. Mycotoxins and Phycotoxins*, p. 12, 1988), attempts at the production of antibodies against MCYST have been made. Kfir et al. (15) have reported a method for the production of monoclonal antibodies against MCYST by immunizing mice with an immunogen in which the leucine-alanine variant of MCYST (MCYST-LA) was conjugated to polylysine. An indirect enzyme-linked immunosorbent assay (ELISA) involving the coating of MCYST-LA directly to the microtiter plate was used to monitor the antibody titers; it

was also used for the MCYST assay. Most recently, polyclonal antibodies against MCYST have been produced by Brooks and Codd (2). They developed a sandwich-type immunoassay for the determination of hepatotoxin in cyanobacteria. However, the sensitivity of the immunoassays developed by both groups was low, and the standard curve for the assay was in the microgram range. In the present study, the MCYST-leucine-arginine variant (MCYST-LR) was used with a different approach to immunogen preparation. Details for the production and characterization of the antibodies as well as protocols for a radioimmunoassay (RIA), an indirect ELISA, and a direct competitive ELISA are described here.

MATERIALS AND METHODS

Materials. The following MCYSTs were obtained from the indicated sources: MCYST-LR (*M. aeruginosa* PCC-7820), MCYST-LA (toxic water bloom of Eau Claire, Wis.), MCYST-arginine-arginine (MCYST-RR; *M. viridis* TAC-44), MCYST-tyrosine-arginine (MCYST-YR; *M. aeruginosa* M-228), and NODLN (*N. spumigena* L575). Some MCYST standards, including MCYST-LR, MCYST-RR, MCYST-LA, and MCYST-leucine-tyrosine (MCYST-LY), were also kindly supplied by H. Siegelman (Brookhaven National Laboratory). The ozonolysis product (cyclic heptapeptide minus the 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-deca-4,6-dienoic acid [ADDA] moiety) of MCYST-LR was provided through the courtesy of A. Dahlem (University of Illinois). It was prepared by the method of Rinehart et al. (17). Bovine serum albumin (BSA; RIA grade), poly-L-lysine (molecular weight, 58,000), *N*-acetylmuramyl-L-alanyl-D-isoglutamine (NAMAG), and 30% hydrogen peroxide were obtained from Sigma Chemical Co. (St. Louis, Mo.). Ethylenediamine (EDA)-modified BSA (EDA-BSA) was prepared by the method of Chu et al. (10). *N*-Hydroxysuccinimide, *N,N'*-dicyclohexylcarbodiimide, and 1-(3-dimethylamino-propyl)-3-ethylcarbodiimide (EDPC) were obtained from Aldrich Chemical Co., Inc. (Milwaukee, Wis.). Complete Freund adjuvant containing *Mycobacterium tuberculosis*

* Corresponding author.

