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Evidence for Two Heat-Stable Enterotoxins Produced by Enterotoxigenic *Escherichia coli*

R. A. KAPITANY[1], A. SCOOT[2], G. W. FORSYTH[2], S. L. McKENZIE[3], AND R. W. WORTHINGTON[4]

Veterinary Infectious Disease Organization, Saskatoon,[1] Western College of Veterinary Medicine, University of Saskatchewan,[2] and National Research Council,[3] Saskatoon, Saskatchewan, Canada S7N OWO, and Department of Physiology, Pharmacology and Toxicology, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, 0110, Pretoria, Republic of South Africa[4]

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Heat-stable enterotoxins from a bovine and porcine strain of *Escherichia coli* were isolated and showed significant differences in amino acid composition and heat stability.

Enterotoxigenic strains of *Escherichia coli* (EEC) were initially recognized as enteropathogens because of their ability to dilate ligated segments of pig and rabbit intestines (6, 15, 16). Shortly thereafter, two toxins, a heat-labile toxin (LT) and a heat-stable toxin (ST) were identified in the cell-free culture supernatants that produced the enterotoxigenic effect (8, 9, 16, 19, 20). Until recently, however, little was known about the structure and mode of action of ST (1), and the question of the heterogeniety of ST has been considered by several workers (1, 5, 10, 14), but never fully resolved. The present paper describes the comparison of the heat stability of two highly purified ST preparations from EEC of swine and bovine origin.

EEC strain 124 (O101:H$^-$:K99) is a K99$^+$ ST only producer received from S. D. Acres (Veterinary Infectious Disease Organization) and isolated from feces of diarrheic calves during a natural outbreak of calf scours. EEC strain 1261 (O138:H$^-$) was received from H. W. Moon (NADC, Ames, Iowa). A single colony was taken from a fresh blood agar plate and inoculated into 300 ml of Syncase medium and cultured overnight at 37°C in a shaking water bath. Strain 124 was inoculated into 10 liters of syncase medium or 10 liters of defined medium (2, 3) in a 12-liter fermentor, and strain 1261 was inoculated into 10 liters of defined medium (2, 3). The defined media was supplanted with 1% sodium lactate as the 60% syrup to stimulate toxin production (2, 3). Growth was for 18 h at 37°C with an air flow of 4 liters/min and a vigorous mixing (300 rpm). The culture broths were clarified in a continuous centrifuge and sterilized through a 0.45-μm filter. The ST (ST-124, ST-1261) were prepared by ultrafiltration, and ion-exchange and gel filtration chromatography was performed by methods to be published elsewhere. Crude ST preparations were UM-2 concentrates prepared from the PM10 filtrate of the culture supernatant. Amino acids were analyzed after hydrolysis in 6M HCl at 110°C for 22 h by gas-liquid chromatography (12, 13). Protein was estimated by the method of Kalb and Bernlohr (11). Toxin for heat-inactivation studies was suspended in 0.1 M NaCl (pH 6.95) and heated at 60, 80, or 100°C for 10 and 30 min. The suckling mouse assay (6) was used to estimate the activity of ST. A ratio of gut weight to whole live body weight greater than 0.08 was defined as a positive response. The titer of the toxin was established as the last of a series of serial twofold dilutions to give a positive response. Four (4) mice were used at each dilution.

Crude ST-124 and crude ST-1261 were both stable to heating up to 100°C for 30 min. However, purified ST-124 was almost completely inactivated when compared to unincubated controls (30 and 6% activity after heating up to 100°C for 10 and 30 min, respectively), whereas purified ST-1261 was stable to heat treatment in both the purified and crude form up to 100°C for 30 min. The amino acid analysis of the two toxins are presented in Table 1, and it can be seen that significant differences are present. The conspicuous lack of cysteine in ST-124 would account for the lack of heat stability for this molecule, and ST-1261 is very similar in composition to the ST-431 of Alderete and Robertson which was also stable to heating at 100°C. Both ST-124 and ST-1261 were purified in a very similar manner and were both active in the suckling mouse test at a few nanograms of protein (1 to 5 ng). The gas-liquid chromatography analysis showed more trace nonprotein peaks for ST purified from complex media than from defined media; however, the yields were more than eight times less from defined media. The lack of methionine and histidine in ST-1261 would suggest that it was more purified than the toxin

TABLE 1. *Amino acid composition*[a]

Amino acid	ST-124	ST-1261
Ala	3.5	16.45
Gly	4.2	10.22
Val	8.3	2.1
The	6.0	3.8
Ser	3.5	2.5
Leu	7.1	6.57
Ile	6.8	1.82
Pro	4.2	7.72
Cys H	0.4	11.78
Meth	0.7	ϕ
Asp	16.3	11.68
Phe	2.6	4.38
Glu	28.6	13.28
Lys	2.6	0.78
Tyr	3.3	5.69
Arg	1.8	1.18
His	1.2	ϕ
Try	ND	ND

[a] Expressed as mole percent.

reported by Alderete and Robertson (1).

It is possible that these differences reflect a genuine host specificity in the toxin plasmids themselves quite analogous to the colonization factors K99 and K88. However, it is just as likely that the origins of the strains are irrelevant and that these differences in composition and characteristics imply the existence of toxins that mediate different diarrheal syndromes. Both ST-124 and ST-1261 appear to be produced by class 1 enteropathogens (16) and to correspond to the STa category of Burgess et al. (4). It would now appear that the mouse-active and pig-active toxins (class 1 or STa) can exist in both a heat-stable and a heat-labile form. It will be interesting to see a systematic examination of other purified toxins produced by these class 1 strains to ascertain what proportions are heat stable and heat labile and the mortality rates associated with both. Only the purification and characterization of STs from other strains will provide an answer to the question.

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