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Detection of T-2 Toxin by an Improved Radioimmunoassay

PAULITO A. FONTELO,[1]* JOANNE BEHELER,[1] DAVID L. BUNNER,[1] AND FUN SUN CHU[2]

Department of Early Diagnosis, Physical Sciences Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21701,[1] and the Food Research Institute and Department of Food Microbiology and Toxicology, University of Wisconsin, Madison, Wisconsin 53706[2]

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T-2 toxin in serum, urine, and saline was analyzed by a modified radioimmunoassay procedure. The specimens were added directly to the assay tubes without extraction steps. The reaction between antibody and ligands was optimal at 1 h. Albumin-coated charcoal was used to separate bound from free radioactivity. Quenching, which occurred with hemolyzed specimens, was corrected by a wet oxidation process with 60% perchloric acid and 30% hydrogen peroxide. The shorter incubation times resulted in an assay that takes less than 6 h to complete. The average affinity constant of the antibody (K_m) was 1.75×10^{10} liters/mol. The sensitivity was 1 ng per assay or 10 ng/ml. Among the other trichothecenes tested, only H-T-2 cross-reacted significantly (10.3%).

The occurrence of mycotoxins depends on such factors as temperature, humidity, food processing methods, and type of food product. Almost all food products can be contaminated with fungi and carry the potential for animal and human intoxication. The ingestion of food products contaminated with toxins from certain fungi, such as *Fusarium* and *Trichoderma*, has been associated with toxic manifestations (4, 6, 9). Epidemics of alimentary toxic aleukia attributed to trichothecene ingestion, specifically T-2, have been reported in certain regions of the U.S.S.R. (11). Because of its potential for mass intoxication, early and rapid methods for the detection of T-2 toxin in the environment, in food products, and in biological samples, such as serum or urine, are essential. The methods that are presently available, such as bioassays, high-performance liquid chromatography, and gas chromatography, are not readily available to smaller laboratories owing to the need for sophisticated instrumentation and skilled personnel. A radioimmunoassay procedure reported by Chu et al. has simplified the testing process (2). We have modified this assay and further characterized the antibody.

MATERIALS AND METHODS

Chemicals. T-2 toxin standards and other trichothecene mycotoxins were purchased from Calbiochem-Behringer Corp., La Jolla, Calif., and Mycolabs Co., Chesterfield, Mo. Tritiated T-2 with a specific activity of 13.5 Ci/mmol was prepared by the procedure of Wallace et al. (10). Rabbit anti-T-2 hemisuccinate antibody was produced by multiple-site injections of T-2 hemisuccinate-bovine serum albumin as described by Chu et al. (2). Known concentrations of T-2 toxin added to pooled human serum and urine were used as biological samples. Albumin-coated charcoal was prepared by adding 1.25 ml of 22% bovine albumin (Sigma Chemical Co., St. Louis, Mo.) to 1.25 g of neutral Norit A Charcoal (Sigma). Distilled water was added to a final volume of 50 ml. The mixture was stirred constantly while being added to the tubes. Radioactivity was counted in a Searle 6880 liquid scintillation counter.

Antibody titration and determination of affinity. The optimal titer of the rabbit anti-T-2 antibody was determined by obtaining the dilution of the antibody that resulted in the binding of 50% of the radioactivity in the radiolabeled ligand, as described previously (8). This dilution was used in all subsequent experiments. The average affinity constant was determined by incubating increasing amounts of radiolabeled T-2 and unlabeled T-2 with constant amounts of the antibody by the method of Odell et al. (7).

T-2 radioimmunoassay. The T-2 standards or the unknown samples (100 μl amounts) in 0.1 M sodium phosphate buffer were added to 10- by 75-mm borosilicate tubes in triplicate. The ^{3}H-labeled T-2 toxin (50 μl, about 20,000 dpm) and 50 μl of the working dilution of rabbit anti-T-2 antibody were then added. After 1 h of incubation at 4°C, 200 μl of the albumin-coated charcoal was pipetted. The tubes were immediately mixed and centrifuged at $2,000 \times g$ for 30 min. The supernatants were decanted into scintillation vials, and the radioactivity was counted. For hemolyzed samples, 200 μl of 60% perchloric acid and 400 μl of 30% hydrogen peroxide were added sequentially. The vials were incubated at 37°C for 30 min and then counted in a refrigerated liquid scintillation counter.

