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Effect of Meat and Isolated Meat Proteins on the Thermal Inactivation of Staphylococcal Enterotoxin B[1]

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The thermal inactivation of staphylococcal enterotoxin B was studied in a phosphate-saline buffer, in the presence of two meat proteins, myosin and metmyoglobin (MetMb), and in a ground-beef slurry. When enterotoxin B was incubated at temperatures from 60 to 110 C, it was shown that the initial thermal inactivation at 80 C was faster than at 100 or 110 C. The heating of enterotoxin B at 60, 80, and 100 C in the presence of either myosin or MetMb resulted in a rapid loss of the enterotoxin. Thermal loss of the enterotoxin B molecule in the presence of meat proteins was more pronounced at 80 C than at either 60 or 100 C. Thermal loss of enterotoxin B in a ground round slurry was rapid when compared to inactivation in a phosphate-saline buffer. The rapid loss of enterotoxin B in the slurry may be due to a combination of thermal inactivation and the binding of enterotoxin molecules to meat proteins.

The thermal inactivation of enterotoxin B has been investigated in Veronal buffer (5) and in milk (6) at temperatures ranging from 96 to 126.7 C. Thus far, no studies have been performed on the fate of enterotoxin B at temperatures normally attained during the cooking of meats. Also, nothing is known about the interaction of proteins in meat with the enterotoxin B molecule.

The purification of enterotoxin B (1) made possible the production of a specific antiserum and development of gel diffusion techniques (8) that can be used for the quantitative determination of the enterotoxin. Extraction procedures (2) made possible the detection of enterotoxin B in food samples.

The purpose of this study was to observe the inactivation of enterotoxin B between 60 and 110 C and to determine what effects isolated meat proteins and meat slurries have on the enterotoxin B molecule during heating.

MATERIALS AND METHODS

The enterotoxin B used was obtained by growing *Staphylococcus aureus* S-6 in a medium consisting of 2% protein hydrolysate powder (PHP), obtained from Mead Johnson and Co., Evansville, Ind., and 0.001% of each of the following vitamins: niacin, thiamine, and panthothenic acid. The culture containing a 1% inoculum was incubated at 37 C for 24 hr with constant stirring. The cells were then removed by centrifugation and the supernatant fluid was dialyzed against cold distilled water for 24 hr. The supernatant fluid was lyophilyzed to dryness and the powder obtained was designated as "crude enterotoxin B." The crude enterotoxin B was further purified by dissolving 500 mg of the crude enterotoxin in 0.010 M phosphate buffer (*p*H 6.7) and passing the crude material through a Sephadex G-100 column (2.5 by 80 cm). The components were eluted from the column, and the enterotoxin B component was collected, dialyzed against cold distilled water, and lyophilyzed to dryness. The powder obtained was designated as "partially pure enterotoxin B." The enterotoxin B content of all samples was determined with a modified Oudin single diffusion technique (8). The Oudin gel diffusion technique was standardized with purified enterotoxin B obtained from M. S. Bergdoll and K. Weiss (Food Research Institute, University of Wisconsin, Madison).

For thermal inactivation studies of the enterotoxin in buffer, either crude or partially pure enterotoxin B was dissolved in a buffer consisting of 0.013 M phosphate (*p*H 7.4) and 0.85% NaCl. The final concentration of enterotoxin B in the buffer was 50 μg/ml. One-milliliter portions of the enterotoxin-buffer solution were placed in small test tubes and heated to each of several temperatures, 60, 80, 100, or 110 C,

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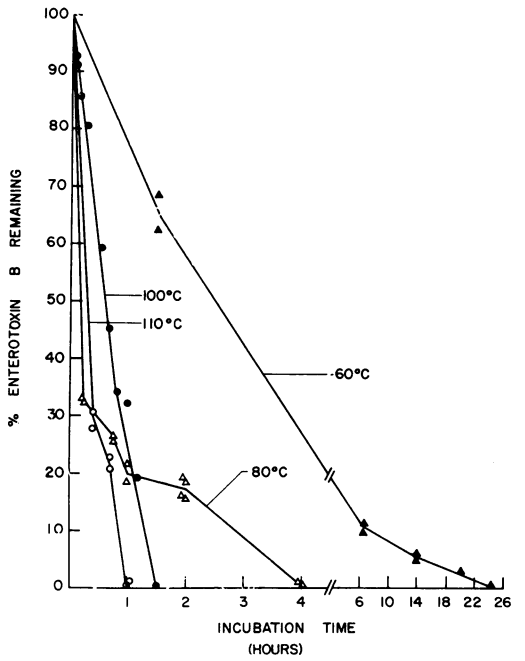

