Vol. 97, No. 2, 1980
November 28, 1980

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
Pages 437-442

LIPOPOLYSACCHARIDES OF GROUP F, A NEW GROUP OF VIBRIOS

Seiichi Kondo, Takehiro Iguchi and Kazuhito Hisatsune

Department of Microbiology, School of Pharmaceutical Sciences
Josai University, Sakado, Saitama 350-02, Japan

Received October 3,1980

Summary The sugar composition of the O-antigenic lipopolysaccharides isolated from Group F vibrios was analysed. 2-Keto-3-deoxy-octonate was totally absent from the lipopolysaccharides. As common component sugars, glucose, galactose, L-glycero-D-mannoheptose, and glucosamine were present. The Group F vibrios examined were found to be divided into two groups, designated tentatively as groups I and II, on the basis of the pattern of the sugar composition of their lipopolysaccharides. As additional sugar components, mannosamine, quinovosamine and two unidentified amino sugars, F1 and F2, were present in group I, while rhamnose, galactosamine, an unidentified amino sugar, F3, and a relatively high content of D-glycero-D-mannoheptose were found in group II.

Introduction Group F, a new group of enteropathogenic vibrios, was named by Furniss et al. in their taxonomic study of Vibrio metschnikovii and related vibrios (1, 2). These vibrios, once classified as Aeromonas, have been isolated not only from patients with diarrhea, many in Bangladesh (1,3), but also from estuarine water and shellfish in large numbers, in Britain and elsewhere (1, 2).

The characterization of the somatic antigen, i.e., the O-antigenic lipopolysaccharides isolated from Group F vibrios has not hitherto been reported. The present communication describes the sugar composition and some serological properties of O-antigenic LPS[1] from this group of vibrios isolated from patients with diarrhea in Bangladesh. For comparison, the sugar composition of LPS isolated from Aeromonas was also analyzed.

Materials and Methods Group F vibrio 305-77, 306-77, 308-77 and 210-73, Aeromonas punctata ATCC 15468 and A. hydrophila ATCC 7966 were provided by Dr. T. Shimada and Dr. R. Sakazaki, the First Department of Bacteriology, the National Institute of Health, Tokyo, Japan. The four strains of the Group F vibrios were originally isolated in Bangladesh by Dr. W. Moseley, Cholera Research Laboratory, Mohakali, Dacca 2, Bangladesh. Vibrio cholerae 569B (Inaba) (4) was used as a source of quinovosamine.

[1] Abbreviations: LPS, lipopolysaccharide; KDO, 2-keto-3-deoxy-octonate; ID_{50}, concentration producing 50% passive hemolysis inhibition.

0006-291X/80/220437-06$01.00/0

The bacteria were grown in broth medium (pH 7.4) containing 2% NaCl with vigorous shaking at 37°C for 16 hr, and killed by exposure to a final concentration of 0.5% phenol at room temperature for 2 hr.

LPS were isolated from the acetone-dried cells by the phenol-water technique of Westphal et al. (5), and highly purified by repeated ultracentrifugation and treatment with ribonuclease (6).

Neutral sugars except fructose were determined after hydrolysis in 2 N trifluoroacetic acid at 120°C for 1 hr by gas-liquid chromatography as alditol acetates according to the method of Laine et al.(7) (column: 3% ECNSS-M coated on Chromosorb W, 3 mm x 2 m) using xylose as an internal standard. Fructose was determined as described by Jann et al. (4).

For amino sugar determination, LPS were hydrolysed in 4 N HCl at 100°C for 8 hr, and the hydrolysates were fractionated on a Dowex 50 (x8) column according to the method of Wheat (8). Amino sugars were then determined as N-acetyl-alditol acetates (7) by gas-liquid chromatography (column: TABSORB [Regis Chemical Co., Morton Grove, Ill.], 3 mm x 2 m) using, as an internal standard, 3-amino-3-deoxy-D-ribose prepared by hydrolysis of puromycin aminonucleoside (Sigma Chemical Co., St. Louis, Mo.) (9). The identities of the sugars were based on retention time values and mass spectra compared with those of the derivatives of authentic standards.

Reducing sugar activity, amino sugar and total phosphorus were determined as described by Hisatsune et al. (10). Total carbohydrate, protein and total lipid were measured as described in our previous report (11). Heptose was determined either by the method of Osborn (12) and gas-liquid chromatography using standard L-glycero-D-manno- and D-glycero-D-mannoheptose, both being prepared as described by Bagdian et al. (13). 2-Keto-3-deoxy-octonate (3-deoxy-D-mannooctulosonic acid was determined by the method of Weissbach (14). KDO purchased from Sigma Chemical Co. (St. Louis, Mo.) was used as a standard.