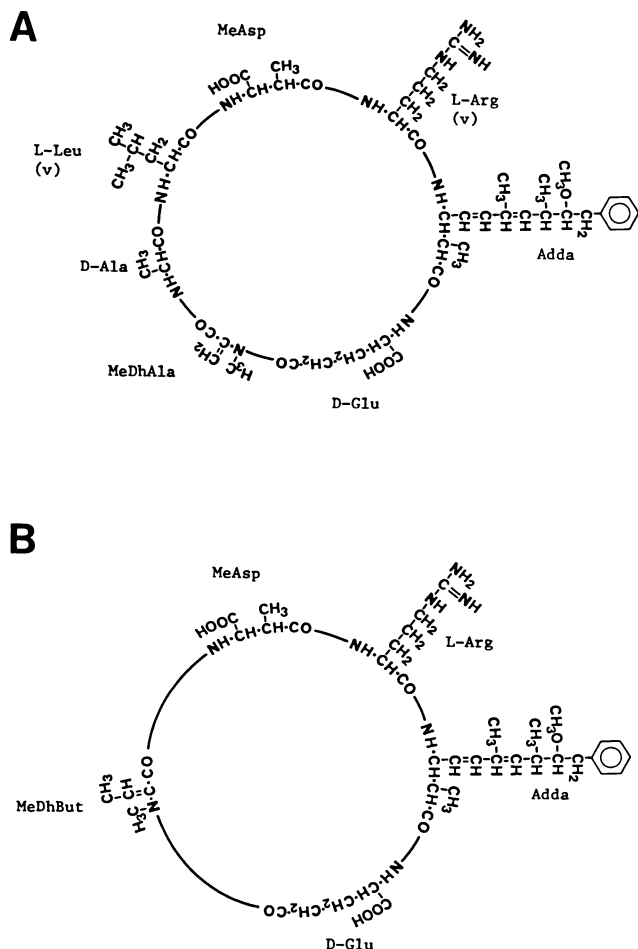


FIG. 1. Structures of microcystin-LR (A) and nodularin (B). Amino acids L-Leu and L-Arg in MCYST variant LR (A) were replaced by L-Arg and L-Arg, L-Tyr and L-Arg, L-Leu and L-Tyr, and L-Leu and L-Ala in MCYST variants RR, YR, LY, and LA, respectively. MeDhAla, Methyl-dehydro-alanine; MeDhBut, methyl-dehydro-butyrine.

H37 Ra and incomplete Freund adjuvant were obtained from Difco Laboratories (Detroit, Mich.). Goat anti-rabbit immunoglobulin G (IgG)-peroxidase conjugate and horseradish peroxidase (HRP; ELISA grade) were obtained from Boehringer Mannheim Biochemicals (Indianapolis, Ind.). ELISA microtiter plates and minisorp RIA tubes were purchased from Nunc (Roskilde, Denmark). The HRP substrate, *ortho*-phenylenediamine tablets (OPD; 4.0 mg per tablet), was kindly supplied by Idexx (Portland, Maine). Female albino rabbits were purchased from Smith's Rabbitry (Seymour, Wis.) and were tested to be free of *Pasteurella* spp. before use. All chemicals and organic solvents were reagent grade or better.

Preparation of tritiated, reduced MCYST. MCYST-LR (0.5 mg) was reduced with 3.4 μ mol of NaB^3H_4 (specific activity, 74.0 Ci/mmol; DuPont, NEN Research Products, Boston, Mass.) in 1 ml of redistilled 2-propanol at room temperature (20°C) overnight. The reaction was terminated by the addition of 0.85 ml of ethanol and 0.15 ml of diluted acetic acid (0.1% glacial acetic acid in ethanol). Reversed-phase thin-layer chromatography (RP TLC) and autoradiography analyses revealed that the toxin was converted to

reduced MCYST-LR. Tritiated toxin was purified by RP TLC (precoated SiC_{18}F TLC plate [J. T. Baker Chemical Co., Phillipsburg, N.J.], developed in *n*-butanol-acetic acid- H_2O ; 40:6:15). The product appeared as a single spot (R_f , 0.88) in the RP TLC and autoradiography analyses. This tritiated, reduced MCYST-LR was used in the RIA for determination of the antibody titers and specificity.

Preparation of immunogens. MCYST-LR was conjugated to EDA-BSA in the presence of a water-soluble carbodiimide (EDPC) under the following conditions. In a typical experiment, 5 mg of MCYST-LR was first dissolved in 0.08 ml of ethanol and diluted with 0.32 ml of distilled water (pH 5.0). This solution, together with an EDA-BSA solution (20 mg in 0.4 ml of water [pH 5.0]) was adjusted to pH 5.0 with a few drops of 0.2 N NaOH and added dropwise to an EDPC solution (1.32 g of EDPC in 1.5 ml of water [pH 5.0]). The reaction was carried out at room temperature for 5 h, with the pH maintained at pH 5.0 throughout the reaction. After the reaction, the mixture was dialyzed against 2 liters of 0.1 M NaCl at 6°C for 24 h and lyophilized.

The method of Kfir et al. (15) was used for the preparation of a MCYST-LR-polylysine (PLL) conjugate for immunization. In this method an adjuvant peptide, NAMAG, was included. In a typical experiment, 3.0 mg of MCYST-LR, 1.48 mg of NAMAG, and 11.9 mg of PLL in 6.0 ml of distilled water were incubated at room temperature in the presence of 600 mg of EDPC. All the reactants were dissolved in 2 ml of distilled water, with the pH adjusted to 5.0 before and after mixing. After 30 min of reaction, another 600 mg of EDPC was added and incubated at 6°C overnight. The solution was dialyzed against 2 liters of distilled water for 3 days, with the water changed once a day.

