JOURNAL OF BACTERIOLOGY, Jan. 1979, p. 670-672
0021-9193/79/01-0670/03$02.00/0

Vol. 137, No. 1

Outer Membrane of *Serratia marcescens*: Apparent Molecular Weights of Heat-Modifiable Proteins in Gels with Different Acrylamide Concentrations

K. B. HELLER

Lehrstuhl für Biologie der Mikroorganismen, Ruhr-Universität Bochum, 4630 Bochum, Germany

Received for publication 31 October 1978

The major proteins from the outer membrane of *Serratia marcescens* SM-6 are heat modifiable. The analysis of their apparent molecular weights in gels with different concentrations of acrylamide and the results obtained by radioactive labeling indicate that the major proteins are covalently linked to carbohydrate moieties.

The cell envelope of *Enterobacteriaceae* consists of an inner, cytoplasmic membrane and an outer membrane separated by a peptidoglycan layer. The biochemical properties of the outer membrane of *Escherichia coli* have been studied extensively (2, 10, 11, 16-18). The physiological importance of the *E. coli* outer-membrane proteins is only partially understood. A few, for example, function as transport-mediating proteins and/or phage receptors (1, 3, 9, 15, 19, 22).

Relatively little is known about the outer membrane of *Serratia marcescens* (2, 10, 21, 23), a gram-negative bacterium, which, in contrast to *E. coli*, releases enzymatically active proteins into the growth medium: the properties and kinetics of a nuclease (13, 20), protease (24), and lipase (25) are under investigation. Furthermore, *Serratia* bacteria are relatively resistant to many antibiotics (4, 25). It is likely that these properties are related to the membrane composition. Preliminary results concerning the correlation between antibiotic sensitivity and surface properties have been reported (25). Antibiotic-hypersensitive mutants of *S. marcescens* show remarkably different binding properties towards various dyes and sensitivity to detergents and adsorb rough-mutant specific phage 6SR.

The outer-membrane proteins of *S. marcescens* separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis after solubilization at 37 or 100°C are shown in Fig. 1, left side. The electrophoretic mobility of the outer-membrane proteins was dependent on the solubilization temperature (heat modification), and the apparent molecular weights of the proteins depended on the concentration of acrylamide in the gels (Fig. 2). The apparent molecular weights of the proteins of *S. marcescens* solubilized at 37°C decreased if the concentration of acrylamide was raised. This result is different from the data obtained with the heat-modifiable protein d of *E. coli* (7). The electrophoretic behavior of protein d can be explained by an overproportional sodium dodecyl sulfate binding to the form solubilized at 37°C due to large beta-helical regions. The apparent molecular weight of the protein d of *E. coli* solubilized at 100°C is independent of the concentration of acrylamide in the gels. However, the molecular weights of the outer-membrane proteins of *S. marcescens* solubilized at 100°C increased by increasing concentrations of acrylamide.

The molecular weight of proteins determined at high concentrations of acrylamide in the gel is proved to be the most reliable (7).

Surprisingly, at high concentrations of acrylamide, the apparent molecular weight of proteins of the outer membrane of *S. marcescens* estimated after boiling the membranes for solubilization was about two times higher than that of the proteins solubilized at 37°C.

The data presented indicate that the outer-membrane proteins of *S. marcescens* bound an unusual amount of sodium dodecyl sulfate before boiling for solubilization (possibly due to large domaines of beta-structure) and/or that the amino acids were not solely responsible for the molecular weight of the major proteins.

Recent results obtained by thin-layer chromatography indicated that the peptidoglycan-associated major proteins (Fig. 1, no. 2 and 3; cf. reference 10) of *S. marcescens* contain at least four different monosaccharides, one of them co-banding with galactose (manuscript in preparation).

