Extraction and partial characterization of enterotoxin produced by Salmonella spp. isolated from sheep

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Abstract

The enterotoxic moiety present in the cell–free culture filtrate of Salmonella spp. was purified to apparent homogeneity by salt precipitation with ammonium sulphate and chromatography through sephadex G-100. It was non dialyzable. The enterotoxin appeared to be of high molecular weight 100 KDa and composed of Glycoprotein. The enterotoxic, rapid and delayed permeability and increase suckling mice intestine weight activities were attributed to single protein moiety.

Introduction

Various investigations have been conducted describing a number of possible Salmonella factors that may contribute to the diarrhea associated with gastroenteritis. One proposed mechanism is related to the ability of Salmonella to invade the intestinal epithelium during the course of the disease.\(^1\) reported that live Salmonella evolved fluid secretion in the ligated rabbit ileum and that the fluid response could not be induced by Salmonella cultures filtrates or concentrates. Although fluid exsorption was demonstrated to accompany mucosal invasion, not all strains of invasive Salmonella tested by the anothers caused fluid secretion. Later investigations by\(^2\) revealed a possible role of adenylate cyclase and it was theorized that the adenylate cyclase activation was due to the local synthesis of prostaglandine induced by the mucosal inflammatory response resulting from the invading Salmonella. An additional factor that might be involved in Salmonella gastroenteritis has been described by\(^3\). They described a heat–labile factor that produced a delayed skin permeability reaction in rabbits, which was identical to the skin reaction evolved by Vibrio cholerae enterotoxin. Evidence for protein enterotoxin associated with the outer membrane of Salmonella was reported by\(^4\), when suckling mice were used for the assay of enterotoxin. The purpose of this investigation was to define and purify enterotoxin from culture supernatant of one Salmonella spp.

Materials and Methods

Samples collection

Fecal and bile samples were collected from 120 sheep, of all ages and sex seen in Basrah slaughter house and in some farms of Basrah. The numbers of study samples were 120 fecal samples and 50 bile samples. Fecal samples were collected directly from rectum of sheep by placing a gloved hand in rectum and obtaining small quantity of feces. Bile samples were collected by sterilize syringe from gall bladder of slaughtered sheep.

Bacteriological analysis:

The isolation and identification of Salmonella isolates were performed according to method of \(^5\). The presence of Salmonella in fecal samples were detected by selective enrichment in tetrathionat and incubated at 37°C for 24hr followed by streaking on Salmonella–shigella agar (SSA) and Brilliant green agar (BGA). With incubation at 37°C for 24hr. Suspected colonies were tested biochemically by hydrogen sulfide production on triple sugar iron Agar (TSI), urea hydrolysis, citrate utilization and detection of motility on semisold medium. The diagnosis of – Salmonella isolates were confirmed serologically by using polyvalent O and H Salmonella antisera.
Preparation of enterotoxin

Cell – free culture supernatants (CFCS) of Salmonella spp. were prepared according to the procedure of (6). Briefly each Salmonella isolate was grown in brain heart infusion (BHI) broth on a shaker incubator at 37°C for 18h and then the culture was centrifuged (1000 rpm, 45 mint at 4°C). The supernatant was collected membrane filtrred (Millipore 0.45µm) and stored at 4°C.

Enterotoxin assay.

Preparation were tested for presence of rapid acting skin permeability factor (PF) on the back of rabbits by the method described by (7). The diameter of the reaction was measured and the area was calculated. A preparation giving reaction of ≥ 78.5 mm² was considered positive for PF. Suckling mice were used for the assay of entertoxin. This test was performed as described by (8).Two sucking mice were used for testing enterotoxicity. A preparation yielding dilatation and increase in the intestinal weight percentage of 0.08 was considered as enterotoxic. Intestinal weight percentage was determined by dividing the average of intestinal weight of two mice by the body weight of these of two mice.