RESULTS

A 1:10 dilution of the antiserum resulted in 50% binding of the radiolabeled T-2 (Fig. 1). At dilutions of 1:100 or more, the antiserum failed to bind any of the ^{3}H-labeled T-2. The average affinity constant (K) was 1.75×10^{10} liters/mol,

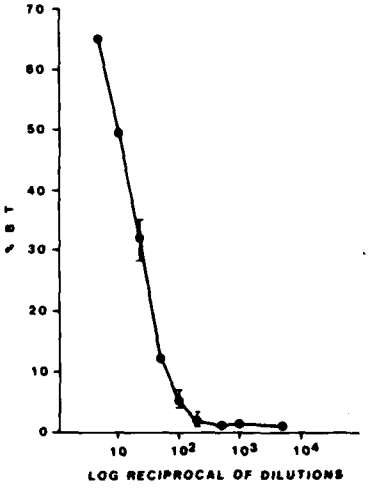

FIG. 1. Primary titration of rabbit anti-T-2 antibody to determine the dilution that will bind 50% of the radioactive ligand. Means ± two standard deviations are shown. % B/T, Percentage of tracer bound.

calculated from the free antigen concentration at 50% saturation (Fig. 2). When known amounts of T-2 toxin were added to serum and urine samples, the recovery of T-2 ranged from 59.8 to 126.8%, with a mean (± standard deviation) recovery of 93.6 ± 16.9%. Hemolyzed serum samples produced colored supernatants after centrifugation. Color quenching reduced the counting efficiency, which was not corrected by preset, automatic quench correction by external standardization in the scintillation counter or by the use of quench correction standards. This resulted in overestimating the measured amount of T-2 toxin added to hemolyzed samples by two to five times. At high concentrations, T-2 added to urine and nonhemolyzed serum samples inhibited the assay, and the samples were brought to the detection range, which was between 1 and 10 ng, by serial dilution (Fig. 3). The interassay coefficient of variation was 9%.

In the cross-reactivity experiments, 2.6 ng of T-2 was needed to inhibit the reaction by 50%, whereas H-T-2, T-2 triol, and diacetoxyscirpenol (DAS) required 25.1, 2,490, and 2,510 ng, respectively, to produce the same degree of inhibition (Fig. 4). The relative potency of the cross-reacting trichothecenes, calculated as a percentage of the T-2 standards at 50% inhibition, was 10.3% for H-T-2 and 0.1% for T-2 triol and DAS. Of the other trichothecenes studied, T-2 Tetraol, Verrucarol, and Vomitoxin showed less than 20% inhibition at 10 μg. Verrucarin exhibited less than 5% inhibition at 10 μg, and Roridin did not inhibit the reaction.

DISCUSSION

Detection methods that are rapid, simple, and sensitive are needed for the determination of mycotoxin contamination and in biological specimens for the assessment of toxin concentration. The ideal assay would be one that is both sensitive and highly specific. The physicochemical methods that have been developed are not widely used because of the need for skilled personnel and sophisticated instrumentation.

The radioimmunoassay for T-2 toxin measures a highly toxic and readily produced member of the trichothecene group (1). We modified this assay, resulting in a total assay time of less than 6 h. The extraction steps used in the previous assays were omitted. Blood and urine samples with T-2 toxin added were used directly in the assay tubes. A 1-hour incubation of the reactants was shown to be as efficient as overnight incubation in the original assay. Albumin-coated charcoal was used to separate bound

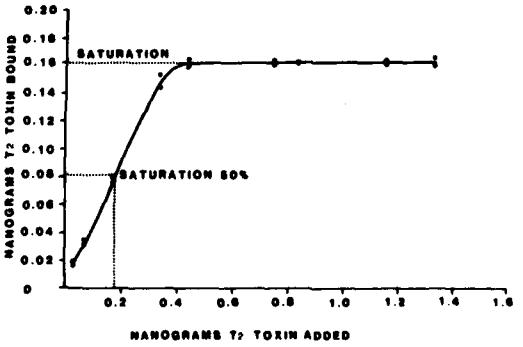

FIG. 2. Saturation curve for antibody against T-2 toxin to determine the average affinity constant. Concentration of antigen at 50% saturation is 1.75×10^{10} liters/mol.

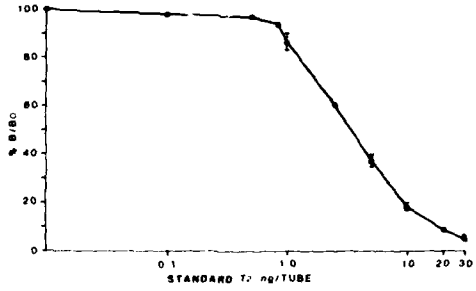

FIG. 3. Typical standard inhibition curve. Sensitivity is 1 ng, two standard deviations greater than the uninhibited tubes. Means ± two standard deviations are shown. % B/B_0 Percentage of tracer bound relative to zero antigen tube.

from free ligand, a much simpler method than the ammonium sulfate precipitation used in Chu's original procedure (2). The sample requirement was minimal, only 100 μl per tube, and the sensitivity was comparable to that of the previously reported assay (2), 1 ng per assay or 10 ng/ml.

