Somatic Antigen 2 Inheritance in Salmonella Groups B and D

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Somatic (O) antigen 2 of Salmonella paratyphi A replaced somatic antigen 4 of an S. typhimurium recipient as the consequence of mating with an S. paratyphi A var. durazzo Hfr strain. The genetic determinants of these O antigens behaved in this cross as alleles of a common O locus, which is linked to the determinant of histidine biosynthesis, his. By employing phage lysates obtained by growth of P22 on an S. typhimurium hybrid which had received his and O-factor 2 determinants from the S. paratyphi A Hfr, it was possible to cotransduce the his and O-antigen 2 genes to both S. typhimurium and S. typhosa. S. typhimurium transductants which received somatic antigen 2 concurrently lost O-antigen 4, and S. typhosa transductants receiving O-antigen 2, lost their native O-antigen 9. These results indicate that the genetic determinants of O-antigens 2, 4, and 9 occupy the same O locus in S. paratyphi A, S. typhimurium, and S. typhosa, respectively, and are probably allelic.

The genes which determine Salmonella somatic (O) antigens 4 (group B) and 9 (group D) are mutually replaceable in genetic transfer experiments between group B and group D Salmonella; in other words, they behave as alleles of the same chromosomal locus (6, 10). This O locus is closely linked to the determinant of histidine biosynthesis, his (6, 10), and has been shown to be cotransducible with his (by phage P22) in both S. typhimurium and S. typhosa (6). It is identical, in all probability, with the locus or gene cluster which, in S. typhimurium, has been called rouB (1), and which has now been redesignated rfb (B. A. D. Stocker, personal communication).

In the present study, conjugation and transduction experiments were employed to examine the genetic basis of somatic antigen 2 of S. paratyphi var. durazzo, a representative of Salmonella group A which differs from S. paratyphi A only in that it lacks O-antigen 1. The results of these experiments indicate that the genetic determinants of O-antigen 2 of S. paratyphi A, 4 of S. typhimurium, and 9 of S. typhosa occupy the same O locus, in their respective species, and are probably allelic.

MATERIALS AND METHODS

Organisms. The bacterial strains employed are described in Table 1. The S. paratyphi var. durazzo strain WR 7000 was obtained from Arthur Abrams of the Walter Reed Army Institute of Research.

Media. The minimal agar selective medium used has been described (4). Selection of prototrophic recombinants was accomplished by omitting the required amino acid from the selective medium; glucose at a concentration of 0.4% served as the carbon source. Streptomycin (625 μg/ml) was employed to counterselect the donor strain WR 4002. Selection of lac" recombinants of WR 7000 was accomplished on minimal agar containing 0.4% lactose.

Technique of bacterial mating and transduction. The bacterial mating procedures (4) and technique of transduction (7) have been described previously. Recombinant clones were purified by streaking on minimal medium of the same composition as that used for initial selection. Unselected auxotrophic marker analysis was accomplished by streaking the purified recombinants on appropriate minimal glucose selective medium. The inheritance of somatic (O) antigens was determined by slide agglutination in appropriate antisera.

