Molec. gen. Genet. 155, 227–229 (1977)

MGG



*Short Communication*

# Identification of the *sid* outer Membrane Receptor Protein in *Salmonella typhimurium* SL1027

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**Summary.** A protein of molecular weight 78,000 daltons, missing in albomycin and phage ES18 resistant mutants, has been identified in the outer membrane of *Salmonella typhimurium* SL1027. Mutants with a *ton*B like resistance and overproduction of outer membrane proteins due to iron shortage were also isolated. The mutation which leads to the protein deficiency maps in the *sid* gene region, the mutation related to overproduction of proteins maps near *trp*. Although the *S. typhimurium* and the *E. coli* protein mediate translocation of the iron complex ferrichrome and the structurally analogous antibiotic albomycin through the outer membrane no cross-reactivity exists in binding the phages T5, T1 and ES18 or colicin M.

A protein in the outer membrane of *Escherichia coli* specified by the *ton*A gene, is the binding site for the phages T5, T1, φ80, colicin M (Braun et al., 1973; Braun et al., 1976; Braun and Wolff, 1973) and is required for the uptake of iron as ferrichrome complex (Hantke and Braun, 1975; Wayne and Neilands, 1975) and the action of the antibiotic albomycin, a structural analogue of ferrichrome. Since structurally very different compounds bind to the same receptor protein, a specificity problem arises. We attempted to resolve this problem by isolating missense mutants which have lost only one or a few binding functions while still retaining the binding capacity for the others. However all spontaneous or mutagen induced *ton*A mutants studied so far were unable to adsorb any of the substrates, and revertants or partial revertants had regained or partially regained all the binding functions (Braun et al., 1976). In this paper we describe another approach. We looked for naturally occuring variants in which only some *ton*A related functions were operating. *Salmonella typhimurium* is such a case. *S. typhimurium* is resistant to the phages, but it is sensitive to albomycin and takes up iron as ferrichrome complex. The question we asked was whether there exists an outer membrane protein in *S. typhimurium* which is structurally and functionally equivalent to the *ton*A receptor protein in *E. coli*. Such a protein could be considered as a variant of the *E. coli* protein operating only in some functions.

B.A.D. Stocker observed a correlation between sensitivity of *S. typhimurium* to albomycin and phage ES18 suggesting a common binding site. From 25 isolated albomycin restistant mutants of *S. typhimurium* SL1027 (Table 1) 3 were resistant to phage ES18. Outer membranes of the albomycin/ES18 resistant mutants were prepared by sucrose density gradient centrifugation and the proteins separated by SDS-slab gel electrophoresis. Compared with the wild type (Fig. 1, rows 1, 4) two of the mutants had lost a protein in the molecular weight region of 78,000 daltons (Fig. 1, rows 2, 6, 7). The electrophoretic mobility of the *Salmonella* protein corresponds with that of *E. coli* AB2847 (Fig. 1, row 8). The T5 resistant *E. coli* strain F470 also lacks this protein (Fig. 1, row 5). It was claimed that this *E. coli* strain is exceptional in that it supports growth of the *Salmonella* specific phage ES18 (B.A.D. Stocker, personal commun.). By cross-streaking ES18 phage and F470 cells we observed some inhibition of cell growth. Few receptor proteins which are below the level of detection by SDS gel electrophoresis suffice for a low phage sensitivity (Braun et al., 1976). In addition our *E. coli* 470 strain was albomycin resistant so that T5, ES18 and albomycin resistance is consistent with the lack of the *ton*A like protein. The third albomycin and ES18 resistant *S. typhimurium* mutant, SL1027/24, contained the *ton*A like protein and in addition increased amounts of three proteins in the molecular weight range between 70,000 and 80,000 daltons (Fig. 1, row 3). This protein pattern is in *E. coli* typical for *ton*B mutants which overproduce