Purification of enterotoxin

The CFCS of Salmonella spp. was precipitated with ammonium sulphate at 60% and 80% saturation level. After adding ammonium sulphate to CFCS, the contents were stirred for 20 minutes and kept at 4°C overnight. The precipitate was collected by centrifugation (10000 rpm for 30 minutes at 4°C) and was redissolved in minimum quantity of distilled water (DW). Thereafter, the preparation was dialyzed in cellophane dialysis tubin (sigma) against DW at 4°C until it became completely free from ammonium sulphat ions

Gel filtration:

The precipitated dialysed preparation (PDP) was gel filtered through sephadex G-100. Two ml of PDP (25mg protein) was placed on column (80 × 1.5cm) of sephadex G- 100 equilibrated with 0.2 M phosphate buffer (pH 6.8). The material was eluted from the gel with same buffer at a flow rate of 15ml/h. Fraction, each of 2.5ml were collected separately. The contents of each peak pooled. The contents of each peak was tested for enterotoxicity by skin permeability tests (Delayed permeability factor).

Protein estimation:

Protein estimation was carried out by method of (9).

Detection of carbohydrate.

To determine the presence of carbohydrate in the entertoxin, Molish reagent was used. To 1ml of active fraction of PD 1 ml of Molish reagent were mixed and allowed to react. Aperance of purpul ring after addition of (10) drops of H₂SO₄ to the mixture considered positive (Presnce of carbohydrate). This test was performeal as described by (10).

Result

Enterotoxicity of the crnde preparation:

The CFCS of Salmonella spp. induced fluid accumulation in the intesten of the two tested mice which lead to increase in their weight. The SFCS also contained rapid acting skin permeability factor (table1).

Purification of enterotoxin:

The enterotoxin activities of the fractions obtained at different levels of purification presented in (table1).

The precipitated dialysed preparation (PDP) of Salmonella spp. was found to be enterotoxic in the suckling mice inteste and delayed permeability factor (Table 1). Increase in saturation level of ammonium sulphate from 60 to 80% did not additionally precipitate appreciable amount of enterotoxin as revealed by the suckling mice test. When the PDP (60%) was chromatographed through sephadex G-100, it fractionated into two peaks (Fig. 1). The first peak (A) which eluted close to the void volumes exhibited delayed PF activity, induced fluid accumulation in suckling mice.
intesten (Table 1). None of these activities was detected in the second peak (B) contents.

**Carbohydrate detection:**

<table>
<thead>
<tr>
<th>Tested material</th>
<th>Prepartion</th>
<th>Protein mg</th>
<th>Suckling mice intestine weight %</th>
<th>Rabbit skin permeability (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella enterotoxin</td>
<td>CFCS</td>
<td>8.00</td>
<td>0.054</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>PDP</td>
<td>6.00</td>
<td>0.065</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>SG</td>
<td>3.00</td>
<td>0.085</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>PA</td>
<td>-</td>
<td>0.085</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>PB</td>
<td>2.00</td>
<td>0.042</td>
<td>7</td>
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<tr>
<td>Controls</td>
<td>BHI Broth</td>
<td>-</td>
<td>0.38</td>
<td>-</td>
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<tr>
<td></td>
<td>PBS</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

CFCS: cell free culture supernatant
PDP: precipitated dialysed preparation
SG: Sephadex G.
P. A: Peak A.
P. B: Peak B.
RPF: Rapid permeability Factor.
DPF: Delayed permeability Factor.

Discussion

The results of the present study indicated that Salmonella spp. isolated from sheep produced and released enterotoxin into the culture supernatants as their CFCS induced fluid accumulation in the suckling mice intestine and increased permeability of the rabbit skin. Enterotoxic activity in the CFCS of Salmonella has also been reported (11)(12), while others failed to detect activity in the extracellular medium (13). Enterotoxicity of the PDP revealed that the enterotoxic moiety was precipitated with ammonium sulphate and was nondialysable. The presence of two peaks on gel filtration (Sephadex G-100), indicated that the purification of enterotoxic moiety
was achieved to apparent homogeneity through salt precipitation and gel filtration. Other have reported partial purification of enterotoxin from several Salmonella serotypes \(^{(14)}\) \(^{(15)}\). The appearance of enterotoxic activity in sephadex G-100 close to void volume indicated that the enterotoxic is of high molecular weight, i.e. approx. 100KDa.\(^{(16)}\) also estimated the M. W. of Salmonella enterotoxin to be of the same order. The presence of rapid and delayed PF in the enterotoxin are not in accordance with the observation made by earlier worker with respect to the absence of rapid PF.\(^{(11)}\) \(^{(14)}\). The presence of enterotoxic activity which is detected by sucking mice test and presence of rapid and delayed in the same peak indicted that entrotoxic activity was due to the single moiety.

References