A Scatchard plot of our experiment gave a biphasic curve, an indication that more than one antibody species was present, all reacting with various avidity to the antigen. We ran a saturation curve for the antibody and determined the average affinity constant by the method of Odell et al. (7) from the concentration of free antigen at 50% antibody saturation. Although the average affinity constant was high (K_m, 1.75×10^{10} liters/mol), the optimal titer for our assay was low (1:10). This is, however, consistent, since the titer also reflects the total specific antibody concentration. The total antibody population most likely contains only a small percentage of specific anti-T-2 antibody, which accounts for the low titer. This small population of specific antibodies, however, exhibits high avidity for the antigen. The narrow saturation range and range of detection also support the theory that only a small portion of the total antibody population is T-2 toxin specific. Useful radioassays have been developed with low-titer antibodies as long as the affinities are adequate (10^9 liters/mol or greater) (3).

During the initial experiments, hemolyzed blood produced quenching that resulted in the overestimation of T-2 in these samples. We used a wet oxidation method, as described by Mahin and Lofberg (5), to correct this problem. The hemolyzed samples we analyzed, which were stored for weeks, cleared completely. Although precipitation occurred in some tubes, this did not contribute to any counting problems. The samples can also be oxidized before incubation with the antibody, although we preferred the postincubation oxidation process. This method of oxidation has also been recommended for tissue samples of 100 mg or less.

H-T-2 cross-reacts significantly with T-2 (10.3%); therefore caution must be exercised in reporting concentrations in the presence of H-T-2. The other trichothecenes examined did not cross-react to any significant degree. The nar-

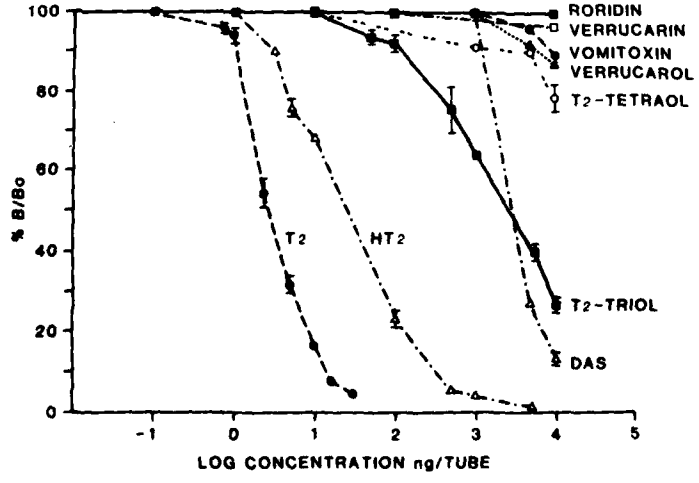

FIG. 4. Cross-reactivity between T-2 and other trichothecene mycotoxins for the binding sites of antibody; 2.6 ng of T-2 inhibited the reaction by 50%, whereas 25.1, 2,490, and 2,510 ng of H-T-2, T-2 Triol, and DAS, respectively, were required to produce the same effect. Means ± two standard deviations are shown. % B/B_0, Percentage of tracer bound relative to zero antigen tube.

row range of detection makes it necessary to dilute specimens serially to bring samples at high concentration to within the sensitivity range of the assay. We routinely diluted samples that were greater than 10 ng/ml to make use of the sensitive portion of the standard curve. Aside from problems common to other isotopic assays, such as isotope disposal, the significant cross-reaction with H-T-2 can make quantitation difficult. The presence of T-2 triol and DAS in very high concentrations, as may occur in multiple massive toxin exposure, can potentially create problems in the interpretation of results. The newer antibody production techniques may offer a solution to the low antibody titer; efforts are being made in other laboratories to produce monoclonal antibodies to T-2 toxins.

The assay, in operation for several months now, has been most useful in measuring T-2 concentrations in animal toxicological studies and evaluating detoxification protocols in experiments in which only T-2 toxin was used. The assay reproducibility and sensitivity are adequate for most laboratory needs. The omission of the extraction steps, the shorter incubation times, and the modification of the separation process have resulted in a more rapid and simpler assay.

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