Antisera against the Group F vibrios were obtained from rabbits essentially according to the method of Shimada and Sakazaki (15). The passive hemolysis-inhibition test was performed according to the method described in our previous report (16).

Results and Discussion The yields of LPS extracted from acetone-dried cells were found to range from 0.8% to 2.3%. There were marked differences in both overall chemical composition and sugar composition between LPS derived from two strains (308-77 and 210-73, tentatively designated as group I) and from the other two strains (305-77 and 306-77, tentatively designated as group II).

The overall chemical composition of LPS from strain 210-73, a member of group I, and of strain 306-77, a member of group II, was respectively as follows (% of total dry weight): total carbohydrate, 52.0 and 15.5%; reducing sugar (including amino sugar), 45.0 and 15.0%; amino sugar, 19.1 and 15.5%; protein, 5.5 and 12.1%; total phosphorous, 2.9 and 6.0%; total lipid, 12.0 and 27.0%. Comparable results were obtained for LPS from strains 308-77 and 305-77. LPS from the members of group I contained markedly higher amounts of

Table 1 Sugar Composition of LPS from Group F Vibrios and Aeromonas (%, w/w)

Organism	Glc	Gal	Rha	Comp. A	Hep L-D	Hep D-D	KDO	GlcN	GalN	ManN	QuiN
Group F vibrio											
308-77	33.8	0.7	-[a]	-	0.9	2.3	-	5.9	-	0.7	+
210-73	47.8	tr[b]	-	-	0.8	1.1	-	3.3	-	0.3	+
305-77	2.6	1.8	0.9	-	1.4	6.7	-	6.6	1.7	-	-
306-77	2.7	1.9	1.1	-	1.0	6.1	-	4.6	1.4	-	-
A. punctata	4.1	-	1.8	1.4[c]	15.2	-	-	9.3	-	-	-
A. hydrophila	7.7	-	-	1.8[c]	20.3	-	-	8.2	-	-	-

a) not detected, b) trace, c) estimated as glucose
Abbreviations: Glc, glucose; Gal, galactose; Rha, rhamnose; Comp.A,"Compound A" an unidentified neutral sugar; Hep L-D, L-glycero-D-mannoheptose; Hep D-D, D-glycero-D-mannoheptose; KDO, 2-keto-3-deoxy-octonate; GlcN, glucosamine; GalN, galactosamine; ManN, mannosamine; QuiN, quinovosamine.

total carbohydrate (52.0 - 72.0%), than were found in LPS from those of group II (15.0 - 16.0%). In contrast, however, twice as much lipid (21.0 - 27.0%) was found in group II LPS than in LPS of the members of group I (11.0 - 12.0%).

The sugar composition of the LPS is presented in Table 1. It is seen that KDO, a regular sugar constituent in the core region of the usual gram-negative bacterial LPS, is totally absent from LPS of the Group F vibrios as well as from the Aeromonas species. As common sugar constituents of the Group F LPS, glucose, galactose, L-glycero- and D-glycero-D-mannoheptose and glucosamine were present. No fructose was detected. Group F vibrios are again divided into groups I and II on the basis of the pattern of their sugar composition, in other words, chemotypes of their LPS. Group I is distinguished from group II by the presence of an unusually high content of glucose (from 34 to 48%) and also by the presence of the amino sugars, mannosamine and quinovosamine. Group II, on the other hand, is characterized by the presence of rhamnose , galactosamine and a relatively high content of D-glycero-D-

mannoheptose. Furthermore, three unidentified amino sugars, designated here as F1, F2 and F3, were found in LPS: F1 and F2 in group I and F3 in group II. Their retention time values relative to that of glucosamine in gas-liquid chromatography were 0.504, 0.624 and 0.597, respectively.

The Aeromonas species were found to be distinct from the Group F vibrios; Aeromonas LPS contained unusually large amounts of L-glycero-D-mannoheptose (15.2 to 20.3%), and also contained an unidentified neutral sugar, designated here as "Compound A." Furthermore, D-glycero-D-mannoheptose, galactosamine, mannosamine, quinovosamine and the three unidentified amino sugars were not found in Aeromonas LPS.

In the passive hemolysis-inhibition test, LPS of both members of group I (strain 308-77 and 210-73) exerted strong and almost equivalent inhibition in the [308-77 LPS-sensitized SRBC/anti 308-77 serum] passive hemolysis system; the concentration producing 50% inhibition was 0.06 and 0.17 μg/ml, respectively. LPS of both members of group II (strains 305-77 and 306-77) also exerted a strong and equivalent inhibition (ID_{50} 0.15 μg/ml) in the [305-77 LPS-sensitized SRBC/anti 305-77 serum] system. However, no significant inhibition ($ID_{50} > 1{,}000$ μg/ml) was observed in either the first hemolytic system with LPS of group II or the second hemolytic system with LPS of group I. These results indicate that the Group F vibrios studied are serologically divided also into the same two groups. Our results are consistent with the unpublished data of Sakazaki and Shimada, in which these four vibrios were slso serologically divided into the same two groups by agglutination and agglutinin absorption tests.