Conjugation of MCYST-LR to PLL for indirect ELISA. A different method was used for the preparation of the MCYST-LR-PLL used in the indirect ELISA. Instead of using the water-soluble carbodiimide EDPC as the coupling reagent, conjugation of MCYST-LR to PLL for the indirect ELISA was achieved by the *N*-hydroxysuccinimide method (8). In a typical experiment, 1.8 mg of MCYST-LR was added to a solution containing 0.28 mg of *N*-hydroxysuccinimide (in 0.28 ml of dimethylformamide) and 0.5 mg of *N,N'*-dicyclohexylcarbodiimide (in 0.5 ml of dimethylformamide). After incubation at room temperature for 30 min, the activated succinimide ester was added dropwise to a PLL solution (2.7 mg in 2 ml of 0.13 M freshly prepared bicarbonate) and stirred at room temperature for 1 h. The reaction mixture was then dialyzed against 2 liters of 0.01 M sodium phosphate buffer (pH 7.5) containing 0.85% NaCl (phosphate-buffered saline [PBS]) for 3 days, with the buffer changed twice, and lyophilized.

Preparation of MCYST-LR-peroxidase. Conjugation of MCYST-LR to peroxidase was achieved by a water-soluble carbodiimide (EDPC) method. In a typical experiment, 0.5 mg of MCYST-LR (0.51 μ mol) in 1 ml of 25% ethanol was mixed with 15 mg of EDPC. A HRP solution (1.12 mg of HRP in 1 ml of 25% ethanol) was added dropwise to this solution, followed by the addition of 15 mg of solid EDPC. After the reaction mixture was stirred at room temperature for 30 min, another 15 mg of solid EDPC was added. The reaction was then carried out in a cold (6°C) room with stirring. After reaction overnight, the mixture was dialyzed against 2 liters of 0.01 M ammonium bicarbonate (pH 7.1) at 6°C for 48 h, with the buffer solution changed twice a day. The dialysate was lyophilized and stored at -20°C.

Production of antibody. The immunization schedule and methods of immunization were essentially the same as those

described previously for T-2 toxin by the multiple injection technique (9). Two immunogens, i.e., MCYST-LR-EDA-BSA and MCYST-LR-PLL, were tested in six rabbits, three for each immunogen. Each rabbit was injected intradermally with 500 μg of the immunogen in 1.0 ml of 0.1 M PBS (pH 7.4) with 2.0 ml of complete Freund adjuvant. For booster injections, 500 μg of antigen in 1.0 ml of PBS and 2.0 ml of incomplete Freund adjuvant was used. The collected antisera were precipitated with $(\text{NH}_4)_2\text{SO}_4$ to a final saturation of 33.3%. The precipitates were redissolved in water and reprecipitated twice. Finally, the precipitates were reconstituted to half of the original volume with distilled water, dialyzed against distilled water for 0.5 to 1 h and then against 0.01 M PBS overnight at 6°C, and lyophilized.

Direct competitive ELISA. The protocol for the direct competitive ELISA was essentially the same as that we have described previously for aflatoxin B₁ (8), except that in the antibody-coating step the antibody was diluted in 0.01 M PBS (1 to 1,000, with 0.1 ml coated onto each well). After the plate was incubated at 6°C overnight, the plate was washed with PBS-Tween (0.35 ml per well; 0.05% Tween 20 in PBS) followed by incubation with BSA-PBS (0.17 ml per well; 0.05% BSA in PBS) at 37°C for 30 min. The plate was washed again with PBS-Tween (0.35 ml per well) four times, followed by incubation with different variants of MCYST (0.05 ml per well) at different dilutions or blank buffer together with the MCYST-LR-HRP conjugate (0.1 ml per well) at 37°C for 60 min. The plate was washed four times with PBS-Tween (0.35 ml) and incubated with 0.1 ml of freshly prepared OPD-substrate solution (one tablet of OPD in 10 ml of substrate buffer, with H₂O₂ added to a final concentration of 0.002% [vol/vol]) at room temperature for 10 min, and then 0.1 ml of stopping reagent was added. The A₄₉₀ was read and samples were run in triplicate.

