A Research Note

Thermal Stability of Staphylococcal Enterotoxins A, B and C in a Buffered System

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ABSTRACT

The heat stability of staphylococcal enterotoxins A, B and C (SEA, SEB, SEC) in phosphate buffered saline solution at a concentration of 100 ng per ml indicated that normal cooking times and temperatures are unlikely to completely inactivate the toxins. The order of heat resistance of the three toxins was SEC>SEB>SEA.

Several studies have been conducted to determine the extent of heating necessary to inactivate the enterotoxins of *Staphylococcus aureus*. In these investigations, 5 to 100 μg of toxin were added per ml of buffer or food extract *(2,7,9,10)*. These concentrations are much higher than would be expected in naturally contaminated foods but were used presumably because the assay systems employed by the investigators were not sensitive enough to detect lower levels of enterotoxin. Humber et al. *(7)* added 5 μg of staphylococcal enterotoxin A per ml of beef bouillon and although it was the smallest amount of enterotoxin studied, they cautioned that their time-temperature end points might not be the same for lower concentrations of enterotoxin found in naturally contaminated foods. This caveat was also mentioned by Denny et al. *(2)* and Fung et al. *(6)*.

Even with the greater sensitivity of the radioimmunoassay (RIA; *(3,4)*, it is impractical to establish thermal inactivaton profiles of staphylococcal enterotoxins A, B and C (SEA, SEB and SEC) using the small amounts of toxin that are normally found in foods implicated in staphylococcal intoxications. In this study, we use 100 ng of toxin per ml as the starting concentration to determine thermal serological inactivation. This quantity of

toxin is 50 times lower than used in previous thermal inactivation studies *(7)* but it is 2 to 25 times higher than that encountered in food poisoning episodes *(8)*.

MATERIALS AND METHODS

Enterotoxin production

Enterotoxin-producing strains of *S. aureus* used were IIN-165 (SEA), S6 (SEB) and ST361 (SEC). The cultures were individually grown in brain heart infusion both (Difco) by the sac culture method of Donnell et al. *(5)*. After incubation at 35°C for 24 h with shaking, the cells were removed by centrifugation and enterotoxin present in the supernatant liquid was determined by RIA *(3,4)*. If necessary, the enterotoxin was concentrated by ultrafiltration using an Amicon UM-10 membrane. The supernatant liquid was filter-sterilized and stored refrigerated.

Heat treatment

Ten-ml volumes of 0.05 *M* phosphate buffer containing 0.15 *M* sodium chloride and 0.05% sodium azide, pH 7.4 *(1)*, were added to screw-capped tubes which had two holes of approximately 1.0 mm in diameter drilled into the cap to expose a resealable rubber liner. The tubes were submerged in an oil bath preset at a specific test temperature and allowed to equilibrate for at least 2 h. Approximately 50 μl of the enterotoxin to be tested was then added to the buffer to obtain a final concentration of 100 ± 10 ng per ml. At the time of adding the enterotoxin, each tube was lifted with a pair of forceps from the oil bath only enough to expose the top of the screw cap. The rubber liner was pierced through one hole with a hypodermic needle to relieve the internal pressure, and through the other hole, enterotoxin was added with the aid of a Hamilton syringe to which was attached a hypodermic needle long enough to deliver the toxin to the middle of the buffer. The tube was reimmersed in the oil bath. At predetermined time intervals, tubes were removed from the oil bath and immediately placed in ice cold water. The amount of enterotoxin remaining after the heat treatment was then determined by RIA *(3,4)*. Unheated controls containing the same amount of enterotoxin were included with each experiment. The amount of enterotoxin in the controls as determined by RIA represented

