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Relationship Between Habs Serotypes and 2-Amino Sugar Composition of *Pseudomonas aeruginosa*

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Thirteen Habs-serotype strains could be classified into 11 groups depending on the characteristic patterns of 2-amino sugar composition; strains of serotypes 2, 5, and 7 had the same pattern, and each other serotype strain had its own distinctive pattern. In our classification, fucosamine and quinovosamine were of importance. We found that fucosamine from strain P14 (Habs serotype 1) was of the D configuration only, unlike DL-fucosamine from strain N10 (Habs serotype 11).

We first succeeded in isolating DL-fucosamine (2-amino-2,6-dideoxy-DL-galactose) from cells of *Pseudomonas aeruginosa* N10 and its lipopolysaccharide in 1969 (14, 15). Subsequently, we isolated D-quinovosamine (2 amino-2,6-dideoxy-D-glucose) from *P. aeruginosa* P14 and found fucosamine and quinovosamine in many *P. aeruginosa* strains, in addition to the principal components of glucosamine, galactosamine, muramic acid, and two unidentified compounds (U-1 and U-2) detected in all *P. aeruginosa* strains so far examined. All of these 2-amino sugars, except muramic acid, were also found in the endotoxic lipopolysaccharides (17). Recently, we reported that 22 *P. aeruginosa* strains could be classified into six groups, depending on the characteristic patterns of 2-amino sugar composition (16).

Chester et al. (3) classified the lipopolysaccharides of Habs-serotype strains into nine chemotypes, based on the presence of mannose, xylose, and eight unidentified amino compounds. Three of these amino compounds were later identified as quinovosamine, fucosamine, and 2-amino-2-deoxy galacturonic acid (8). Therefore, it seemed of interest to see if our 2-amino sugar pattern scheme was applicable to Habs-serotype (5) strains. We also studied fucosamine configuration.

MATERIALS AND METHODS

Organisms. *P. aeruginosa* strains P1 to P20 (except P18), N10, P14 PyR^r-2, P14M, 5936, A443, U3433, and T6370 were described previously (11, 16). Strains 4821, 13264, A486, 121, 5933, 5939, 5940, and 5945 were obtained through the collection of the Institute of Medical Science. Strain 6092, Veron's O-13, was kindly provided by P. V. Liu (Department of Microbiology and Immunology, University of Louisville, Louisville, Ky.). The O antigen of this strain corresponds to Sandvik's type II (13) and is closely related to type O-5 of Verder and Evans.

Serological methods. Serological methods were practically the same as those described previously (12). Cross-absorption tests were performed when an antigen was agglutinated significantly by two or more antisera against different serotypes.

Analyses of 2-amino sugar composition. Each strain was grown on two slants containing 50 ml of nutrient agar medium for 18 h at 37°C. Cells harvested were hydrolyzed with 4 M HCl for 3 h at 100°C. The hydrolysate was then filtered through a sintered-glass filter (G2) and dried in vacuo at 55°C to remove HCl. The residue was dissolved in 0.3 ml of water and placed on a column (1.3 by 50 cm) of Dowex 50-HW-X8 (200 to 400 mesh). Elution was carried out with 0.33 M HCl. Fifty fractions (10 ml) were collected at a flow rate of 10 ml/h. A part (3 ml) of each fraction was analyzed for 2-amino sugar content with the Boas modification (2) of the Elson-Morgan reaction, using galactosamine as a standard. Fractions from a single peak of 2-amino sugar were pooled and dried in vacuo at 55°C. 2-Amino sugar in the residue was separated from other ninhydrin-positive substances by preparative thin-layer chromatography on Silica Gel G (Merck) with *n*-butanol-pyridine-water-acetic acid (60:40:30:3, by volume) and then identified in comparison with authentic samples by thin-layer or paper chromatography as described previously (14, 15, 17).