Indirect ELISA. The protocol for the indirect ELISA was similar to that described by Fan et al. (11). In general, 0.1 ml of MCYST-LR-PLL (1 $\mu\text{g}/\text{ml}$ in bicarbonate buffer at pH 9.5) was added to each well of a 96-well ELISA microdilution plate (plate 2-69620; Nunc). The plate was kept at 6°C overnight. After the solution was removed, the wells were washed twice with 0.16 ml and twice with 0.35 ml of the PBS-Tween buffer per well. This was followed by incubation with 0.17 ml of 0.1% gelatin in PBS (which served as the blocking agent) at 37°C for 30 min. The plate was washed four times with 0.35 ml of PBS-Tween per well to remove the excessive blocking agent. To each well, 0.1 ml of diluted anti-MCYST-LR antiserum was added, and the wells were mixed gently; the plate was incubated at 37°C for 1 h. The plate was washed with 0.32 ml of PBS-Tween four times, and 0.1 ml of goat anti-rabbit IgG-HRP conjugate at a 1:20,000 dilution in PBS containing 0.1% BSA was added to each well. After incubation at 37°C for 60 min, the plate was washed, and 0.1 ml of freshly prepared OPD substrate solution was added. After 10 min of color development in the dark at room temperature, the reaction was terminated by adding 0.1 ml of 1 N HCl. The A₄₉₀ was determined in an automatic reader (MR 600; Dynatech Industries, Inc., Alexandria, Va.).

RIA. Protocols for RIA were essentially the same as those described previously for T-2 toxin, in which an ammonium sulfate precipitation method was used to separate free and bound toxin (9). In general, 50 μl of radioactive marker ligand (10,000 to 12,000 dpm) was incubated with 0.15 ml of antiserum solution of various dilutions in PBS at room temperature for 30 min and then in a cold (6°C) room for 1 h or longer. Separation of bound and free ligand was achieved

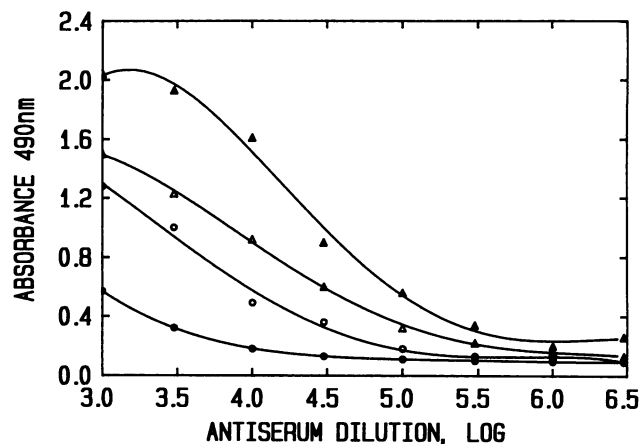


FIG. 2. Determination of antibody titers for a rabbit after immunization with MCYST-LR-EDA-BSA by indirect ELISA. Serum was obtained from rabbits after 0 (●), 4 (○), 8 (△), and 15 (▲) weeks of immunization. The numbers on the x axis are the dilution factors (in log units).

by an ammonium sulfate precipitation method described previously (9). The antibody titer was defined as the reciprocal of the amount of antiserum (in milliliters) required to give 50% binding of tritiated toxin under the conditions described. For the determination of antibody specificity, 50 μl of various concentrations of different MCYSTs was present in the assay system, in addition to the antiserum and radioactive marker.

Determination of radioactivity. Radioactivity was determined in a liquid scintillation spectrometer (model LS-5801; Beckman Instruments, Inc., Fullerton, Calif.) in 4.5 ml of Ecolume (ICN Biochemicals, Inc., Irvine, Calif.).

RESULTS AND DISCUSSION

Production of antibody. In the initial studies, the antibody titers were monitored by an RIA in which the reduced tritiated MCYST-LR was used as the marker ligand. Although the apparent antibody titers for some rabbits were very low, we found that MCYST-LR-EDA-BSA was a better immunogen than the MCYST-LR-PLL. The RIA titers for rabbits immunized with MCYST-LR-PLL were in the range of 10 to 20 compared with the titers in those immunized with MCYST-LR-EDA-BSA, which were in the range of 300-2,500. Consequently, only the antiserum obtained from the rabbits that were immunized with MCYST-LR-EDA-BSA were characterized further.