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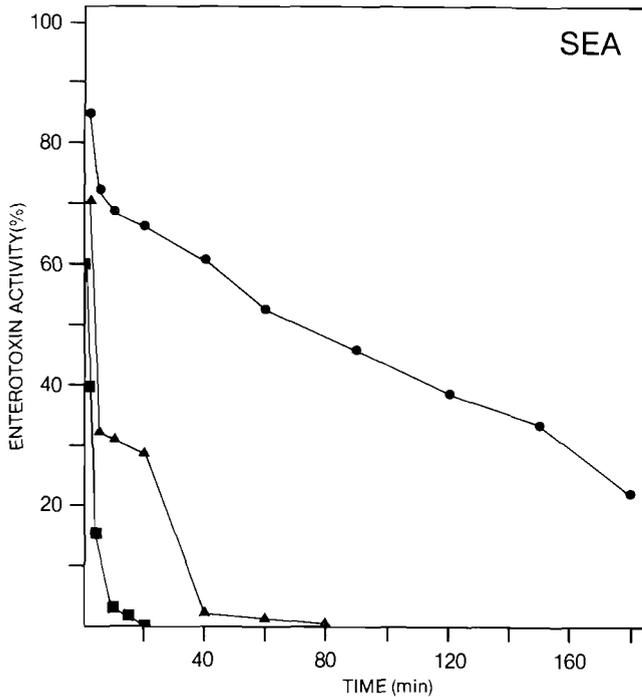
Figure 1. *Heat stability of staphylococcal enterotoxin A (SEA) with time at different temperatures. SEA was at an initial concentration of 95 ±5 ng per ml of buffer (pH 7.4), representing 100% enterotoxin activity at zero time. Zero time was not plotted in this or any of the subsequent figures. Circles = 80°C; triangles = 100°C; squares = 120°C.*

100% serological activity for purposes of calculating the thermal stability of the toxins.

Each experiment was done twice and duplicate tubes were used for each temperature-time determination. The unheated controls comprised duplicate tubes which were held in ice water for the duration of the heating period.

RESULTS AND DISCUSSION

Figure 1 shows the percent SEA activity remaining after each sampling interval. After 180 min of heating at 80°C, there was still 22% of SEA activity. At 100 and 120°C, SEA was completely inactivated (<0.78 ng per ml) in 90 and 30 min, respectively.

The pattern of heat inactivation of SEB at the three test temperatures is shown in Fig. 2. As reported by Fung et al. *(6)*, Jamlang et al. *(9)* and Satterlee and Kraft *(10)*, destruction of SEB activity was initially more rapid at 80 than at 100 and 120°C. This anomalous behavior was ascribed to the formation of heat-sensitive complexes or aggregates of the toxin at 80°C but not at higher temperatures *(9)*. Approximately 15% of SEB activity remained after heating at 80°C for 180 min, and as observed with SEA, virtually all SEB activity was destroyed after heating at 100 and 120°C for 90 min and 30 min, respectively.

Staphylococcal enterotoxin C was the most heat-stable of the three enterotoxins. At 80°C, approximately 30% of SEC activity remained after 180 min, and there was
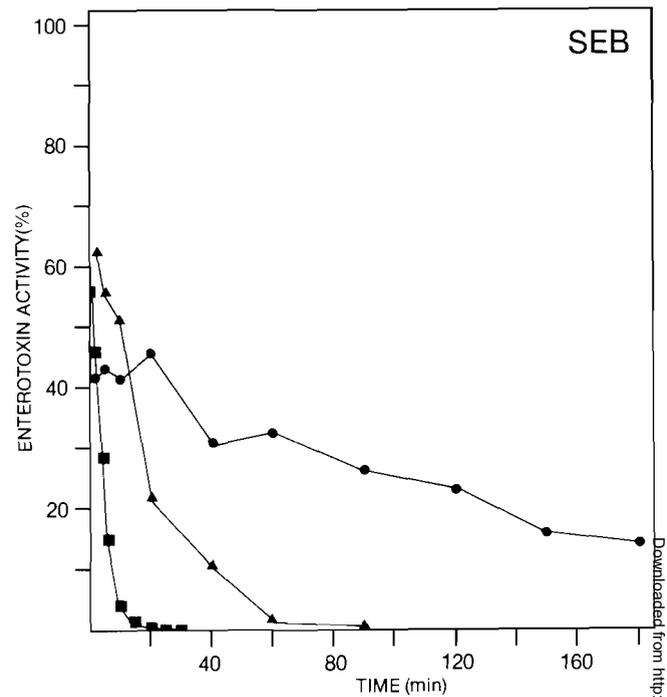

