Degradation of Muramyl Dipeptide by Mammalian Serum

J. HARRISON and A. FOX*

Department of Microbiology and Immunology, University of South Carolina School of Medicine, Columbia, South Carolina 29208

Received 29 April 1985/Accepted 26 June 1985

Muramyl dipeptide, N-acetylmuramyl-\(L\)-alanine-\(D\)-isoglutamine (MDP), is the minimal biologically active subunit of bacterial peptidoglycan and elicits an acute inflammation in vivo. We now report that MDP is degraded by normal rat serum into its constituents, N-acetylmuramic acid and \(L\)-alanine-\(D\)-isoglutamine. The dipeptide is further degraded into its components \(L\)-alanine and \(D\)-isoglutamine. These results may help to explain how inflammation elicited by MDP is terminated in vivo.

An elucidation of the events involved in the processing and elimination of peptidoglycan (PG) by the mammalian host is important in determining how inflammation elicited during bacterial infection is terminated. For example, high-molecular-mass (ca. \(10^6\) to \(10^9\) daltons or greater) group A streptococcal PG-polysaccharide complexes will induce perpetuating polyarthritis (4, 6). The size of PG-polysaccharide fragments is one factor in determining the severity and duration of inflammation they elicit (6), which is related to their dissemination and persistence in tissues (5, 8). Fragments of PG-polysaccharide of lower molecular mass (<\(5 \times 10^3\) daltons), in contrast, induce only a mild, transient edema (3). Soluble monomers and oligomers of PG which are generated during growth or processing of certain bacteria also display some toxicity for mammalian cells (9, 12, 16, 19). One possible degradation product of PG is muramyl dipeptide, \(N\)-acetylmuramyl-\(L\)-alanine-\(D\)-isoglutamine (MDP), its smallest component known to have biological activity (2). MDP elicits a mild inflammation of limited duration only in exceptional circumstances, including administration in an oil vehicle (14), in aqueous solution when given intravenously on multiple occasions (20), or as a single, large intraocular aqueous dose (7). The question of why inflammation elicited by MDP is so rapidly terminated remains unanswered. Two possibilities are readily apparent. First, MDP is soon degraded into inactive by-products, or, second, it is rapidly excreted in the urine or elsewhere. A serum enzyme, \(N\)-acetylmuramyl-\(L\)-alanine amidase, which hydrolyses the lactylamide bond between the glycan and peptide portions of PG oligomers, has been described previously (13, 18). However, it has been suggested on the basis of indirect evidence that MDP is apparently not a substrate for \(N\)-acetylmuramyl-\(L\)-alanine amidase (11) and that the demonstrated urinary excretion of intact MDP (1, 15) is thus the major means of elimination of MDP activity in vivo. PG oligomers have also been shown to be excreted in the urine (10, 17). We now report that MDP is in fact cleaved by mammalian serum into its constituents, \(N\)-acetylmuramic acid and the dipeptide \(L\)-alanine-\(D\)-isoglutamine. Furthermore, the dipeptide is lysed, producing \(L\)-alanine and \(D\)-isoglutamine.

Blood was obtained aseptically from female Lewis rats by cardiac puncture and allowed to clot at 4°C for several hours, and serum was isolated by centrifugation. The serum was frozen at \(70°C\) in aliquots until further use. Thawed serum was dialyzed under sterile conditions against 0.05 M Tris hydrochloride-0.02 M \(MgCl_2\) buffer (pH 7.9) three times over a 24-h period at 4°C. Dialyzed serum alone or containing 8 mg of MDP per ml was incubated for 24 to 72 h at 37°C. In some experiments, as an additional control for spontaneous (nonenzymatic) hydrolysis MDP was also incubated in buffer alone. Portions of each incubation mixture (3 \(\mu\)l for primary amine demonstration and 10 \(\mu\)l for secondary amine demonstration) and appropriate standards for possible digestion products (\(L\)-alanine-\(D\)-isoglutamine, \(L\)-alanine, \(D\)-isoglutamine, muramic acid, and \(N\)-acetylmuramic acid [Research Plus, Bayonne, N.J.]) were spotted on silica gel thin-layer chromatography plates with preabsorptive areas (Whatman, Inc., Clifton, N.J.) and analyzed in a butanol-ethanol-water (50:32:18) system. Plates were dried with hot air, sprayed with 0.2% ninhydrin in butanol, and heated at 110°C for 1 min to detect primary amines (\(L\)-alanine-\(D\)-isoglutamine, \(L\)-alanine, \(D\)-isoglutamine, and muramic acid) or at 250°C for 10 min to detect secondary amines (\(N\)-acetylmuramic acid and MDP). Primary amines were blue or pink, while secondary amines were black or brown, which, along with \(R_f\) values (Table 1), aided in their distinction.

Primary amines with \(R_f\) values corresponding to \(L\)-alanine-\(D\)-isoglutamine, \(L\)-alanine, and \(D\)-isoglutamine were detected in serum samples that had been incubated with MDP for periods ranging from 24 to 72 h. The amount of degradation products increased with extended incubation (Fig. 1A). Muramic acid was not identified as a digestion product of muramyl dipeptide. Secondary amines with \(R_f\) values corresponding to \(N\)-acetylmuramic acid and residual MDP were also detected. (Fig. 1B). MDP incubated alone in Tris

<table>
<thead>
<tr>
<th>Substance</th>
<th>(R_f)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(L)-Alanine-(D)-isoglutamine</td>
<td>0.09</td>
</tr>
<tr>
<td>(D)-Isoglutamine</td>
<td>0.17</td>
</tr>
<tr>
<td>(L)-Alanine</td>
<td>0.30</td>
</tr>
<tr>
<td>MDP</td>
<td>0.37</td>
</tr>
<tr>
<td>Muramic acid</td>
<td>0.40</td>
</tr>
<tr>
<td>(N)-Acetylmuramic acid</td>
<td>0.43</td>
</tr>
</tbody>
</table>

* Corresponding author.
hydrochloride buffer did not exhibit spontaneous breakdown.

The observations suggest that serum displays N-acetylmuramyl-L-alanine amidase activity capable of lysing MDP into its constituents N-acetylmuramic acid and L-alanine-D-isoglutamine. Furthermore, serum also displays peptidase activity capable of degrading L-alanine-D-isoglutamine into its constituents L-alanine and D-isoglutamine. The excretion of intact MDP in the urine on systemic administration is probably an important route for its elimination. However, it is not inconceivable that in certain other situations enzymatic degradation of MDP may play a significant role in terminating inflammatory events.

This work was supported by Public Health Service grant EY04715 from the National Institutes of Health.

LITERATURE CITED