An indirect ELISA was subsequently developed and was also used for monitoring the antibody titers. The typical ELISA titration curves for the determination of antibody titers are shown in Fig. 2. Since some nonspecific binding of preimmune serum to the coated microdilution plate was observed, a preselected absorbance of 1.0 was arbitrarily used in the estimation of antibody titers. Thus, the antibody titer was defined as the antiserum dilution that would give an absorbance of 1.0 under the indirect ELISA conditions described above. Under these conditions, the antibody titers for the preimmune sera varied from 140 to 2,000. Results for the antibody titers for three rabbits immunized with MCYST-LR-EDA-BSA, as determined by both RIA and ELISA, are shown in Fig. 3. The rabbits started to elicit antibodies as early as 4 weeks after immunization, and the titers increased after each booster injection. In general, the

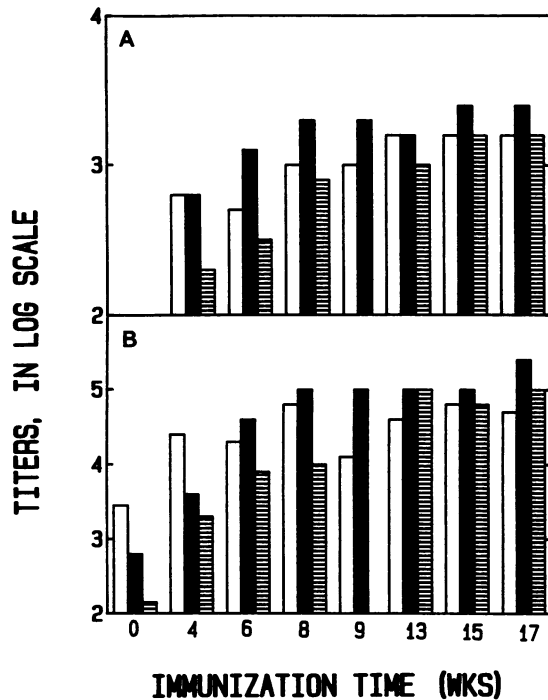


FIG. 3. Antibody titers of three rabbits after immunization with MCYST-LR-EDA-BSA determined by RIA (A) and indirect ELISA (B). Booster injections were given at 7 and 12 weeks. For three rabbits, antibody titers after immunization with MCYST-LR-PLL, as determined by RIA, were too low (in the range of 10 to 20) and so are not shown here.

antibody titers determined by ELISA were consistent with those obtained by RIA.

Antibody specificity. Both RIA and a competitive ELISA in which MCYST-LR conjugated to HRP was used as the marker were used to determine the specificity of the antibodies obtained from rabbits that were immunized with the MCYST-LR-EDA-BSA. The RIA results (Fig. 4) indicate that the antibodies were most reactive with MCYST-RR, MCYST-LR, and MCYST-YR and less so with NODLN. Cross-reactivities were least with MCYST variants MCYST-LY and MCYST-LA. The concentrations causing 50% inhibition of binding of radiolabeled, reduced MCYST-LR to the antibodies by MCYST-RR, MCYST-LR, MCYST-YR, NODLN, MCYST-LA, and MCYST-LY in the RIA were 43, 105, 112, 503, 671, and 1,920 ng/ml, respectively. The results for the competitive ELISA are shown in Fig. 5. The antibodies were most reactive with MCYST-RR, MCYST-LR, and MCYST-YR and less so with NODLN. Cross-reactivities were least with MCYST variants MCYST-LY and MCYST-LA. Very little cross-reactivity was found when the ozonized MCYST-LR was tested. The concentrations causing 50% inhibition of binding of MCYST-LR-HRP to the antibodies by MCYST-RR, MCYST-LR, MCYST-YR, NODLN, MCYST-LY, and MCYST-LA in the ELISA were 1.75, 2.2, 3.4, 4.6, 50, and 114 ng/ml, respectively. Ozonized MCYST gave only 20% inhibition of binding when 5 µg/ml was tested. Two different preparations of MCYST-RR, MCYST-LR, and MCYST-LA obtained from two different laboratories were used in this study. Results for two different preparations of MCYST-RR and MCYST-LR agreed. However, there was a slight difference in the displacement curve for MCYST-LA for the preparations ob-