FIG. 1. *Thermal inactivation curves for crude enterotoxin B. The enterotoxin B concentration was 50 μg/ml of a buffer consisting of 0.013 M phosphate (pH 7.4) and 0.85% NaCl.*

for a specified time. Timing was begun when the solution reached the temperature of the incubation bath. After the specified time, the tube was placed in ice and kept at 4 C until it was analyzed for enterotoxin B content.

The thermal inactivation in the isolated meat proteins consisted of dissolving 26.2 μg of partially pure enterotoxin B along with either pure myosin or metmyoglobin (MetMb) in the phosphate-saline buffer. The enterotoxin-meat protein-buffer solution was incubated at 23, 37, 60, 80, or 100 C for a specified time and then cooled in ice. All insoluble protein was removed by centrifugation, and the enterotoxin B content was determined as described previously (8).

A meat slurry, consisting of equal weights of ground round and water, was prepared and cooked by boiling for 10 min; 100 μg of enterotoxin B was added to 10 ml of the slurry (*p*H 6.6) in a screw-cap test tube, and the mixture was heated at either 80 or 100 C for a specified period of time. Timing was begun when the slurry reached the temperature of the bath. After incubation at either 80 or 100 C, the enterotoxin was removed from the slurry samples by a modification of the extraction and isolation procedure of Casman and Bennett (2). The extraction was performed with 0.10 M phosphate buffer (*p*H 7.6) containing 0.2 M NaCl. Enterotoxin B was separated from other soluble extractives by placing the crude meat extract on an Amberlite IRC-50 (XE-64) column equilibrated with 0.01 M phosphate buffer (*p*H 6.2) and then eluting the enterotoxin from the column with 0.50 M phosphate buffer (*p*H 6.8).

RESULTS

Thermal inactivation of enterotoxin B in phosphate-saline buffer. Data were obtained on the thermal inactivation of both crude and partially pure enterotoxin B in 0.013 M phosphate buffer (*p*H 7.4) containing 0.85% NaCl. The thermal inactivation curves for both crude and partially pure enterotoxin B are shown in Fig. 1 and 2. Loss of biological activity occurred rapidly for both crude and partially pure enterotoxin B until 65 to 75% of the activity had been lost, then the slope changed and the remaining 25 to 35% of the activity was lost less rapidly. The initial loss of activity at 80 C was more rapid than at 100 or 110 C. Crude enterotoxin B, when incubated at 80 C, lost 67.5% of its activity in the first 15 min and the remaining 32.5% of the activity in 225 min.

Another illustration of the rapid initial inactivation of enterotoxin B at 80 C is shown in Table 1, in which the time required to inactivate 50% of the enterotoxin is given for each temperature. Time for inactivation of 50% of the enterotoxin at 80 C is shorter than the times at

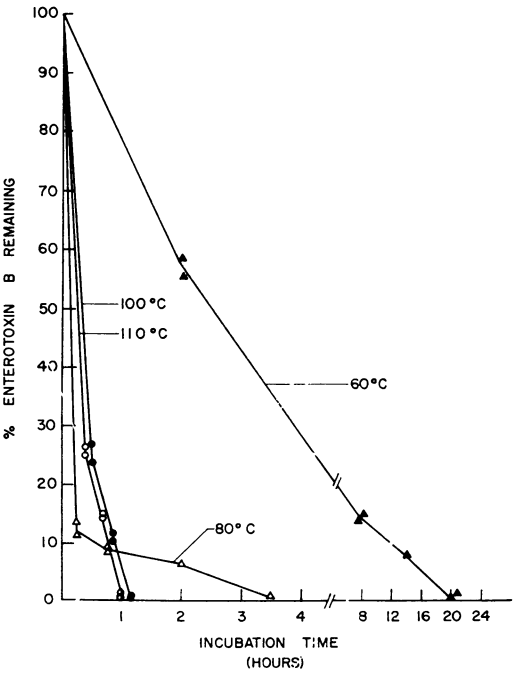

FIG. 2. *Thermal inactivation curves for partially pure enterotoxin B. The enterotoxin B concentration was 50 μg/ml of the same phosphate-saline buffer as in Fig. 1.*