The results from radioactive-labeling experiments of isolated outer membranes by the galactose oxidase-sodium-boro[^{3}H]hydride technique (5) before the solubilization of the membranes for electrophoresis supported the suggestion that carbohydrate moieties were covalently

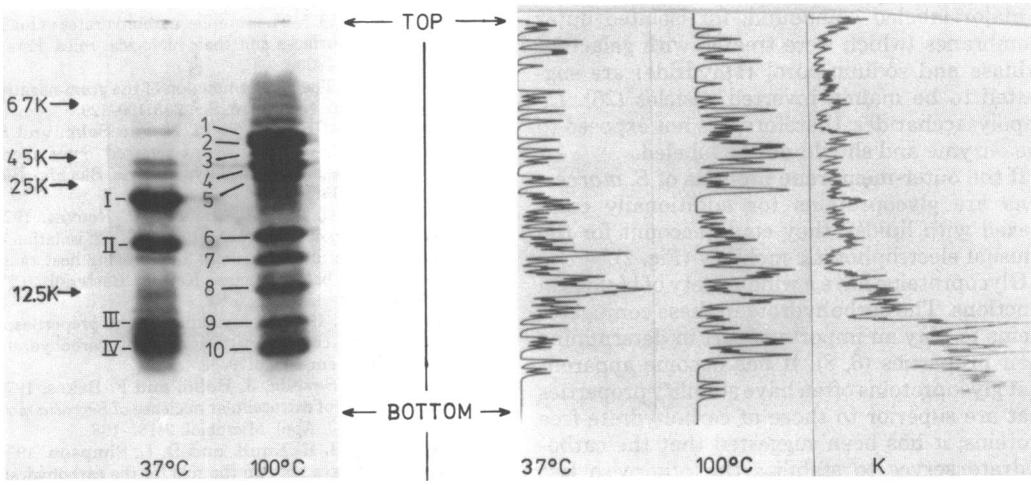

FIG. 1. *(Left side) Electrophoretic profile of ^{3}H-labeled isolated outer membranes (25) of S. marcescens SM-6. In a buffer described by Rosenbusch (16), one aliquot was solubilized for 30 min at 37°C, and the other was solubilized for 5 min at 100°C. Electrophoresis was performed as described by Winkler et al. (25). The gel was stained for proteins, and the designation of protein bands was done arbitrarily. (Right side) Scan traces of the radioactivity incorporated in outer-membrane proteins of S. marcescens SM-6. Isolated membranes were labeled with the galactose oxidase sodium-boro[^{3}H]hydride technique (5) before electrophoresis. The scans were obtained from the gel shown at the left side by means of a beta-scanner (Berthold, Karlsruhe, Germany). (K) Control, isolated membranes were labeled without preincubation with galactose oxidase before electrophoresis. In the control, a 10-fold-higher amount of protein was applied to detect radioactivity.*

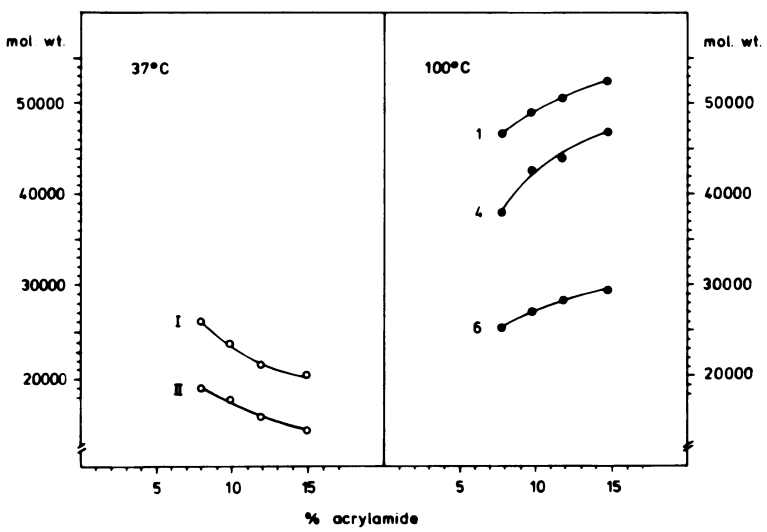

FIG. 2. *Apparent molecular weights of representative outer-membrane proteins of S. marcescens SM-6 estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. For designation of proteins, see Fig. 1. Experiments were performed as previously described (7). The protein samples of the isolated outer membranes (25) were solubilized for 30 min at 37°C (○) or for 5 min at 100°C (●) in a buffer described by Rosenbusch (16).*

linked to the proteins (Fig. 1, right side). Most of the radioactive label could be detected with the major proteins independently of the solubilization procedure.