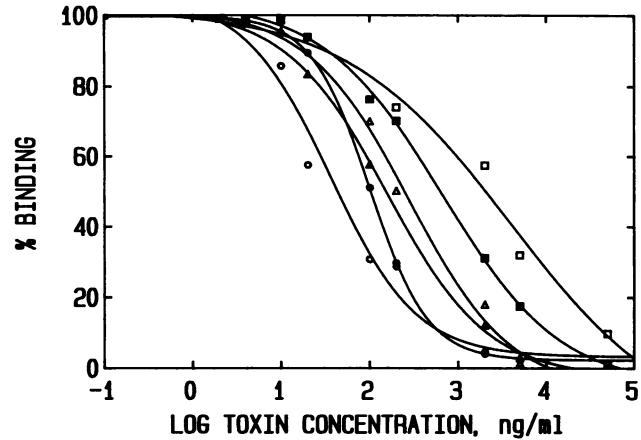


FIG. 4. Cross-reactivity of anti-MCYST-LR with different MCYST variants in a competitive RIA. ³H-labeled and reduced-MCYST-LR (12,000 dpm) was used as the marker ligand. Separation of the free and bound ligand was achieved by an ammonium sulfate precipitation method. Fifty microliters of a standard solution was used in each assay. All results were obtained from the average of three sets of experiments. The concentrations causing 50% inhibition of binding of reduced MCYST-LR to the antibodies by MCYST-RR (○), MCYST-LR (●), MCYST-YR (▲), NODLN (△), MCYST-LA (■), and MCYST-LY (□) were 43, 105, 112, 503, 671, and 1,920 ng/ml, respectively.

tained from the two laboratories. Therefore, average data are reported here.

The results indicate that the arginine residue between the two unusual amino acids, i.e., erythro-β-methyl-*d*-isopartate and the β-amino acid residue of ADDA, plays an important role in immunogenicity. In both analyses, variants

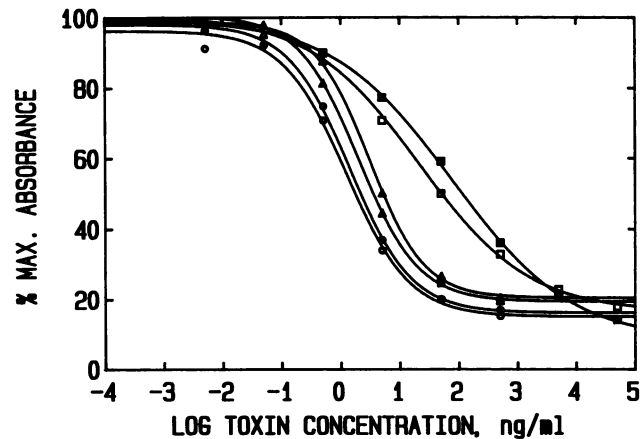


FIG. 5. Cross-reactivity of anti-MCYST-LR with different MCYST variants in a competitive direct ELISA. The microtiter plate was coated with anti-MCYST-LR-EDA-BSA antiserum (50 µl of a 1:1,000 dilution) and MCYST-LR-HRP (0.05 µg per well) was used as the marker enzyme. Fifty microliters of toxin was used in each assay. The concentrations causing 50% inhibition of binding of MCYST-LR-HRP to the antibodies by MCYST-RR (○), MCYST-LR (●), MCYST-YR (▲), NODLN (△), MCYST-LY (□), and MCYST-LA (■) in the ELISA were 1.75, 2.2, 3.4, 4.6, 50, and 114 ng/ml, respectively. Ozonized MCYST gave only 20% inhibition of binding when 5 µg/ml was tested. The concentrations (0.05 to 50,000 ng/ml or 2.5 pg to 2,500 ng per assay) shown on the x axis are in the log scale.