Figure 2. *Heat stability of staphylococcal enterotoxin B (SEB) with time at different temperatures. SEB was at an initial concentration of 105 ±5 ng per ml of buffer (pH 7.4), representing 100% enterotoxin activity at zero time. Circles = 80°C; triangles = 100°C; squares = 120°C.*
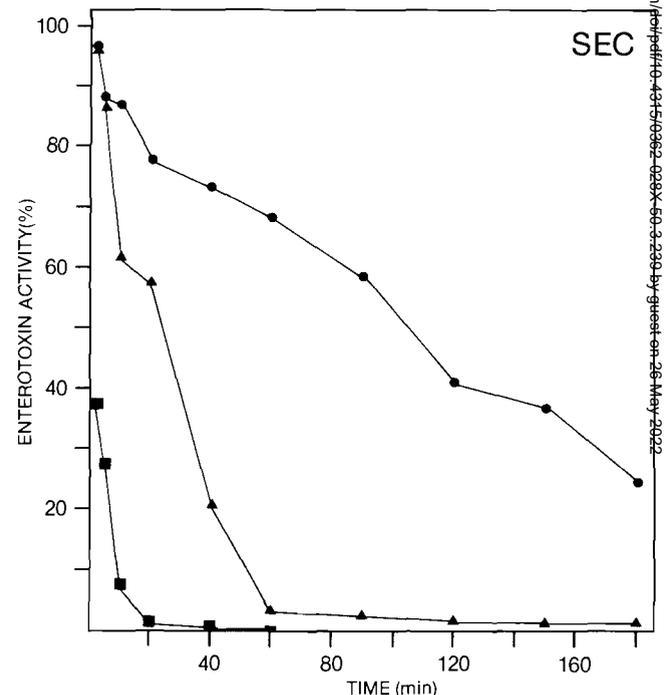

Figure 3. *Heat stability of staphylococcal enterotoxin C (SEC) with time at different temperatures. SEC was at an initial concentration of 110 ±10 ng per ml of buffer (pH 7.4), representing 100% enterotoxin activity at zero time. Circles = 80°C; triangles = 100°C; squares = 120°C.*

detectable activity after heating the toxin at 100°C for 180 min (Fig. 3). The toxin was completely inactivated in 60 min at 120°C.

TABLE 1. *Rate of loss of activity per minute of staphylococcal enterotoxins A, B and C.*

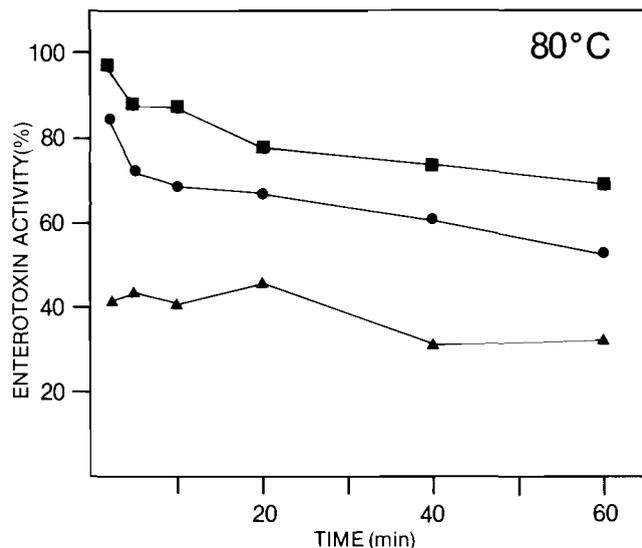| Staphylococcal enterotoxin	Rate of loss of activity (% per min)		
	80°C	100°C	120°C
A	0.54	7.74	27.84
B	0.63	5.78	21.48
C	0.50	5.12	17.59