Without preincubation with galactose oxidase, there was only little hydration by tritium of fast-migrating material (possibly unsaturated fatty acids were hydrated) independent of the solubilization temperature (Fig. 1, right side, K). It is not very likely that the lipopolysaccharide is

a major labeled compound, for isolated outer membranes (which were treated with galactose oxidase and sodium-boro[^{3}H]hydride) are suggested to be mainly inverted vesicles (26). Lipopolysaccharides, therefore, are not exposed to the enzyme and should not be labeled.

If the outer-membrane proteins of *S. marcescens* are glycoproteins (or additionally complexed with lipids), they could account for the unusual electrophoretic mobility (Fig. 2).

Glycoproteins have a wide variety of biological functions. The carbohydrate in these conjugates seems to play an important part in determining their properties (6, 8). It has become apparent that glycoproteins often have stability properties that are superior to those of carbohydrate-free proteins; it has been suggested that the carbohydrate serves to stabilize the otherwise less stabile polypeptide chain (12, 14).

The glycosylation (which still is hypothetical) of the membrane proteins of *S. marcescens* might be one reason for a structural change of the membrane organization. An altered selectivity of the hydrophilic pores (the major proteins are postulated to build transmembrane channels [cf. reference 19]) could result in an exclusion of antibacterial agents. The surface properties have been shown to be involved in the resistance towards antibiotics and sensitivity to detergents, as well as in phage adsorption (25). Possibly, the liberation of extracellular enzymes, which also seem to be glycoproteins (J. Sossinka, personal communication), from the outer membrane of *S. marcescens* is affected, too.

I thank L. Bohne and U. Winkler for helpful discussion and Th. Whitmore for critical reading of the manuscript. The radioactive labeling was carried out by U. Stirnberg in partial fulfillment of her M.Sc.