Preparation and use of antisera. Group B O-antiserum (factors 4, 5, 12), group A O-antiserum (factors 1, 2, 12), single-factor somatic 2 antiserum, and single-factor somatic 5 antiserum were obtained from Arthur Abrams of the Walter Reed Army Institute of Research. Single-factor somatic 9 antiserum was prepared by absorption of anti-S. typhosa serum (factors 9, 12) with S. paratyphi var. durazzo, following the procedure described by Edwards and Ewing (2). It was not necessary to absorb factor 12 antibody from the group B antiserum, since its activity was too weak to be detected. The group B antiserum was used, therefore, as an anti-4, 5 serum. Hybrids agglutinating in group B antiserum were checked in single-factor 5 antiserum for the presence of antigen 5. Group A (factors 1, 2, 12) antiserum was employed along with group B antiserum, in preliminary screening, as an anti-2 reagent. With this antiserum, the presence of antigen 12, common to all strains employed, produced a weak, delayed positive reaction which was
TABLE 1. Characteristics of the bacterial strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Former designation</th>
<th>Salmonella species</th>
<th>Antisomotic characters</th>
<th>Carbohydrate utilization</th>
<th>O antigens</th>
<th>Mating polarity</th>
</tr>
</thead>
<tbody>
<tr>
<td>WR 4000</td>
<td>TD-7</td>
<td>S. typhosa</td>
<td>Cys, Trp</td>
<td>+ + + + +2, 12</td>
<td>9, 12</td>
<td>S Hfr</td>
</tr>
<tr>
<td>WR 4002</td>
<td>None</td>
<td>S. paratyphi var. durazzo</td>
<td>- - - - -</td>
<td>+ + + + +2, 12</td>
<td>9, 12</td>
<td>S Hfr</td>
</tr>
<tr>
<td>WR 4205</td>
<td>643WS/HM</td>
<td>S. typhosa</td>
<td>Cys, Trp, His, Met</td>
<td>+ + + + - 4, 5, 12</td>
<td>- + + +</td>
<td>Recipient</td>
</tr>
<tr>
<td>WR 5000</td>
<td>HMX♂</td>
<td>S. typhimurium LT2</td>
<td>His, Met</td>
<td>+ + + + +2, 12</td>
<td>4, 12</td>
<td>S Hfr</td>
</tr>
<tr>
<td>WR 7000</td>
<td>None</td>
<td>S. paratyphi var. durazzo</td>
<td>+ + + + +2, 12</td>
<td>+ + + + +2, 12</td>
<td>9, 12</td>
<td>S Hfr</td>
</tr>
</tbody>
</table>

* Abbreviations and symbols: Cys, cystine; Trp, tryptophan; His, histidine; Met, methionine; Ara, arabinose; Rha, rhamnose; Xyl, xylose; Fuc, fucose; Lac, lactose; Str, streptomycin; S, sensitive; R, resistant; +, utilized; -, not utilized.

b The genetic locus controlling this character is metaA.

distinguishable from the strong, immediate positive reaction characteristic of strains possessing antigen 2. Nevertheless, single-factor 2 antigen was used in all instances to confirm the presence of somatic antigen 2.

RESULTS

Derivation of the S. paratyphi var. durazzo donor strain. The S. paratyphi A donor strain, WR4002, was obtained by mating WR7000 with the S. typhosa Hfr strain WR4000 and selecting for those S. paratyphi A hybrids which inherited the terminal lac+ marker of WR4000. WR4000, formerly designated TD-7 (5), possesses a chromosomally integrated lac+ marker and linked sex factor, F, of Escherichia coli origin; its derivation by terminal marker hybridization (3) has been described previously (5). Lac+ S. paratyphi A hybrids were obtained at a frequency of 10-7 per donor cell from the WR4000 × WR7000 cross, and most of these also carried the closely linked sex factor F. One such hybrid, an Hfr which transferred its chromosomal determinants in the same order as WR4000 (origin, proA, metaA, his, lac, F), was selected for further use; it was designated WR4002. As shown in Table 1, WR4002 acquired, in addition to lac and F, the fuc− marker of WR4000.

Transfer of somatic antigen 2 to S. typhimurium by conjugation. The S. paratyphi A Hfr strain WR4002 was mated with S. typhimurium LT2 recipient strain WR5000. Selection was made for WR5000 hybrids which inherited the his marker (transferred at a frequency of 6 × 10-2 per donor cell) of WR4002. Serological examination of 100 WR5000 his+ hybrids revealed that 70 of these agglutinated in single-factor 2 antisera and displayed no reaction when tested in group B O-antisera. The remaining 30 gave positive reactions in group B O-antisera and did not react with somatic 2 antisera (Table 2).

The 70% inheritance of the O-antisens 2 determinant with his parallels the previously observed 73 to 80% linkage of O-antisens 4 and 9 with his in reciprocal crosses between S. typhimurium and S. typhosa (6). These percentages are indicative of exceptionally close linkage of the genetic determinants involved. In interspecies Salmonella crosses, unlinked proximal markers are inherited with the selected marker at only 4 to 10% (6), and the present cross proved no exception. The unlinked metaA marker, which is transferred proximal to his by WR4002, was inherited by only 6 of the 100 WR5000 his+ hybrids (Table 2).