Other analytical procedures. Infrared spectra were taken in potassium bromide pellets with a Hitachi infrared recording spectrophotometer. Optical rotatory dispersion measurements were made with a JASCO automatic recording spectropolarimeter, model J-20. Nuclear magnetic resonance spectra were recorded at 100 MHz on a Varian HA-100 spectrometer, at room temperature, in D_2O, with tetramethylsilane as an external standard. Chemical shift (δ) was expressed in parts per million relative to tetramethylsilane (0 ppm). Spectra for analysis were recorded at sweep widths of 250 and

500 Hz; however, the sweep offset was calibrated from spectra measured at the 1,000-Hz sweep width.

RESULTS AND DISCUSSION

Table 1 gives the 2-amino sugar patterns and serotypes for the 22 *P. aeruginosa* strains mentioned above. Strains that had the same pattern of 2-amino sugar composition belonged to the same serotype, except pattern III strains, which were of two serotypes, 8 and 11. Strain P14 PyR^r-2 was an autoagglutinable rough mutant, as suggested previously (16). Strain P14M (pattern VI), which could be a semirough mutant (16), belonged to the same serotype as its parent strain, P14.

The results of 2-amino sugar analyses for Habs-serotype strains are given in Fig. 1 and Table 2. Habs-serotype strains of the same serotypes as the strains in Table 1 (i.e., 1, 5, 6, 8, and 11) were found to fall into the same 2-amino sugar composition patterns (II, IV, I, III, and III, respectively). Each other Habs-serotype strain had its own distinctively different 2-amino sugar composition pattern, except that serotypes 2 and 7 were the same as serotype 5. Each strain had a similar quantity of glucosamine and galactosamine (plus muramic acid) in comparison to U-2 (i.e., unknown no. 2) amount. The molar ratios of glucosamine to U-2 were in the 3 to 5 range, except for serotypes 3 (9.1), 10 (7.0), and 13 (7.9). The molar ratios of galactosamine (plus muramic acid) to U-2 were in the 2 to 4 range, except for serotype 1 (8.8). In contrast, fucosamine and quinovosamine contents varied greatly for each serotype strain. In fact, their quantity was the factor determining each serotype, along with an abundance of glucosamine in serotypes 3, 10, and 13 and of galactosamine in serotype 1. Serotype 8 could be distinguished from serotype 11 by its lower molar ratio of fucosamine to U-2. Seven new patterns were designated no. VII to XIII. Strains P7 and P13 could be rearranged into pattern VIII. Thus, we could conclude that all serotypes, except 2, 5, and 7, in the Habs classification correspond to patterns of 2-amino sugar composition of *P. aeruginosa*. This correlation of serotypes and 2-amino sugar patterns could mean a double check in identification of *P. aeruginosa* strains.

TABLE 1. *Habs serotypes and patterns of 2-amino sugar composition of P. aeruginosa*

Strain	Pattern[a]	Serotype
P4	I	6
P9	I	6
P15	I	6
P20	I	6
P2	II	1
P5	II	1
P6	II	1
P10	II	1
P11	II	1
P14	II	1
P7	III	8
P13	III	8
P16	III	11
P19	III	11
N10	III	11
P1-III	IV	5
P3	IV	5
P8	IV	5
P12	IV	5
P17	IV	5
P14 PyR^r-2	V	Rough
P14 M	VI	1

[a] See reference 16.

Clearly, fucosamine and quinovosamine were of primary importance in our classification. However, their configuration has not yet been clarified, with the exception of our early work on DL-fucosamine (14, 15) and D-quinovosamine (17). Recently, we obtained a high-molecular-weight polysaccharide fraction (F-A) consisting of galactosamine, quinovosamine, fucosamine, and an unidentified compound (U-7), possibly the O side chain from the lipopolysaccharide of strain P14 (16). The approximate molar ratio of 2:2:1:1 suggested the possibility of repeating units. (The unidentified compound, U-7, may be the same compound as one of the unidentified reducing compounds of the lipopolysaccharide of strain ATCC 7700 [1]. The O antigen of this strain was identified as Habs serotype 1 by Matsumoto [unpublished data].) If the fucosamine of F-A were of the DL configuration, similar to that isolated from strain N10, the repeating unit of F-A would contain 12 saccharide residues or more. Such a large number of saccharides would not correspond to the number determined for the repeating units of *Enterobacteriaceae* (for a review, see 10). Also, we could not overlook this question of configuration because of the possibility of distinguishing serotypes 2, 5, and 7 by differences in their fucosamine configuration. As a preliminary step, we tested for fucosamine of the D or L configuration only, using strain P14.

