Studies on Amino-hexoses. XII. Crystalline Muramicitol and Its Use in a Study of the Lysozyme Action on the Cell Wall of Micrococcus Lysodeikticus

By Saburo HARA and Yoshio MATSUSHIMA

Department of Chemistry, Osaka University, College of Science, Toyonaka, Osaka

(Received June 11, 1966)

It has been concluded that the egg white lysozyme splits the polysaccharide part of the bacterial cell wall and that the di- and tetra-saccharides released have muramic acid as their reducing ends.[1] One of the structure analyses of these oligo-saccharides consisted of the reduction of the saccharides with borohydride and the examination of the subsequent hydrolysis mixture on chromatograms. As a result of a lack of the authentic muramicitol, 2-amino-3-*O*-(D-1'-carboxyethyl)-2-deoxy-D-glucitol, however, the above conclusion was drawn from the facts that muramic acid disappeared or diminished and that a new ninhydrin positive compound appeared on the chromatograms.

In order to gain a straight and positive conclusion, we have now prepared crystalline muramicitol and used it as the reference standard in a study of lysozyme action on the cell wall of M. lysodeikticus. As is shown in Table I, egg white lysozyme splits *N*-acetyl-muramide linkages only, leaving *N*-acetyl-glucosaminide linkages intact; this seems somewhat peculiar, because the same enzyme splits *N*-acetyl-glucosaminide linkages in chitin,[2] glycol chitin and carboxymethyl chitin.[3]

TABLE I. THE COMPOSITION OF THE ACID HYDROLYSATES (μmol./g.)

	Intact cell wall	Lysozyme digest treated with sodium borohydride
Muramic acid	597	169
Glucosamine	628	649
Muramicitol	0	360
Glucosaminitol	0	0
Glutamic acid	740	731
Glycine	765	729
Alanine	1635	1615
Lysine	697	688
Ammonia	763	832

The Preparation of Muramicitol.—Muramic acid[4] (150 mg.) was dissolved in 5 ml. of water, and to this solution there was then added 50 mg. of sodium borohydride while it was being stirred at room temperature. After the mixture had stood for 2 hr., its pH was adjusted to 5.6 by adding Amberlite IR-120 (H^+ form). The solution separated from the resins was concentrated to dryness in vacuo. To the residue 10 ml. of dry methanol was added, and the solution was evaporated to dryness in order to remove the methyl borate formed. This process was repeated three times. The residue was dissolved in 0.5 ml. of water, and to this solution 10 ml. of methanol was added. After the mixture had stood in a refrigerator, 90 mg. of colorless crystals were obtained. The specimen melted at 196°C (uncorr.) ($[\alpha]_D^{26}$ +44.9 (*c*, 0.423, water)). Found: C, 42.29; H, 7.72; N, 5.33. Calcd. for $C_9H_{19}O_7N$: C, 42.68; H, 7.56; N, 5.53%. The muramicitol thus obtained showed no reducing power against a ferricyanide reagent.[5] The ninhydrin color reaction was very weak, and the molar extinction coefficient was about 6% that of muramic acid. In a Stein-Moore column,[6] the specimen eluted before aspartic acid at pH 3.25 (0.2 N citrate).

The Lysozyme Action on the M. Lysodeikticus Cell Wall.—A cell wall (4.4 mg.) isolated from M. lysodeikticus by the usual bead-shaking method[7] was incubated with 0.5 mg.- of crystalline egg white lysozyme for 24 hr. at 37°C in 1.5 ml. of a M/15 phosphate buffer of pH 7.0. After the incubation had ended, the pH of the solution was adjusted to 9.0 by adding 0.1 N sodium hydroxide; then the mixture was treated with sodium borohydride for 24 hr. at room temperature. After lyophilization and hydrolysis with 6 N hydrochloric acid for 16 hr. at 100°C, an appropriate amount of the hydrolysate was run on a Stein-Moore column using an automatic instrument (Hitachi KLA-3). In Table I the results are shown and compared with the hydrolysate of the intact cell wall.

No correction was made for the degradation during acid hydrolysis, and the data for the minor-constituent amino acids are omitted.

This investigation was supported by U. S. Public Health Service Grant AI-04586-04.

1) M. R. J. Salton and J. M. Ghuysen, *Biochim. Biophys. Acta*, **36**, 552 (1959); ibid., **45**, 355 (1960); H. R. Perkins, *Biochem. J.*, **74**, 182 (1960); N. Sharon, T. Osawa, H. M. Flowers, and R. W. Jeanloz, *J. Biol. Chem.*, **241**, 223 (1966).

2) L. R. Berger and R. S. Weiser, *Biochim. Biophys. Acta*, **26**, 517 (1957).

3) Y. Matsushima, S. Hara, T. Miyazaki, and Y. Umemura, The 38th Annual Meeting of The Japanese Biochemical Society, October, 1965.

4) Y. Matsushima and J. T. Park, *J. Org. Chem.*, **27**, 3581 (1962).

5) J. T. Park and M. J. Johnson, *J. Biol. Chem.*, **181**, 149 (1949).

6) S. Moore and W. H. Stein, ibid., **192**, 663 (1951).

7) M. R. J. Salton and R. W. Horne, *Biochim. Biophys. Acta*, **7**, 177 (1951).