SHORT COMMUNICATION

A Receptor for *Proteus morganii* Bacteriocin 336

By J. A. SMIT AND L. A. STOCKEN,

Division of Life Sciences, Atomic Energy Board, Pretoria, South Africa

AND H. C. DE KLERK

Department of Microbiology, University of Pretoria, South Africa

(Accepted for publication 30 November 1970)

The walls of *Escherichia coli* and *Proteus mirabilis* have a rigid peptidoglycan foundation with lipopolysaccharide and lipoprotein as interlinking macromolecules (Hofschneider & Martin, 1968). These polymers carry phage and bacteriocin receptors whose structural details are unknown (see Smit, Hugo & de Klerk, 1969). Recently, bacteriocins were shown to adsorb to walls (Weltzien & Jesaitis, 1969), to the lipopolysaccharide fraction (Smit *et al.* 1969), or to the cytoplasmic membrane (Šmarda & Taubeneck, 1968). The properties of a receptor for the *P. morganii* bacteriocin 336 (Smit, de Klerk & Coetzee, 1968) are described here.

Bacterial strains and media. The bacteriocinogenic *Proteus morganii* strain MR336 and the *P. morganii* indicator strain MR47 have been described (Coetzee, 1967). Difco brain-heart infusion broth was used.

Assay of receptor activity. A dilution of bacteriocin 336 (Smit *et al.* 1968) was selected (0.1 ml.) which would inhibit a logarithmically growing culture (4.0 ml.) of strain MR47 with an absorbance of 0.19 to 0.21 at 600 nm. Serial fivefold dilutions of cell wall or receptor fractions (see below) were preincubated at 37° for 10 min. with the bacteriocin before addition to the cultures. Growth at 37° was then monitored at 10 min. intervals. Receptor activity (degree of neutralization of bacteriocin) was determined from rate of growth of strain MR47 expressed as a percentage of the rate for control, to which no bacteriocin was added.

Isolation and fractionation of cell walls. Walls were prepared from strain MR47 as previously described (Smit *et al.* 1969). Lipoprotein, lipopolysaccharide, polysaccharide and murein fractions were isolated from these walls with phenol (Westphal & Jann, 1965) or trichloroacetic acid (Staub, 1965).

Isolation of receptor. Walls (9.3 g. dry wt) were suspended in 0.1 M-tris-HCl buffer, pH 8.0 (500 ml.), and treated with pronase (1 mg./ml.; Seravac Laboratories Ltd) for 16 h. at 37° to allow self-digestion of the enzyme. The suspension was centrifuged (30,000 g for 30 min.). The pellet was washed three times with cold water (500 ml.) to remove residual proteolytic activity and lyophilized (1.5 g. dry wt). This material was suspended in water (150 ml.) and defatted with 20 % (v/v) butan-1-ol at 0°. After centrifugation (100,000 g for 1 h.) the pellet was re-extracted twice with butan-1-ol (760 mg. dry wt). Receptor activity was then extracted with 50 mM-sodium pyrophosphate, pH 7.9 (12 ml.), by stirring at 0° for 16 h. The suspension was centrifuged (10,000 g for 30 min.) and the pellet extracted a further five times. The supernatant

solutions were combined and lyophilized. This material was dissolved and chromatographed in 25 mM-ammonium formate, pH 7.5, on Sephadex G-200 and Sephadex G-25, the latter step being repeated with water as solvent. At each stage fractions with the highest receptor activity were combined and lyophilized; final yield 9 mg (0.1 %).

Analysis of receptor. Methods for the determination of inorganic phosphate, lipid, carbohydrate, amino acids, nucleic acid and proteins and the electron microscopy techniques have been described (de Klerk & Smit, 1967; Smit *et al.* 1968). Hexosamines were determined by gas chromatography.

Isolated walls of strain MR47 contain receptor activity for bacteriocin 336. Lipopolysaccharide, polysaccharide, lipoprotein and the peptidoglycan extracted with phenol or trichloroacetic acid did not neutralize the bacteriocin. The superficial non-covalently bound lipid also lacked receptor activity. Pronase-treated wall suspensions retained receptor activity. This treatment reduced the absorbance of wall suspensions

Fig. 1(a) Separation on Sephadex G-200 exclusion gel of receptor for *Proteus morganii* bacteriocin. The receptor was isolated with sodium pyrophosphate from pronase treated defatted walls. (b) Further purification of combined receptor fractions from Sephadex G-200 columns on Sephadex G-25. Fractions (5 ml.) were assayed for protein content (E_{280}) (—) and receptor activity (---).

by 45 % within 2 min. Braun & Rehn (1969) postulated that the reduced absorbance reflects disruption of covalent bonds of those proteins which stabilize the wall. Receptor activity might have been protected by complexed lipids during proteolysis because in order to preserve receptor activity the cell walls had to be treated with pronase in the presence of their lipids. After defatting, receptor was quantitatively removed from the remaining sedimentable material by repeated extraction with sodium pyrophosphate. This treatment disrupts salt linkages and hydrogen bonds. Exclusion chromatography on Sephadex G-200 separated the receptor from contaminating cell wall material which moved with the void volume of the column (Fig. 1a). Separation was performed in the presence of ammonium formate to prevent reaggregation. Subsequent exclusion chromatography on Sephadex G-25 removed low molecular weight contaminants (Fig. 1b). Repassage through Sephadex G-25 with water freed the isolate of ammonium formate and yielded receptor activity which moved in a single band. The final product was 333 times more active than the starting material.

Purified receptor was white powder which appeared as amorphous material in the electron microscope. It behaved as a lipoprotein and contained 16.5 % (w/w) tightly bound lipid which could be released only by acid hydrolysis. The amino acid compo-

sition was as follows (moles %): aspartate, 8.39; threonine, 6.27; serine, 7.49; glutamate, 12.25; proline, 2.59; glycine, 10.64; alanine, 17.50; 1/2 cystine, 0.43; valine, 5.26; methionine, 0.98; isoleucine, 3.01; leucine, 5.01; tyrosine, 2.25; phenylalanine, 2.27; glucosamine, 4.43; diaminopimelate, 4.15; histidine, 0.93; lysine, 2.58; arginine, 3.12; and 3-methyl-histidine, 0.45. Purified receptor passes through dialysis membrane and probably has a molecular weight below 10,000. After treatment with pronase the dialysis diffusate had no activity. This testifies to the importance of the protein component. No muramic acid was detected but α , ϵ -diamino pimelic acid and glucosamine were present. Tests for phosphate, carbohydrate and nucleic acids were negative. Receptor was not affected by lysozyme, DNase or RNase.

Fractionation of walls of *Proteus vulgaris* (Kandler, Hund & Zehender, 1958) and *P. mirabilis* (Martin, 1964) showed that a protein could be removed from the peptidoglycan of these strains. The interlinking peptide bridge of these polymers contains alanine, glutamic acid and diaminopimelic acid. The presence of these amino acids in relatively high concentrations in the *P.morganii* receptor suggests that it may include a similar peptide bridge. It is unlikely that the glucosamine moiety of the receptor originated from the glycan polymer as a result of pronase treatment. It is probably an integral part of the lipoprotein. Braun & Rehn (1969) isolated a protein fraction from the peptidoglycan of *Escherichia coli* with trypsin and concluded that it was a structural protein. The lipoprotein isolated here may have a stabilizing effect on the murein sacculus and act as a receptor for bacteriocin 336.

This work was aided by grants to Professor J. N. Coetzee from the South African Medical Research Council.

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