Fucosamine was isolated and crystallized by the same method as that used for DL-fucosamine from strain N10 (14, 15). Cells harvested from a 7.2-liter culture of strain P14 were used. A total of 44 mg of crystalline material was obtained. The melting point was 173°C (decomposition). (Analysis – calculated for $C_6H_{14}NO_4Cl$: C, 36.10; H, 7.06; N, 7.01; Cl, 17.76. Found: C, 36.32; H, 6.99; N, 7.04; Cl, 17.61.) The values of elementary analyses cor-

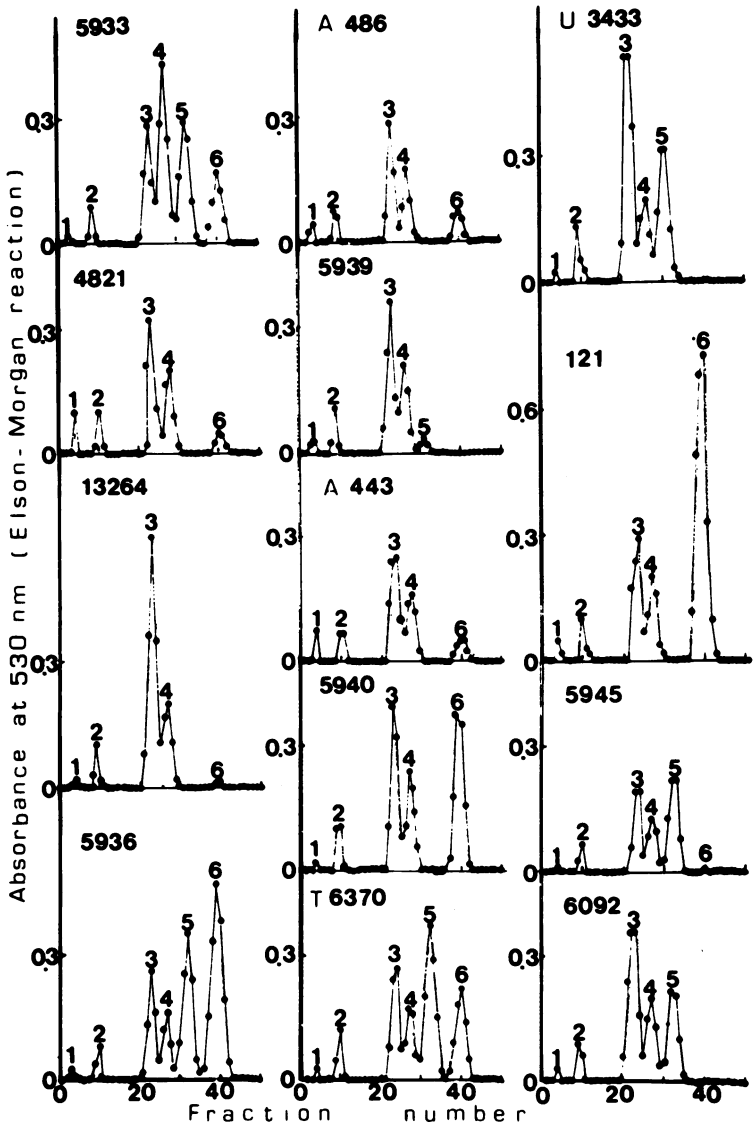

FIG. 1. *Chromatographic patterns of the hydrolysates of P. aeruginosa strains of the Habs-serotype classification. Experimental conditions were as described in Materials and Methods. Peaks are numbered as described previously (17): peak 1, unidentified compound U-1; peak 2, unidentified compound U-2; peak 3, glucosamine; peak 4, galactosamine (plus muramic acid); peak 5, quinovosamine; peak 6, fucosamine.*