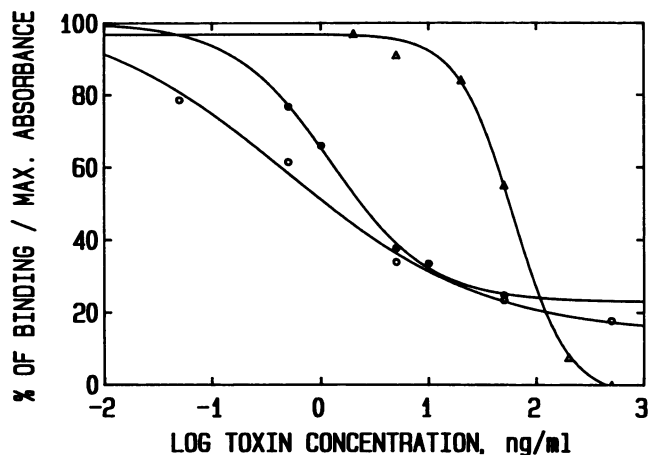


FIG. 6. Standard curves for immunoassay of MCYST-LR. In the RIA (Δ), reduced tritiated MCYST-LR was used as the marker ligand. MCYST-LR-labeled HRP was used in the direct ELISA (\bullet), whereas HRP-labeled goat anti-rabbit IgG was used in the indirect ELISA (\circ). Fifty microliters of sample was used in each assay. All data were obtained from the average of three sets of experiments. The concentrations (0.05 to 500 ng/ml or 2.5 to 25,000 pg per assay) shown on the x axis are in the log scale.

RR, LR, and YR gave similar relative cross-reactivities with the antibody. It is interesting that NODLN, the first pentapeptide in the MCYST series that also contains an arginine residue between the two unusual amino acids, cross-reacted well with the antibodies. Whereas NODLN competed favorably with the MCYST-LR-HRP conjugate in the ELISA, NODLN was less reactive in the RIA, in which the reduced MCYST-LR was used as the marker ligand. Ozonolysis of MCYST, which caused an extensive structural change in the molecule by removing the ADDA residue, resulted in a structure that the antibodies failed to recognize. Thus, present results support the recent finding of Rinehart et al. (19) that the configurations of ADDA in both toxins are similar. The overall results suggest, perhaps, that an integral part of these 2-3 amino acids, i.e., erythro- β -methyl-*d*-isoaspartate, Arg, and ADDA, plays a dominant role in expressing antibody specificity. In contrast to the present results, Kfir et al. (15) found that the monoclonal antibody raised against MCYST-LA cross-reacts with most MCYST variants LR, leucine-asparagine, LY, phenylalanine-arginine, alanine-tyrosine, and YR. Using an immunogen similar to that used in the present study, Brooks and Codd (2) found that the antibodies did not cross-react with toxic peptide extracts from *Osillatoria* or *Anabaena* cyanobacteria but gave a positive response in all cases with toxic water blooms from *M. aeruginosa* algae. The ELISA formats of two studies mentioned above differed considerably from those used in the present one. It is possible that those investigators were not able to demonstrate such differences because their assay systems were less sensitive than those used in the present study. In the later study (2), only toxic extracts were used in the tests.

The standard curves for the immunoassay of MCYST by both direct and indirect ELISA and RIA are compared in Fig. 6. The linear responses of inhibitions of binding by MCYST in the RIA and the direct and indirect competitive ELISAs were in the range of 20 to 500 (1 to 25 ng per assay), 0.5 to 10 (25 to 500 pg per assay), and 0.05 to 10 (2.5 to 500 pg per assay) ng/ml, respectively. Assuming that the concentration of MCYST to cause a 20% inhibition of binding

was the minimum detection level in those immunoassays, the minimum detection levels for MCYST-LR in RIA and the direct and indirect ELISAs would then be 1.15 ng, 20 pg, and 2.85 pg per assay, respectively. Although RIA was less sensitive than either of the ELISAs, the sensitivity could be improved by using a high-specific-radioactivity marker. Particularly, an iodinated MCYST, e.g., by using MCYST-YR, could be prepared for such purposes. Nevertheless, since ELISAs do not require radioactive compounds, they easily could be adapted for field studies. Between the two ELISAs, the direct ELISA is more versatile than the indirect one because it is less time consuming. Because all the standard curves established in the experiment were carried out in buffer solutions, the present protocols could be easily adapted for analysis of the toxin in water. However, the matrix interference problems should not be overlooked. For example, using algae collected from Lake Mendota (Madison, Wis.) in September 1988, we found (F. S. Chu, unpublished data) that an extract equivalent to 50 μ g of lyophilized algae in the assay solution interfered with the direct ELISA. The sensitivity for the analysis of MCYST in an untreated algal extract was in the parts-per-million range. Therefore, to apply the direct ELISA for MCYST in a complex matrix and to improve the sensitivity, development of sample treatment methods warrants further investigation. Research efforts in our laboratory are currently directed to these areas.

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