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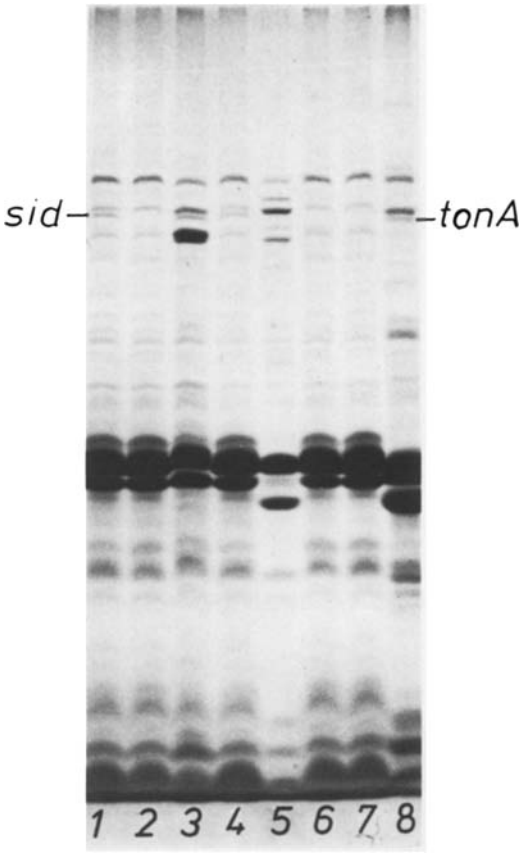


**Fig. 1.** Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the outer membranes of *Salmonella typhimurium* strains SL1027 wild type (row 1 and 4), the albomycin and phage ES18 resistant *sid* mutant SL1027/1 (row 2), the albomycin and phage ES18 resistant *ton*B like (*chr*) mutant SL1027/24 (row 3), the *Escherichia coli ton*A mutant F470 (row 5), the albomycin and phage ES18 resistant *sid* mutant of *Salmonella typhimurium* SL1027/23 (row 6 and 7) and *Escherichia coli* AB2847, wild type (row 8). Cells were grown in nutrient broth to an adsorbance of 0.5 at 578 nm. The outer membranes were prepared by the method of Osborn et al. (1972). Gel electrophoresis followed basically the method of Lugtenberg et al. (1975). The spacer gel contained 5.5% acrylamide. The current was first held constant at 12 mA until the tracking dye bromphenol blue had passed through the spacer gel and then adjusted to 15 mA and kept at this level. This resulted in straight protein bands which with higher currents became wavy. Between 30 and 60 μg protein were applied per gel

these proteins in response to intracellular iron shortage (Braun et al., 1976). It is known that in *S. typhimurium* these proteins are coordinately regulated by the concentration of iron in the medium (Bennet and Rothfield, 1976).

The mutations in the *ton*A like strain SL1027/23 and the *ton*B like strain SL1027/24 were maped by P1 transduction. For this purpose phage FO resistant mutants from both strains were isolated among which SL1027/231 and SL1027/243 proved to be P1 sensitive. The *ton*A like gene was transduced into the recipient strain *S. typhimurium* CW111 (Table 1) which

**Table 1.** Properties of Bacterial strains

| Strain | Genetic markers | Source or reference |
|---|---|---|
| *S. typhimurium* | | |
| *SL1027* | trp, met | B.A.D. Stocker |
| SL1027/1 | trp, met, sid | this paper |
| SL1027/23 | trp, met, sid | this paper |
| SL1027/24 | trp, met, chr | this paper |
| SL1027/231 | trp, met, sid, P1 sensitive | this paper |
| SL1027/243 | trp, met, chr, P1 sensitive | this paper |
| TA2167 | his C3076, Gal E506 | G.F.L. Ames |
| CW111 | dap-106, metA, trpB, etc. | Graham and Stocker (1977) |
| *E. coli* | | |
| F470 | aro, pro, his, met, mtl, rfb | G. Schmidt |
| AB2847 | aroB, tsx, malT | Braun et al. (1976) |

is mutated either in the dap C or the dap D gene, a locus close to *ton*A in *E. coli*. Of 182 selected colonies able to grow on a plate without addition of diaminopimelate 99 were ES18 resistant. The high cotransduction frequency of 54% indicates that, like in *E. coli*, the protein in the outer membrane of *S. typhimurium* conferring albomycin, phage ES18 sensitivity and uptake of iron as ferrichrome complex maps close to the dap C (D) locus. The *ton*B like phenotype in strain SL1027/243 could be reversed, measured as ES18 sensitivity, in 51 out of 80 transductants using a P1 lysate of *S. typhimurium* TA2167 (Table 1) and selecting for tryptophane prototrophy. Mutations conferring chromium sensitivity were mapped in the same gene region (Sanderson, 1972). Chromium reduces iron solubility which becomes effective in *ton*B like mutants, called for this reason *chr* in *S. typhimurium*, which are impaired in iron uptake. In fact SL1027/24 was found to be chromium sensitive. *Chr* mutants of *S. typhimurium* and *ton*B mutants in *E. coli* show the same phenotypes: chromium sensitivity, hyperexcretion of the iron chelator enterochelin and induction of outer membrane proteins due to iron shortage. Phage *ES*18 still binds to the *chr* mutant (Fig. 2) as does T1 or ϕ80 to *E. coli ton*B mutants (Garen, 1954; Hancock and Braun, 1976), but in both cases binding is reversible and does not lead to infection. The adsorption kinetics of phage ES18 to the *chr* mutant SL1027/24 and likewise to the wild type strain SL1027 (Fig. 2) clearly differ from the resistant mutant SL1027/1 which lacks the receptor protein.