TABLE 1. *Time required to inactivate 50% of the enterotoxin B present in a phosphate saline buffer*[a]

Temp	Time required for 50% inactivation	
	Crude enterotoxin B	Partially pure enterotoxin B
C	*min*	*min*
60	204	204
80	14	12
100	37	24
110	17	16

[a] Enterotoxin concentration was 50 μg/ml of a buffer containing 0.013 M phosphate (*p*H 7.4) and 0.85% NaCl.

TABLE 2. *Thermal loss of enterotoxin B in the presence of MetMb and myosin at five different incubation temperatures*

Partially pure enterotoxin B (26.2 μg) plus	Enterotoxin B lost[a] (incubation temp/incubation time)				
	23 C/120 min	37 C/60 min	60 C/60 min	80 C/6 min	100 C/15 min
	%	%	%	%	%
1.03 mg of MetMb...	2.7	0.0	50.4	84.5	86.7
1.8 mg of Myosin...	5.6	4.6	84.5	100.0	83.8

[a] Figures are averages from three samples.

either 100 or 110 C. The crude enterotoxin is more resistant to thermal inactivation than is the partially pure enterotoxin B (Table 1, Fig. 1 and 2).

Effect of myosin and MetMb on the thermal inactivation of enterotoxin B. The effects of the presence of either MetMb or myosin during thermal inactivation of partially pure enterotoxin B were studied by heating the enterotoxin in the presence of either meat protein. When enterotoxin B was incubated with pure MetMb, little thermal loss occurred at 23 and 37 C, but at 60, 80, and 100 C a large amount of the toxin was lost. Thermal loss was more rapid at 80 C than at 60 or 100 C. Similar results were obtained when pure myosin was incubated with enterotoxin B. At 23 and 37 C, the amount of enterotoxin lost was small; at 60, 80, and 100 C, large amounts were lost. Again, the thermal loss of enterotoxin B at 80 C was most rapid when compared to the loss at 60 and 100 C. Table 2 shows the amount of enterotoxin B lost in the presence of both myosin and MetMb at the various incubation temperatures.

Thermal inactivation of enterotoxin B in a meat slurry. When ground round slurries containing

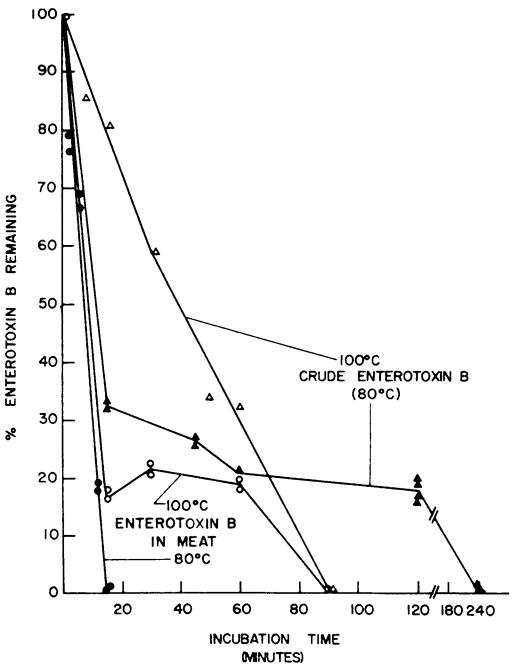

FIG. 3. *Thermal loss of enterotoxin B at 80 and 100 C in a ground round slurry. The thermal inactivation curves for crude enterotoxin B in phosphate-saline buffer at 80 and 100 C are also shown.*

