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Stability of T-2 Mycotoxin in Aqueous Media

LYNN R. TRUSAL

Pathology Division, U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, Frederick, Maryland 21701-5011

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^{3}H-labeled T-2 mycotoxin was dissolved in various aqueous media and stored for up to 3 weeks at 4, 22, and 37°C. At periods ranging from 1 to 21 days, aliquots were assayed by thin-layer chromatography. Thin-layer chromatography plates were scanned for breakdown products by use of a radioisotope scanner, and breakdown products were identified based on their comigration with known standards. Results indicated that T-2 toxin was more stable in tissue culture medium with or without serum, than in Hanks balanced salt solution (HBSS), at all temperatures. The metabolites HT-2, T-2 triol, and T-2 tetraol were detected as early as 1 day (HBSS; 37°C) and as late as 3 weeks (HBSS; 4°C) after storage. Stability of the toxin in aqueous media decreased with increasing temperature.

Many investigators who use mycotoxins in their work find it necessary to dilute stock toxin solutions for various experimental protocols. While toxins generally come lyophilized from the manufacturer, they are usually reconstituted in methanol or ethanol for storage at 4°C or a lower temperature. Studies conducted in vitro often call for adding toxins to aqueous media for extended time periods. Since such protocols are prevalent in our laboratory, we were interested in examining the stability of T-2 mycotoxin in several aqueous media.

^{3}H-labeled T-2 mycotoxin (0.1 μg/ml) (New England Nuclear Corp., Boston, Mass.) was dissolved in sterile solutions of Hanks balanced salt solution (HBSS) and in tissue culture medium with Hanks salts (H-199) (GIBCO Laboratories, Grand Island, N.Y.) with and without 10% fetal calf serum. All samples were then stored for up to 21 days at 4, 22, and 37°C. At each of the following time periods (i.e., 1, 3, 5, 7, 14, and 28 days), aliquots of each sample were spotted on thin-layer chromatography plates with known standards. The thin-layer chromatography plates were developed and scanned for radioactivity with the Bioscan BID100 radioisotope scanner (Bioscan Inc., Washington, D.C.). Breakdown products were identified based on their comigration with known standards which included the following trichothecene mycotoxins: T-2, HT-2, T-2 triol, and T-2 tetraol.

We chose to examine HBSS and tissue culture medium since the majority of our experiments are conducted in one of these aqueous solutions. Since these experiments use tissue culture media which contain fetal calf serum, we examined the effect of its deletion from one of our experiments. This is important because serum is the least-defined component of tissue culture media.

We found that T-2 toxin was most stable at 4°C and least stable at 37°C (Table 1). This was not surprising, since all metabolic reactions are slowed considerably at low temperatures. T-2 toxin was as stable in medium with serum as it was without serum at both 4 and 22°C. At 37°C, the presence of serum did speed the breakdown of the toxin. In this sample, HT-2 was present after 1 week with serum but was not evident after 3 weeks without serum (Table 1). This indicates that unknown components of fetal calf serum may play a role in T-2 breakdown at physiological temperatures. Since most experiments are not run for 7 days at physiological temperatures, this should not have an adverse effect.

What was not expected was the greater stability of T-2 toxin in tissue culture medium compared with HBSS. Both H-199 and HBSS contain eight inorganic salts plus D-glucose. Formulations for each are based on Hanks formula of inorganic salts with slight differences in the specific salt or the amount of the salt that is employed. The major difference is that H-199 contains many more ingredients than HBSS does. These include 23 amino acids or their derivatives, 17 vitamins, and other added components as described by the manufacturer (GIBCO Laboratories).

TABLE 1. T-2 mycotoxin stability in aqueous media

Temp	Aqueous medium	Toxin	First appearance of a breakdown product (wk)	% of breakdown product in medium
37°C	HBSS	HT-2	1 day	8
		T-2 triol	1	3
		T-2 tetraol	2	12
		Unknown	2	4
	Tissue culture medium + serum	HT-2	1	5
	Tissue culture medium − serum	T-2	3[a]	100
22°C	HBSS	HT-2	1	16
		T-2 triol	1	1
		T-2 tetraol	3	1
	Tissue culture medium + serum	HT-2	2	2
		Unknown	3	10
	Tissue culture medium − serum	HT-2	2	2
4°C	HBSS	HT-2	3	5
	Tissue culture medium + serum	T-2	3[a]	100
	Tissue culture medium − serum	T-2	3[a]	100

[a] Only T-2 toxin was evident after 3 weeks.

Breakdown of T-2 occurred at least 1 week sooner in HBSS than in H-199 at all temperatures. Therefore, we conclude that the ingredients in H-199 which are not found in HBSS increase the stability of toxin in aqueous solutions.

Although the total amount of a breakdown product did not exceed 16%, workers in the field should be aware of toxin degradation based on their own experimental parameters. These results should be of interest to investigators who use T-2 mycotoxin in in vitro studies at physiological temperatures. If studies conducted over several days involve dilution of toxin in aqueous solutions, metabolic breakdown products may appear. This may be checked by the use of pilot studies, under experimental conditions, using thin-layer chromatography to monitor toxin stability. Therefore, T-2 toxin stored in ethanol or methanol should be freshly diluted just before use. Other mycotoxins may be similarly affected.

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