LITERATURE CITED

1. **Bassford, P. J., Jr., D. L. Diedrich, C. A. Schnaitman, and P. Reeves.** 1977. Outer membrane proteins of *Escherichia coli*. VI. Protein alteration in bacteriophage-resistant mutants. J. Bacteriol. **131**:608–622.
2. **Braun, V.** 1975. Covalent lipoprotein from the outer membrane of *Escherichia coli*. Biochim. Biophys. Acta **415**:335–377.
3. **Chai, T.-J., and J. Foulds.** 1977. *Escherichia coli* K-12 *tolF* mutants: alterations in protein composition of the outer membrane. J. Bacteriol. **130**:781–786.
4. **Cooksey, R. C., G. M. Thorne, and W. E. Farrar, Jr.** 1976. R factor-mediated antibiotic resistance in *Serratia marcescens*. Antimicrob. Agents Chemother. **10**: 123–127.
5. **Gahmberg, C. G., and S. Hakomori.** 1975. Surface carbohydrates of hamster fibroblasts. J. Biol. Chem. **250**:2438–2446.
6. **Gottschalk, A.** 1972. Glycoproteins, 2nd ed. Elsevier, Amsterdam.
7. **Heller, K. B.** 1978. Apparent molecular weights of a heat-modifiable protein from the outer membrane of *Escherichia coli* in gels with different acrylamide concentrations. J. Bacteriol. **134**:1181–1183.
8. **Hughes, R. C.** 1975. The complex carbohydrates of mammalian cell surfaces and their biological roles. Essays Biochem. **11**:1–36.
9. **Leive, L.** 1974. The barrier function of the gram-negative envelope. Ann. N.Y. Acad. Sci. **235**:109–129.
10. **Lugtenberg, B., H. Bronstein, N. van Selm, and R. Peters.** 1977. Peptidoglycan-associated outer membrane proteins in gram-negative bacteria. Biochim. Biophys. Acta **465**:571–578.
11. **Manning, P. A., A. Puspurs, and P. Reeves.** 1976. Outer membrane of *Escherichia coli* K-12: isolation of mutants with altered protein 3A by using host range mutants of bacteriophage K3. J. Bacteriol. **127**: 1080–1084.
12. **Marshall, J. J.** 1978. Manipulation of the properties of enzymes by covalent attachment of carbohydrate. Trends Biochem. Sci. **3**:79–83.
13. **Nyeste, L., B. Sevella, J. Holló, and F. Békés.** 1976. Kinetic study of extracellular nuclease of *Serratia marcescens*. Eur. J. Appl. Microbiol. **2**:161–168.
14. **Pazur, J. H., H. R. Knull, and D. L. Simpson.** 1970. Glycoenzymes: a note on the role for the carbohydrate moieties. Biochem. Biophys. Res. Commun. **40**: 110–116.
15. **Randall, L. L.** 1975. Quantitation of the loss of the bacteriophage lambda receptor protein from the outer membrane of lipopolysaccharide-deficient strains of *Escherichia coli*. J. Bacteriol. **123**:41–46.
16. **Rosenbusch, J. P.** 1974. Characterization of the major envelope protein from *Escherichia coli*. J. Biol. Chem. **249**:8019–8029.
17. **Schnaitman, C. A.** 1970. Protein composition of the cell wall and cytoplasmic membrane of *Escherichia coli*. J. Bacteriol. **104**:890–901.
18. **Schweizer, M., H. Schwarz, I. Sonntag, and U. Henning.** 1976. Mutational change of membrane architecture. Mutants of *Escherichia coli* K-12 missing major proteins of the outer cell envelope membrane. Biochim. Biophys. Acta **448**:474–491.
19. **Steven, A. C., B. ten Heggeler, R. Müller, J. Kistler, and J. P. Rosenbusch.** 1977. Ultrastructure of a periodic protein layer in the outer membrane of *Escherichia coli*. J. Cell Biol. **72**:292–301.
20. **Timmis, K., and U. Winkler.** 1973. Isolation of covalently closed circular deoxyribonucleic acid from bacteria which produce exocellular nuclease. J. Bacteriol. **113**:508–509.
21. **Tsang, J. C., D. A. Brown, and D. M. Kranz.** 1976. Detection of lipopolysaccharide components in isolated outer membranes of *Serratia marcescens*. Microbios Lett. **1**:209–217.
22. **Van Alphen, W., Lugtenberg, B., and W. Berendsen.** 1976. Heptose-deficient mutants of *Escherichia coli* K-12 deficient in up to three major outer membrane proteins. Mol. Gen. Genet. **147**:263–269.
23. **Weber, D. A., M. J. Nadakavukaren, and J. C. Tsang.** 1976. Effect of polymyxin B on outer membranes of *Serratia marcescens*: morphological alterations of the outer membranes and their lipopolysaccharide components. Microbios **17**:149–161.
24. **Winkler, U.** 1967. Extrazelluläre Nuklease-, Protease- und Bacteriocin-Aktivität beim Wildtyp und Mutanten von *Serratia marcescens* HY. Zentralbl. Bakteriol. Abt. Parasitenkd. Infektionskr. Hyg. Orig. Reihe A **205**: 62–63.
25. **Winkler, U., K. B. Heller, and B. Folle.** 1978. Pleiotropic consequences of mutations towards antibiotic-hypersensitivity in *Serratia marcescens*. Arch. Microbiol. **116**:259–268.
26. **Yamato, I., M. Futai, Y. Anraku, and Y. Nonomura.** 1978. Cytoplasmic membrane vesicles of *Escherichia coli*. II. Orientation of the vesicles studied by location of enzymes. J. Biochem. **83**:117–128.