responded to the formula for fucosamine HCl. The infrared spectrum (Fig. 2) was identical to that previously determined for D-fucosamine HCl (18) but was different from the spectrum of DL-fucosamine HCl (15). The optical rotation was determined as $[\alpha]_D^{20}$ = +116° (6 min) → +93 ± 2° (final, C = 0.2, H_2O). D-Fucosamine HCl from *Chromobacterium violaceum* had $[\alpha]_D^{20}$ = +91 ± 2° (C = 1.0, H_2O) (4), and synthetic D-fucosamine HCl had $[\alpha]_D^{23}$ = +117° (3 min) → +92 ± 1.2° (final, C = 0.2, H_2O) (19). The optical rotatory dispersion curve of the material indicated the D configuration, unlike DL-fucosamine HCl from strain N10, which had no optical activity from 200 to 700 nm (14, 15). Consequently, the material isolated from strain P14 (Habs serotype 1) can be characterized as 2-amino-2,6-dideoxy D-galactose (D-fucosamine) HCl. It seems likely that the repeating unit of F-A has a similar number of saccharides to that

TABLE 2. *2-Amino sugar composition of strains of the Habs-serotype classification*

Strain	Serotype	2-Amino sugar composition (%)[a]						Pattern
		U-1	U-2	Glucosamine	Galactosamine[b]	Quinovosamine	Fucosamine	
5933	1	1.2	3.9	18.6	34.5	26.7	15.1	II
4821	2	6.9	9.5	41.7	33.1	0	8.8	IV
13264	3	1.3	7.4	67.5	22.4	0	1.4	XI
5936	4	0.8	3.4	16.4	10.6	26.6	42.2	VII
A486	5	4.4	10.7	40.2	28.8	0	15.9	IV
5939	6	3.1	9.6	49.1	32.3	5.9	0	I
A443	7	2.4	8.6	44.5	32.7	0	11.8	IV
5940	8	0.7	7.8	31.5	21.3	0	38.7	VIII[c]
T6370[d]	9	1.0	5.8	21.7	15.5	33.3	22.7	IX
U3433	10	0.6	6.6	45.9	16.3	30.6	0	X
121	11	1.8	3.5	14.3	9.9	0	70.5	III
5945	12	0.6	5.8	29.8	20.9	42.3	0.6	XII
6092	13	1.1	5.7	45.2	20.5	27.5	0	XIII

[a] 2-Amino sugar content detected in the frist 50 fractions of chromatography regarded as 100%.
[b] Contained muramic acid.
[c] Distinguished from pattern III and redesignated.
[d] Verder and Evans strain.

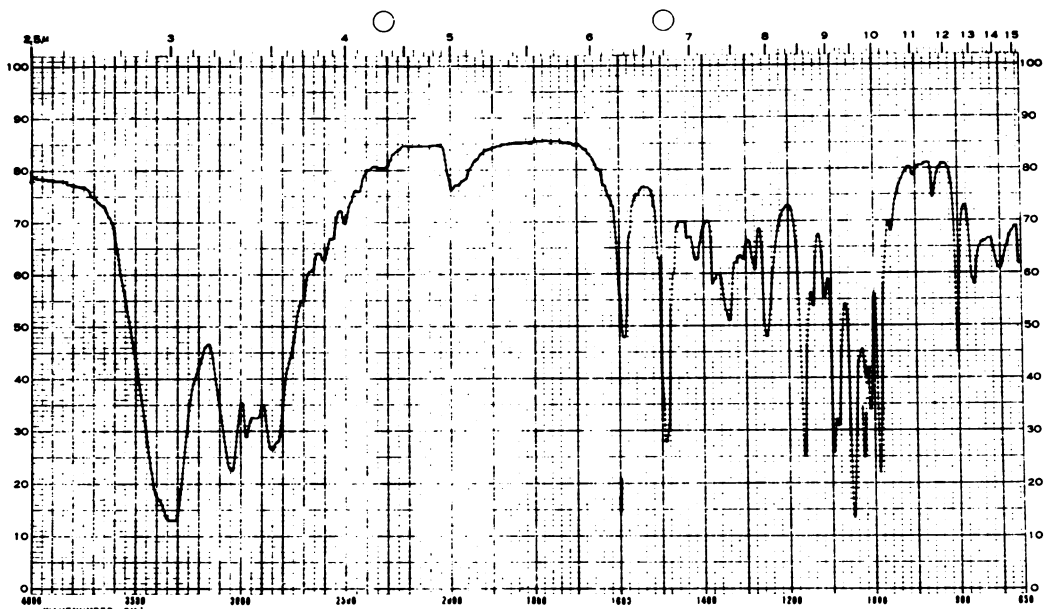

FIG. 2. *Infrared spectrum of fucosamine isolated from P. aeruginosa P14.*

of *Enterobacteriaceae*. There seems to be ample reason to conduct further studies on the configuration of fucosamine in serotypes 2, 5, and 7.