All of the 70 WR5000 his+ hybrids which inherited the somatic antigen 2 determinant concurrently lost somatic antigen 4, whereas none of those hybrids which retained antigen 4 expressed antigen 2. As in group D × group B crosses (6, 10), no intermediate types expressing both or neither of the antigens were detected. These findings are consistent with the view that the genetic determinants of O-antisens 2 and 4, as well as of 9, occupy the same chromosomal locus, in their respective Salmonella species, and are probably allelic.

Somatic antigen 5 was not detected in the 70 WR5000 his+ hybrids with somatic antigen 2. Some of these hybrids perhaps lost antigen 5 as a consequence of acquisition of a negative factor 5 allele from the S. paratyphi A donor. However, previous crosses between S. typhimurium donors and S. typhosa recipients have shown that the donor antigen 5 determinant, O-5, which is less closely linked to his than is the antigen 4 determinant (6, 7, 10), is inherited by only 20% of the his+ hybrids which receive the donor antigen 4 (7). Therefore, it appears that, in most of the...
### Table 2. Analysis of hybrids obtained from the cross between Salmonella paratyphi var. durazzo Hfr WR 4002 and S. typhimurium recipient WR 5000

<table>
<thead>
<tr>
<th>No. obtained</th>
<th>Characteristics examined</th>
<th>His</th>
<th>O-2</th>
<th>O-4</th>
<th>O-5</th>
<th>Met</th>
</tr>
</thead>
<tbody>
<tr>
<td>66</td>
<td></td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
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<td>25</td>
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<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* All hybrids were selected for acquisition of the donor his+ marker.

antigen 2-containing hybrids, the reason for the absence of antigen 5 is the same as that previously demonstrated in group D × group B crosses; i.e., antigen 5 appears only when antigen 4 is present also (6, 7, 10). This interpretation was confirmed subsequently by transduction experiments (see below). The 30 WR5000 his+ hybrids which retained antigen 4 were tested in factor 5 antisem, and only 3 of these were found to have lost antigen 5, in this instance presumably as the consequence of receipt of a negative (or missing) S. paratyphi A allele (Table 2).

Transfer of somatic antigen 2 to S. typhimurium and S. typhosa by transduction. Phage P22 was grown on a WR5000 his+ hybrid which had received the somatic antigen 2 determinant from WR4002. The resulting phage lysate, containing $5 \times 10^{14}$ plaque-forming units (PFU) per ml, was used to transduce the his+ marker to S. typhimurium WR5000 and to the S. typhosa strain WR4205. Transduction of his+ to WR5000 occurred at a frequency of $10^{-2}$ per PFU. Of 100 WR5000 his+ transductants tested, 14 had acquired somatic antigen 2 and concurrently lost O-antigen 4. Although the O-5 determinant is not cotransducible with his (6, 7), all of the 13 his+ transductants which received the antigen 2 determinant lost antigen 5, as well as antigen 4. It is inferred that the loss of antigen 5 is caused by the loss of antigen 4, and does not involve, in this instance, replacement of the S. typhimurium O-5 determinant. Possible reasons for the nonappearance of antigen 5 in strains lacking antigen 4 have been discussed by Mäkelä (10).

Transduction of his to S. typhosa WR4205 occurred at a frequency of $6 \times 10^{-3}$ per PFU, and 72 his+ transductants were examined. Eight of these had acquired O-antigen 2 and, as expected, failed to agglutinate in single-factor 9 antisem. The remainder agglutinated in factor 9 antisem and displayed no reaction in anti-2 serum. As with the WR5000 recombinants and transductants, none was detected which displayed both or neither of the antigens in question. The findings again indicate that the genetic determinants of somatic antigens 2, 4, and 9 are probably alleles of the same O locus in S. paratyphi A, S. typhimurium, and S. typhosa, respectively.

