Occurrence of Neuraminidase and Acylneuraminate Pyruvate Lyase in a Strain of Vibrio Falling into Heiberg’s Group II Isolated from a Patient with Diarrhea

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The enzymes neuraminidase (EC 3.2.1.18) and acylneuraminate pyruvate lyase (EC 4.1.3.3) were found in a strain of vibrio falling into Heiberg’s group II isolated from a patient with diarrhea. The neuraminidase of this strain resembles the neuraminidase of Vibrio cholerae. The pH-optimum, activation by Ca-ions and heat inactivation were studied. There is also some indication of an immunologically common antigenicity. The occurrence of this neuraminidase in the Heiberg’s group II vibrio stain is discussed especially with reference to the relationship with the well known enzyme of Vibrio cholerae.

After the first finding of the “receptor destroying enzyme” (RDE) in Vibrio cholerae by Burnet and Stone (3) this enzyme was studied in following years by virologists, bacteriologists, and biochemists. But the occurrence of neuraminidase and also of the acylneuraminate pyruvate lyase was not investigated in the close relatives of V. cholerae, i.e., in the vibrios belonging to Heiberg’s groups. In the past, neuraminidases were found in many bacteria such as Bacteroidaceae (16, 25), Brucellaceae (9, 19), Clostridium (7, 8, 14), Corynebacteriaceae (1, 5, 10, 11, 13), Lactobacillaceae (6, 12), Mycoplasma (15, 21), and Streptomyces (17). Until now the only member of the Pseudomonadaceae known to possess this enzyme was V. cholerae. Some previous descriptions of neuraminidases in this family could not be confirmed. Therefore, it was interesting to investigate a representative of the so-called non-agglutinatable vibrios falling into Heiberg’s group II, isolated by Bader (2) from a patient with diarrhea coming from a cholera endemic region of India.

MATERIALS AND METHODS

Bacterial strain. The Heiberg’s group II vibrio strain (31876/1970) was obtained through the courtesy of R. E. Bader, Tübingen, Germany. The strain has been previously described (2).

Preparation of crude enzyme. The organisms, cultivated for two days on sheep blood agar plates at 37°C, were harvested and washed in saline. The cells were lysed by repeated freezing and thawing and the cell-free supernatant fluid, after centrifuging, was studied as a crude extract.

Paper chromatography. The ascending chromatography was performed on paper (Schleicher & Schüll 2043 b) with an ethanol-water-ammonia solution (79.5:19.5:1) as described in previous papers (9, 10).

Colorimetric assay. The N-acetylneuraminic acid (NANA) liberated by the enzyme neuraminidase from different substrates such as glycoproteins of human or rabbit serum was determined by Warren’s thiobarbituric method (24).

Immunoelectrophoresis. The study of the enzyme activities of the vibrio strain against human plasma proteins was performed in a previously described manner (8). The specific monovalent rabbit antisera, directed against the different human proteins which were used in this investigation, were also obtained from Behringwerke/Marburg. They are listed in Table 1.

RESULTS

The occurrence of neuraminidase (= sialidase = mucopolysaccharide N-acetylmuraminyldrolose, EC 3.2.1.18) was proved by paper chromatography by using the neuraminidase specific substrate N-acetylmuramylactose (left side of Fig. 1). This substance was cleaved into two fragments, i.e., lactose and NANA. The lactose was split into glucose and galactose by a galactosidase-like enzyme. The NANA was...
TABLE 1. Enzyme actions of vibrio falling into Heiberg's group II on 19 human plasma proteins

<table>
<thead>
<tr>
<th>Plasma proteins</th>
<th>Enzyme actionsa</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Prealbumin</td>
<td>O</td>
</tr>
<tr>
<td>2 Albumin</td>
<td>O</td>
</tr>
<tr>
<td>3 α1-Lipoprotein</td>
<td>L</td>
</tr>
<tr>
<td>4 Acid α1-glycoprotein</td>
<td>N</td>
</tr>
<tr>
<td>5 α1-Antitrypsin</td>
<td>N</td>
</tr>
<tr>
<td>6 α1-Antichymotrypsin</td>
<td>N</td>
</tr>
<tr>
<td>7 Haptoglobin</td>
<td>N + P</td>
</tr>
<tr>
<td>8 Ceruloplasmin</td>
<td>N + P</td>
</tr>
<tr>
<td>9 α2-Macroglobulin</td>
<td>N</td>
</tr>
<tr>
<td>10 α2HS-glycoprotein</td>
<td>N + P</td>
</tr>
<tr>
<td>11 β-Lipoprotein</td>
<td>L</td>
</tr>
<tr>
<td>12 Transferrin</td>
<td>N + P</td>
</tr>
<tr>
<td>13 β1c/β1x-globulin</td>
<td>P</td>
</tr>
<tr>
<td>14 Hemopexin</td>
<td>N</td>
</tr>
<tr>
<td>15 Fibrinogen</td>
<td>P</td>
</tr>
<tr>
<td>16 β2-glycoprotein-I</td>
<td>P</td>
</tr>
<tr>
<td>17 IgA</td>
<td>O</td>
</tr>
<tr>
<td>18 IgM</td>
<td></td>
</tr>
<tr>
<td>19 IgG</td>
<td>P</td>
</tr>
</tbody>
</table>

a Abbreviation: O, no action; N, neuraminidase action; L, lipolytic action; P, proteolytic action.

The neuraminidase-altered human glycoproteins show characteristic patterns due to the loss of the negatively charged NANA (20) as listed in Table 1. In addition to the neuraminidase action on the different glycoproteins there is also a proteolytic activity corresponding with Bader's report of the gelatinase activity of this strain (2). Furthermore, the enzyme neuraminidase was studied by Warren's thiobarbituric acid assay (24). The results are given in Fig. 2 to 4. There are some similarities between the neuraminidases of V. cholerae and the strain falling into Heiberg's group II regarding pH-optimum, activation by Calcium ions and inactivation by heating at 60 C.

**DISCUSSION**

It is interesting to note that there is some relationship between V. cholerae and so-called...
non-agglutinable vibrios. Ohashi and co-workers recently described the exotoxin of NAG vibrios by using this as identity with the choleragen of V. cholerae (18). Now the existence of neuraminidase is an additional similarity. In both cases the activities are markedly lower in the NAG vibrios than in V. cholerae. There is a long standing discussion as to whether the cholera-like gastroenteritis produced by non-choleravibrios were caused primarily by vibrios belonging to Heiberg's group II (2, 22, 23). The question of the pathogenic importance of these vibrios has been investigated during the past few years testing for a rise of agglutinin titers in the sera of patients after choleriform gastroenteritis. However, the role of all these substances as pathogenic factors is still unclear.

In the other hand, the presence of the enzyme neuraminidase in the vibrio strain falling into Heiberg's group II helps to show that vibrios belonging to Heiberg's group II are related closely to V. cholerae, as is demonstrable also by some other properties. The importance of the enzymes neuraminidase and acylneuraminate pyruvate lyase in these vibrios may reside in the similar ecology and nutritional requirements of both species.

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LITERATURE CITED