100 μg of enterotoxin B were heated at 80 C for specified periods of time, a rapid loss of enterotoxin was noted. Within 15 min after the sample reached 80 C, no enterotoxin B was detectable in the meat slurry. When slurries containing the same amount of enterotoxin were heated at 100 C, the loss was not as rapid as at 80 C. After 30 min at 100 C, 80% of the enterotoxin was lost, and after 90 min at 100 C, no enterotoxin was detectable. The thermal loss of enterotoxin B in meat slurries was also more rapid at 80 and 100 C than was the thermal inactivation of the crude enterotoxin B at the same temperatures. The loss of enterotoxin B in meat slurries at 80 and 100 C were compared with the thermal inactivation of crude enterotoxin B at the same temperatures (Fig. 3).

DISCUSSION

One of the goals of this study was to determine whether the heat inactivation of enterotoxin B in a buffer solution corresponded to the heat inactivation of the enterotoxin in a meat sample. Earlier work (5, 6) showed that loss of biological activity of enterotoxin B, when assayed by a gel diffusion technique, was in substantial agreement with data obtained from the injection

of the same samples into cats. Denny et al. (3) studied the thermal inactivation of crude enterotoxin A at temperatures from 212 to 250 F (100 to 121.11 C). By using cats and monkeys as assay animals for the presence of enterotoxin A, Denney et al. demonstrated that crude enterotoxin A is less resistant to thermal inactivation when compared to enterotoxin B and that the thermal inactivation of enterotoxin A is complete in 16.4 min at 250 F (121.11 C) and 65 min at 212 F (100 C). Hilker et al. (4) used the Oudin single-diffusion technique to study the thermal inactivation of enterotoxin A in Veronal buffer (*p*H 7.2) between 212 and 250 F (100 to 121.11 C). Hilker et al. demonstrated that the rate of thermal inactivation was in direct proportion to the initial enterotoxin A concentration in the sample. The Oudin single diffusion technique (8) was used in this study to determine the amount of enterotoxin remaining in each sample after a specific heat treatment.

The inactivation of enterotoxin B in a phosphate-saline buffer demonstrated that the crude enterotoxin B preparation was more heat stable when compared with the partially pure preparation. This heat stability should be expected since the presence of other proteins, which are very abundant in the crude preparation, would give stability to the enterotoxin B molecule. The unusual instability of the enterotoxin at 80 C may be the result of an enterotoxin B complex forming at this temperature but unable to form at higher temperatures (100 or 110 C). This complex could be the enterotoxin B molecules binding with other enterotoxin molecules or with other proteins or inorganic ions in the preparation. Both enterotoxin B preparations, crude and partially pure, were contaminated with large amounts of other proteins. Smith and Gardner (7) demonstrated that a lecithinase of *Clostridium perfringens* was more unstable to heat at 65 than at 100 C. This abnormal heat inactivation was due to a complex formation at 65 C which was not formed at 100 C. The complex that formed was between lecithinase molecules and Ca^{++} or Mg^{++} ions in the solution.

When the partially pure enterotoxin B preparation was heated in the presence of either pure MetMb or myosin, very little thermal loss occurred at 23 or 37 C, but excessive loss did occur at 60, 80, and 100 C. The most rapid loss of enterotoxin B was at 80 C. The loss of enterotoxin B in a heated meat slurry was more rapid than the thermal inactivation of crude enterotoxin B in the phosphate-saline buffer. The rapid thermal loss of enterotoxin B in the meat slurry, when compared to the loss in the phosphate-saline buffer, is not due to the initial enterotoxin B concentration, since the meat slurry samples contained twice as much enterotoxin as did the buffer samples. The small *p*H difference (0.8 *p*H unit) between the slurry sample and the buffer sample could influence the inactivation, but only to a slight degree. The rapid loss of enterotoxin B in the meat protein solutions and in the meat slurry may be due to two factors: (i) some of the enterotoxin may bind to meat proteins and is then undetectable by the gel diffusion technique; and (ii) the toxin that is not bound may be inactivated rapidly by the heat. The combination of both effects could contribute to the very rapid loss of enterotoxin B in the meat protein solutions and in the meat slurry.

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