Figure 4. *Comparative heat stability of staphylococcal enterotoxins A (circles), B (triangles), and C (squares) at 80°C in buffer, pH 7.4. The initial concentrations of SEA, SEB, and SEC, each of which represented 100% enterotoxin activity at zero time, were the same as given in Fig. 1, 2 and 3, respectively.*

As observed by others *(6,9,10)*, thermal inactivation of the three toxins was greatest during the first 5 to 10 min of heating; however, the rate of loss of activity as determined by plotting log of activity against time (not shown) was constant after the initial 2 min of heating. In Table 1, these rates are given for each toxin at each of the three test temperatures. In Fig. 4 and 5, the thermal inactivation profiles of SEA, SEB and SEC are compared over the first 60 min of heating at 80 and 100°C. The initial rapid inactivation of SEB relative to SEA and SEC is most evident in Fig. 4. Within the first 2 min of heating at 80°C, more than 50% of SEB activity was destroyed. After this initial period, the inactivation curves for SEA, SEB and SEC were close to parallel, indicating a constant rate of loss of activity as shown in Table 1. The order of heat stability of the toxins in the first 60 min of heating at 80°C was SEC>SEA>SEB.

At 100°C, SEC was again the most heat-stable toxin; however, there was little difference in the heat inactivation profile of SEA and SEB at this temperature (Fig. 5). The greater heat stability of SEC compared with that of SEB was also observed by Fung et al. *(6)*. We are not aware of any published work in which the heat stability of SEA, SEB and SEC is compared.

At 120°C, the three toxins were equally affected and were completely inactivated in 20 to 30 min. Overall,
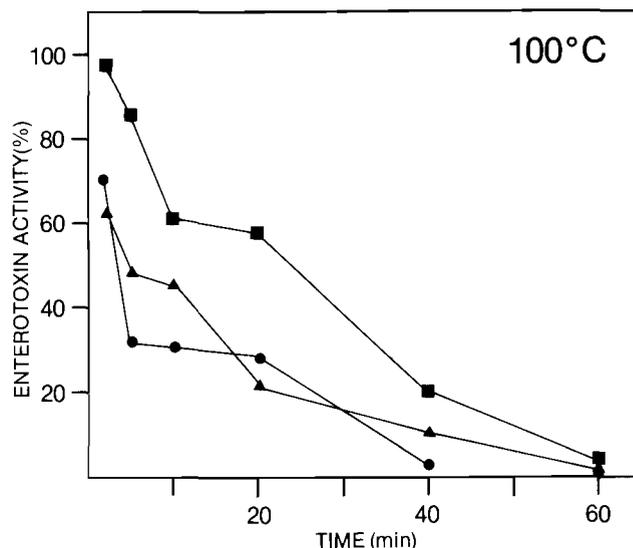

Figure 5. *Comparative heat stability of staphylococcal enterotoxins A (cricles), B (triangles) and C (squares) at 100°C in buffer, pH 7.4. The initial concentrations of SEA, SEB and SEC each of which represented 100% enterotoxin activity at zero time, were the same as given in Fig. 1, 2 and 3, respectively.*

the order of heat stability of the toxins was SEC>SEB>SEA (see Table 1).

Our results are not directly comparable with those of other researchers because of the small quantity of enterotoxin used, but they do bear some similarities to published data on staphylococcal enterotoxin heat stability. These include the more rapid inactivation of SEB at 80 than at 100 or 120°C, and the greater heat stability of SEC relative to SEA and SEB.

Denny et al. *(2)* reported greater thermal stability of SEA in beef bouillon (pH 6.2) than in phosphate buffer (pH 7.2), also Fung et al. *(6)* noted that at 100°C, SEC in phosphate buffered saline solution was inactivated in 60 min, whereas, in culture medium, it took 180 min to inactivate the toxin. If, as it would appear from the above results, staphylococcal enterotoxins are more heat resistant in complex media than in simple buffers, then our results indicate that normal cooking times and temperatures used in a home might be insufficient to completely detoxify and make safe for human consumption a food that is contaminated with staphylococcal enterotoxin.

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