### Discussion

The O-antigenic specificity of Salmonella cell wall lipopolysaccharides is determined by repeating oligosaccharide units which form their O-specific side chains (14-16). Although the lipopolysaccharide “core” structure to which these O-specific side chains are attached is apparently the same in all members of this genus (8), there is diversity in the chemical constitution of the side chains themselves. It now has become apparent from several studies (1, 6, 10, 11, 17), including the present, that a chromosomal O locus, linked to his, contains the genetic determinants which are responsible to a large extent, for the chemical diversity of these side chains and, consequently, for the existence of the variety of Salmonella O serotypes.

The O-specific side chain of S. typhosa is composed of repeating units of D-galactose, D-mannose, and L-rhamnose, with the species-specific dideoxyhexose, tyvelose, linked to D-mannose, being responsible for factor 9 specificity (16). In the similar S. typhimurium O-specific side chain, abequose replaces tyvelose, and determines factor 4 specificity (16). S. paratyphi A is believed to contain a repeating unit similar to that of S. typhosa, except that paratose (the dideoxyhexose which determines factor 2 specificity) is present in the place of tyvelose (9). Since the chromosomal O locus must contain some of the genes determining the synthesis of the monosaccharides of these repeating units, as well as the transferases required to carry these sugars to the repeating units, its genetic structure is rather complex (13).

When the inheritance of somatic antigens at the O locus is examined, it appears that single-gene replacement is a rare occurrence. Most frequently, it seems that a complex of closely linked donor genes is transferred and integrated as a single unit. Mäkelä (10) pointed out, in the case of the mutual replacement of antigens 4 and 9, that, in all probability, more than one donor gene must be inherited to enable the recipient to synthesize the donor-specific dideoxyhexose and transfer it to the repeating unit. Similarly, in the case of replacement of antigen 4 by antigen 2, it would seem that more than one donor enzyme (and, therefore, more than one transferred gene) would be required to synthesize paratose from cytidine diphosphate (CDP)-4-keto-6-deoxyglucose (the common precursor of the group A, B, and d dideoxyhexoses) and to transfer it to the repeating unit.
With regard to the replacement of antigen 9 (tyvelose) by antigen 2 (paratose), it is known that CDP-paratose is the precursor of CDP-tyvelose, and that the interconversion of these dideoxyhexose nucleotides is catalyzed by a specific 2-epimerase (12). Thus, it is conceivable that the substitution of factor 9 by factor 2 might have been accomplished simply by the replacement of an epimerase allele. However, this notion would presume that S. typhosa already possessed a transference capable of transporting paratose to its repeating unit. Here again it is perhaps more reasonable to suspect that such a transference gene was inherited from the donor.

Multiple-gene substitution at the O locus has been shown to occur also in crosses between S. montevideo (group C, O antigens 6, 7) and S. typhimurium (11). Although the exact structure of the O-specific side chain of group C, Salmonella has not been determined, it contains, in contrast to that of S. typhimurium, only D-mannose as an O-specific sugar (9). Also, the demonstration of a difference in O locus-determined enzymes in S. montevideo and S. typhimurium has indicated that the genetic composition of their O loci differs (13). Nevertheless, in the crosses between S. montevideo donors and S. typhimurium recipients, the group C, determinants replaced their group B alleles in the same manner as do those of groups A and D. That is, no new combinations of group B and group C, antigens were observed. Rough hybrids, resulting from rare recombination (crossing over) within the O locus, were observed in less than 1% of the recombinants examined (11).

In the present study, the mutual replacement of antigens 2, 4, and 9, and the absence, in the hybrids, of any combination of these factors, indicated that their respective genetic determinants are probably allelic. Moreover, among 100 recombinants and 172 transductants examined, no rough hybrids were detected. In fact, the detection of rough hybrids has yet to be reported in genetic transfer experiments involving only the O-antigen determinants of groups A, B, and D (6, 7, 10). However, since the number of recipient genes which must be replaced by donor alleles in these crosses probably is greater than one, it seems reasonable to suspect that recombination within the O-antigen gene cluster might be detected if larger numbers of hybrids were examined.

LITERATURE CITED