Figure 3A shows the nuclear magnetic resonance spectrum of the material from strain P14, and Fig. 3B shows that of DL-fucosamine HCl from strain N10. The two were superimposable. The chemical shifts of the C-1 proton were 5.87 ppm (doublet, J = 3.4 Hz) and 5.31 ppm (doublet, J = 8.6 Hz), similar to data of other researchers (6, 9). The signals of the methyl proton appeared at 1.76 ppm (doublet, J = 6.1 Hz) and 1.72 ppm (doublet, J = 6.8 Hz). This fact provides additional evidence that fucosamine from strain N10 (Habs serotype 11) was of the DL configuration (14, 15). Recently, Horton and Liav reported that an amino sugar obtained from antigenic lipopolysaccharide of *P. aeruginosa* strain 2 (classification of Fisher et al.) was a mixture of D- and L-fucosamine (7).

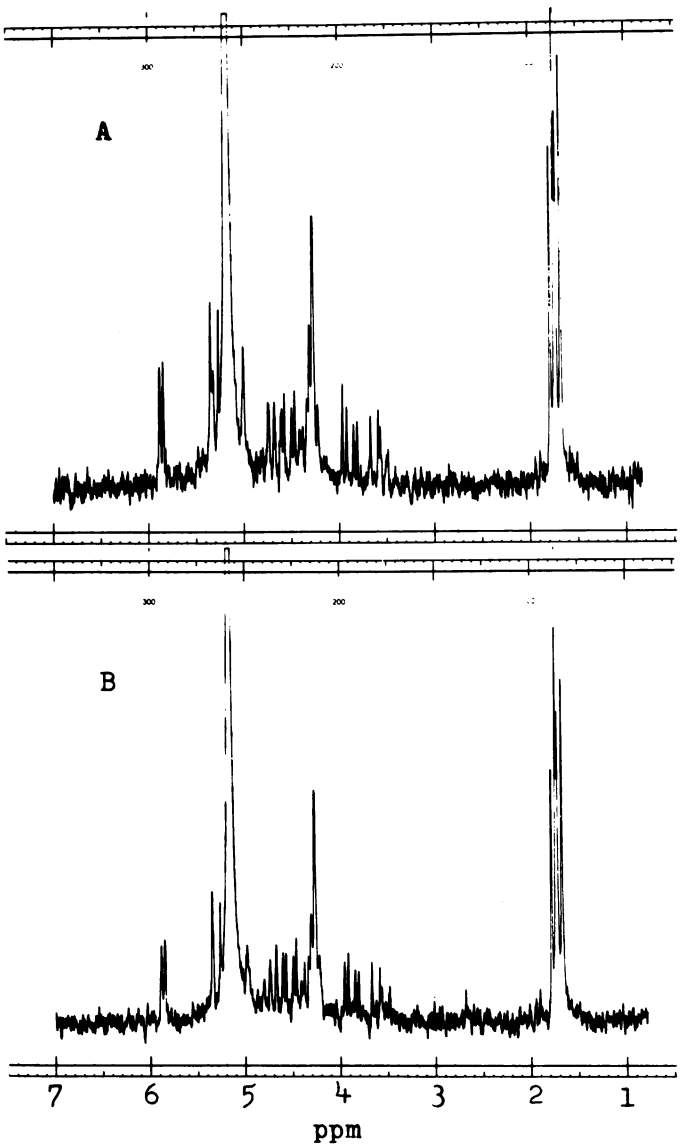

FIG. 3. *Nuclear magnetic resonance (100 MHz) spectra of (A) fucosamine from P. aeruginosa P14 (3.3% in D_2O) and (B) DL-fucosamine HCl from P. aeruginosa N10 (3.2% in D_2O).*

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