The simultaneous resistance of the two *S. typhimurium* mutants lacking the *ton*A like protein against albomycin and phage ES18 point to a common binding site. Indeed infection of cells by phage ES18 can be completely inhibited by 100 μM concentrations of

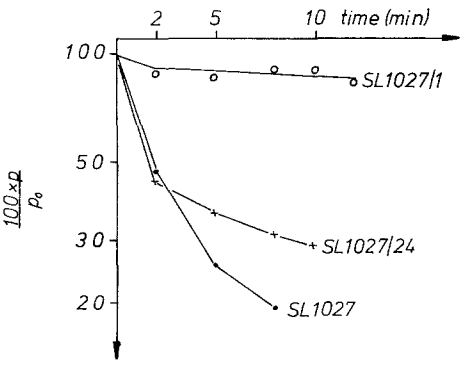


**Fig. 2.** Adsorption of phage ES18 to *Salmonella typhimurium* SL1027 wild type strain, the *sid* mutant SL1027/1 lacking the receptor protein and to the *ton*B like (*chr*) mutant SL1027/24 which is albomycin and ES18 resistant but contains normal amounts of the receptor protein. Cells were grown in tryptone-yeast medium to late logarithmic phase. They were spun down and resuspended in the same medium to an optical density of 1.0 at 578 nm and shaken for 5 min at 37° C. Then at 0 time 0.1 ml ES18 (titer $2 \times 10^9$/ml) was added. Samples of 0.05 ml were withdrawn at 2, 4, 5, 8 and 10 min and diluted into 5 ml ice cold medium. The cells were spun down and $10^{-3}$ dilutions of the supernatants were plated on strain SL1027. $P_0$ denotes the input phage number, P the number of phages remaining in the supernatant after adsorption has been allowed to the cells

ferrichrome, a result published also by Luckey and Neilands (1976) during the course of this work.

The results presented show that *S. typhimurium* contains in the outer membrane a protein similar in size and function to the *ton*A protein of *E. coli*. We propose to call it *sid* protein because it is most likely coded by one of the *sid* genes described earlier in connection with siderochrome promoted growth and ferrichrome uptake (Luckey et al., 1972). In addition the properties of the third albomycin and phage ES18 resistant mutant point to a *ton*B like function in *Salmonella*. Graham and Stocker (1977) transferred an F′-trp$^+$ of *S. typhimurium* LT2 origin into a *trp*, *ton*B mutant of *E. coli* B and restored sensitivity to phage T1 and colicin M which depend on the *ton*B function. In addition, transfer of the *ton*A gene of *E. coli* into *S. typhimurium* LT2 rendered the latter T5 sensitive and partially T1 sensitive showing again a *ton*B equivalent function in *S. typhimurium*. The *ton*A and the *sid* proteins can apparently both become coupled to the *ton*B (*chr*) function. The difference between *E. coli* and *S. typhimurium* with regard to this receptor system is only that the former binds phage T1 and T5 and excludes phage ES18, the other binds ES18 and excludes T1 and T5. Both proteins function in facilitating diffusion of ferrichrome and albomycin across the outer membrane. The *E. coli* and the *S. typhimurium* outer membrane protein form the presumptive pore through the outer membrane for ferrichrome and albomycin. Because there is no cross-reactivity for phage or colicin binding it appears that the stereospecific requirements for forming the pore are less narrow than for binding phages or colicins.

*Acknowledgement.* This research was supported by the Deutsche Forschungsgemeinschaft (SFB 76). We thank B.A.D. Stocker, Stanford University, California and G. Schmidt, Max-Planck-Institut für Immunbiologie, Freiburg for providing phage and bacterial strains. The technical assistance of Mrs. Ursula Holzwarth is gratefully acknowledged.

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Communicated by H.G. Wittmann

*Received June 21, 1977*