Effect of Peptidelipids Produced by Bacillus on the Enzymatic Lysis of Gram-negative Bacterial Cells

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Received June 23, 1976

Bacillus subtilis YT-25 was reported to produce lytic enzymes which attacked the cell wall of Pseudomonas aeruginosa. In general, native cells of gram-negative bacteria are resistant to lytic enzymes produced by microorganisms. Lytic enzymes purified from the culture filtrate of B. subtilis YT-25 were also unable to lyse the native cells of Ps. aeruginosa, but the culture filtrate remarkably lysed its native cells. From these findings, the presence of another factor was suggested in the culture filtrate of B. subtilis YT-25, and this factor was designated as Native Cell-Lytic Factor (NLF-I**), which was purified from the culture filtrate of this strain through Amberlite IRC-50, silicic acid and Sephadex G-25 column chromatographies. NLF-I itself brought about either no or less lysis of native cells of Ps. aeruginosa, but in the presence of NLF-I lytic enzymes markedly lysed its native cells. Such effect that promotes the lysis of native cells by lytic enzymes is hereafter called NLF-effect.

Further, we found a new peptidelipid in the culture filtrate of B. circulans AJ 3902, newly isolated from soil, which showed the same promoting effect as NLF-I on the lysis of Ps. aeruginosa. This NLF-like substance was designated as NLF-II and was purified as follows. B. circulans AJ 3902 was aerobically cultured in a nutrient broth at 30°C for two days. NLF-II was extracted from 5 liters of the culture filtrate with equal volume of n-butanol three times. n-Butanol layer was concentrated and dried in vacuo, and the residue was dissolved in 100 ml of water. Five grams of sodium heliantate was added to this solution, wherein NLF-II was precipitated as its heliantate. The heliantate of NLF-II was collected by centrifugation and was dissolved in 90 ml of dimethylformamide. To this heliantate solution, 1.5 liters of water

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**TABLE I. PHYSICAL AND CHEMICAL PROPERTIES OF NLF-I HYDROCHLORIDE AND NLF-II HYDROCHLORIDE**

<table>
<thead>
<tr>
<th></th>
<th>NLF-I·HCl</th>
<th>NLF-II·HCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight(1)</td>
<td>1000 ~ 15000</td>
<td>1000 ~ 1500</td>
</tr>
<tr>
<td>Melting point (°C)</td>
<td>219 ~ 221</td>
<td>210 ~ 220</td>
</tr>
<tr>
<td>Elemental analyses (%)</td>
<td>C; 48.90, H; 8.29, N; 13.45, Cl; 10.81</td>
<td>C; 50.46, H; 7.92, N; 12.86, Cl; 9.62</td>
</tr>
<tr>
<td>Ultraviolet absorption</td>
<td>End absorption and a series of very weak maxima between 245 and 270 nm</td>
<td>Typical of a peptide</td>
</tr>
<tr>
<td>Infrared absorption</td>
<td></td>
<td></td>
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<tr>
<td>Amino acid analyses (%)</td>
<td>Phe; 5.9, Leu; 17.5, 2,4 Dab; b) 56.4</td>
<td>Ileu; 5.8, Ser; 6.4, Val; 8.2, Phe; 9.2, Leu; 18.6, 2,4 Dab; 39.6</td>
</tr>
</tbody>
</table>

(1) Molecular weight was estimated by gel-filtration on Sephadex G-25.
(2) 2,4-Diaminobutylic acid.

** NLF-I is the same as NLF previously reported.**
was added to precipitate the heliantate of NLF-II again, which was redissolved in 50 ml of 0.36 N HCl to release NLF-II. NLF-II thus obtained was chromatographed twice on a Sephadex G-25 column equilibrated with 0.02 N HCl. NLF-II-containing fractions were dried in vacuo to obtain white powder of NLF-II. This sample showed single-spot in thin layer chromatography in a number of solvent systems.

Physical and chemical properties of NLF-II and NLF-I isolated as previously reported are shown in Table I. Hydrolysis of both NLFs in 6 N HCl at 110°C for 20 hr gave a mixture of amino acids. Fatty acids as other component of NLFs were obtained by hydrolysis in 6 N HCl at 110°C for 30 min, and their methylesters were examined by mass spectrometry, after being separated by gas chromatography. A major component of fatty acids of NLF-I had formula C_{18}H_{35}COH-CH_{2}COOH, and that of NLF-II had formula C_{16}H_{17}CHOHCH_{2}COOH. From amino acid and fatty acid contents, NLF-I seems to be very similar to EM 49 which was reported as peptide antibiotics obtained from B. circulans. Amino acid composition of NLF-II is similar to a peptide antibiotic, polypeptin, but no threonine was detected and serine was contained in NLF-II. To our knowledge, compound such as NLF-II have never been reported as product from B. circulans.

Polymyxin B and colistin, peptidelipid antibiotics produced by Bacillus, showed the same effect as NLFs as shown in Fig. 1. Whereas peptide antibiotics which does not contain fatty acid residue, such as gramicidin D and bacitracin, did not show any NLF-like effect. From these results, substances showing the NLF-effect are similarly consisted about ten amino acid residues and a long chain fatty acid, and the presence of fatty acid residue seemed to be essential for the lysis promotion of native gram-negative bacterial cells. Surfactin, a surfactive peptidelipid which was produced by B. subtilis, was reported to promote the lysis of gram-negative bacteria and to be also consisted of seven amino acid residues and C_{18}-iso-β-hydroxy-fatty acid.

NLF-I and NLF-II seem to have some effect on membrane structure as does polymyxin B which was reported to denature the cell membrane structure. The lysis of native bacterial cells by the cooperation of lytic enzymes and NLF seems to be firstly caused by alteration of outer membrane of bacteria, followed by the penetration of lytic enzyme to peptidoglycan layer of the cell wall of Ps. aeruginosa. As these peptidelipids are considered to have different effect on various membranes such as outer or cytoplasmic membranes of gram-negative bacteria and cytoplasmic membranes of gram-positive bacteria, these peptidelipids seem to be useful for the studies of bacterial cell membrane. Comparison of the function and the structure
of these peptidelipids are now under investigation, which will be reported in another paper.

REFERENCES
6) A. Kakinuma and K. Arima, Kagaku to Seibutu, 6, 606 (1968).