The present study demonstrates the absence of KDO in LPS of Group F vibrios and Aeromonas species. The absence of KDO in LPS of the genus Vibrio was first reported by Jackson and Redmond for V. cholerae 569B (Inaba) (17) and it was also demonstrated in our previous studies of V. cholerae (both OI and non-OI groups) (11, 18), V. parahaemolyticus (19) and V. alginolyticus (20). These results can probably be interpreted as support for the contention

that the absence of KDO in LPS is one of the characteristic features generally possessed by the genus Vibrio as well as related bacteria including Aeromonas, and it is thus of taxonomical significance.

The unusually high content of glucose was found in LPS from the members of group I. The characterization of the glucose residue as well as their O-specific polysaccharide region is now under investigation in our laboratory.

The Group F vibrios were once classified as Aeromonas (2). Furniss's group distinguished them taxonomically from Aeromonas and other related vibrios on the basis of their biological and biochemical properties including susceptibility to the vibriostatic agent O/129 (2,4-diamino-6,7-diisopropyl-pteridine phosphate), growth in the absence of sodium chloride, and DNA base composition (1, 2). We have shown that the Group F vibrios are also clearly distinct from Aeromonas on the basis of the sugar composition of their LPS.

This is believed to be the first report of the sugar composition of O-antigenic LPS isolated from the Group F vibrios.

Acknowledgment We thank Dr. T. Shimada and Dr. R. Sakazaki, the National Institute of Health, Tokyo, Japan, for their courtesy in providing the Group F vibrios and Aeromonas strains used in this study, and Professor K. Takeya, Department of Bacteriology, School of Medicine, Kyushu University, Fukuoka, Japan, for his support and encouragement.

References

1. Furniss, A. L., Lee, J. V., and Donovan, T. J. (1977) Lancet 2, 565-566.
2. Lee, J. V., Donovan, T. J., and Furniss, A. L. (1978) Int. J. Syst. Bacteriol. 28, 99-111.
3. Spira, W. M., Daniel, R. R., Ahmed, Q. S., Huq, A., Yusuf, A., and Sack, A. A. (1979) Proceedings of the 14th Joint Cholera Research Conference, U.S.-Japan Cooperative Medical Science Program, Karatsu, 1978. pp.137-153, Japanease Cholera Panel, U.S.-Japan Cooperative Medical Science Program.
4. Jann, B., Jann, K., and Beyaert, G. O. (1973) Eur. J. Biochem. 37, 531-534.
5. Westphal, O., Lüderitz, O., and Bister, R. (1953) Z. Naturforsch. 7b, 148-155.
6. Fensom, A. H., and Gray, G. W. (1969) Biochem. J. 114, 185-196.
7. Laine, R. A., Stelner, K., and Hakomori, S. (1974) Methods in Membrane Biology, Vol. 2, pp.228-244, Plenum Press, New York.
8. Wheat, R. W. (1969) Methods in Enzymology 8, pp.60-75, Academic Press, New York.
9. Reinhart, K. L. Jr., Woo, P. W. K., and Argoudelis, A. D. (1958) J. Am. Chem. Soc. 80, 6461-6462.

10. Hisatsune, K., DeCourcy, S. J. Jr., and Mudd, S. (1967) Biochemistry 6, 586-594.
11. Hisatsune, K., and Kondo, S. (1980) Biochem. J. 185, 77-81.
12. Osborn, M. J. (1963) Proc. Natl. Acad. Sci. 50, 499-506.
13. Bagdian, G., Droge, W., Kotelko, K., Lüderitz, O., and Westphal, O. (1966) Biochem. Z. 344, 197-211.
14. Weissbach, A., and Hurwitz, J. (1959) J. Biol. Chem. 234, 705-709.
15. Shimada, T., and Sakazaki, R. (1973) Japn. J. Med. Sci. Biol. 26, 155-160.
16. Hisatsune, K., Kondo, S., and Kobayashi, K. (1978) Japn. J. Med. Sci. Biol. 31, 181-184.
17. Jackson, G. D. F., and Redmond, J. W. (1971) FEBS Lett. 13, 117-120.
18. Hisatsune, K., Kondo, S., Nozaki, S., and Kawata, T. (1978) Chem. Pharm. Bull. 26, 3597-3599.
19. Hisatsune, K., Kiuye, A., and Kondo, S. (1980) Microbiol. Immunol. 24, 691-701.
20. Hisatsune, K., Kiuye, A., and Kondo, S. (1980) Microbiol. Immunol. in press.