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A NEUTRAL SUGAR IS RESPONSIBLE FOR SEROVAR SPECIFICITY OF THE ANTIGENIC DETERMINANT OF *LEPTOSPIRA INTERROGANS* SEROVAR *CANICOLA*

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ABSTRACT

To provide information on the chemical structures of antigenic determinants of leptospira, glycolipids of *Leptospira interrogans* serovar *canicola* strain Hond Utrecht IV (Ut-IV) and its antigenic variant selected in the presence of a serovar-specific monoclonal antibody were compared physicochemically. Gas-liquid chromatography-mass spectrometry analysis revealed that the glycolipid of Ut-IV contained 6 neutral sugar species; rhamnose, mannose, galactose, glucose, and unknown sugars III and IV, in addition to unknown sugars I and II that had been previously reported. On the other hand, the glycolipid of the variant lacked unknown sugar III, suggesting that this sugar is responsible for the serovar-specific antigenic determinant.

Key words: *Leptospira*, glycolipid, antigen, determinant, sugar

INTRODUCTION

The serovar-specific antigenic determinants of *Leptospira interrogans* have not been defined, despite numerous studies^{1,2,5,6,9,12,13}. Glycolipid antigen was purified from *Leptospira interrogans* serovar *canicola* strain Hond Utrecht IV (Ut-IV) and shown to be suitable for analysing the structure of the serovar-specific antigenic determinants¹⁰.

Several monoclonal antibodies against the TM antigen of *Leptospira interrogans* serovar *canicola* were produced⁸. Of those, serovar-specific antibody CT3 recognizing the sugar moiety, has been used for analysis of the structure of the antigenic determinant^{9,10}. An antigenic variant, CV (CT3)-1 that did not react with the CT3 antibody, was selected from Ut-IV by cultivation in the presence of antibody CT3. This was not identical to any leptospire of the known serovars of serogroup

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Canicola¹¹).

In the present study, the neutral sugar compositions of the glycolipids of Ut-IV and CV (CT3)-1 were compared to clarify the structures of the serovar *canicola*-specific antigenic determinants.

MATERIALS AND METHODS

Strains and medium: *Leptospira interrogans* serovar *canicola* strain Ut-IV was provided by the Leptospirosis Reference Laboratory, National Institute of Health, Tokyo, Japan. The antigenic variant, CV (CT3)-1, selected from Ut-IV in the presence of anti-*canicola* monoclonal antibody CT3¹¹ was used. These strains were grown in protein-free medium³ at 30°C for one week and harvested by continuous-flow centrifugation at 12,000 × g.

Purification of glycolipids: Glycolipids of leptospire were purified by the method of Ono *et al.*¹⁰.

Monoclonal antibody: Monoclonal antibody CT3 against serovar *canicola* TM antigen⁸ was used. The agglutination titre of CT3, in the form of mouse ascitic fluid, with Ut-IV was 1: 5, 120, and negative (<1: 40) with CV (CT3)-1.

Oligosaccharide fraction CN-1: Oligosaccharide fraction CN-1 (the CN-1 fraction), prepared from serovar *canicola* TM antigen by formic acid and successive sulphuric acid hydrolysis⁹ was used.

Thin-layer chromatography (TLC)-enzyme immunostaining: One hundred nanograms of purified glycolipid was applied to each lane of a Polygram Sil G TLC plate (Macherey-Nagel, West Germany) and chromatographed as shown in Figure 1. After that, enzyme immunostaining of glycolipids separated on the TLC plate was carried out according to the method of Higashi *et al.*⁴.

Determination of the neutral sugar compositions of glycolipids and the CN-1 fraction: Neutral sugar compositions of glycolipids and the CN-1 fraction were determined by gas-liquid chromatography-mass spectrometry (GLC-MS) as described previously⁷. Samples (200 μg) were hydrolyzed with 5% (w/v) HCl/methanol at 80°C for 24 hr. After the fatty acid methyl esters were extracted with hexane, the methylglycosides obtained from the methanol layer were trimethylsilylated. Per-trimethylsilylated glycosides were chromatographed on a 2.6 × 1,000mm glass column of 2% OV-1 on 60-80 mesh Chromosorb W by increasing the temperature from 125°C to 180°C at a rate of 2°C/min, with 0.6kg/cm² He gas pressure, and analysed in a JMS-HX110 GLC-MS apparatus (Nihondenshi, Tokyo, Japan). Ion energy was 23 eV.

RESULTS

Reactivity of the purified glycolipids with antibody CT3: The purified glycolipids of Ut-IV and CV (CT3)-1 were chromatographed on a TLC plate and immunostained with serovar-specific monoclonal antibody CT3. On the TLC plate, the glycolipids migrated

as a single spot that was visualized by anthrone/H₂SO₄ reagent (Fig. 1). Monoclonal antibody CT3, which reacted with the glycolipid of Ut-IV, did not react with the glycolipid of CV (CT3)-1 (Fig. 1), indicating that the glycolipid of CV (CT3)-1 lacked the serovar-specific antigenic determinant recognized by the CT3 antibody.

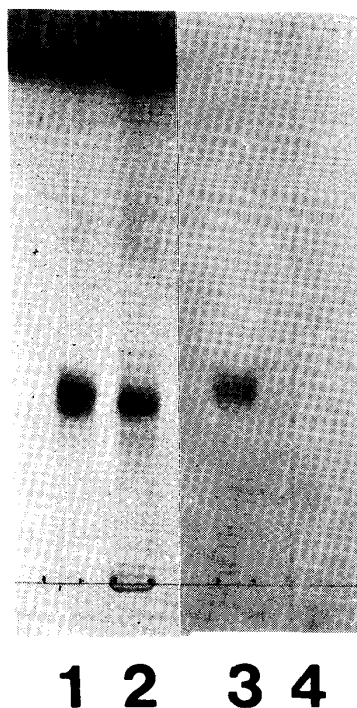


Fig. 1. Thin-layer chromatograms of the glycolipids of Ut-IV and CV (CT3)-1. Lanes 1 and 3, the glycolipid of Ut-IV; lanes 2 and 4, the glycolipid of CV (CT3)-1. The glycolipids in lanes 1 and 2 were visualized with anthrone/H₂SO₄ reagent and lanes 3 and 4 were immunostained with monoclonal antibody CT3. The chromatograms were developed with chloroform/methanol/water (50 : 40 : 10, by vol.).

Neutral sugar composition of the glycolipids and the CN-1 fraction: The neutral sugar compositions of the glycolipids of Ut-IV and CV (CT3)-1 were analysed by GLC-MS. The glycolipid of Ut-IV contained 4 authentic sugars (rhamnose, mannose, galactose, and glucose) and 4 unknown sugars (I, II, III, and IV) (Table 1). The glycolipid of CV (CT3)-1 lacked unknown sugar III, and the molar ratio of glucose was much higher than that of Ut-IV (Table 1).

The oligosaccharide fraction CN-1 obtained from Ut-IV, which reacted with antibody CT3, also contained unknown sugars II, III, and IV, but not rhamnose,

Table 1. Neutral sugar compositions of the glycolipids of Ut-IV and CV (CT3)-1, and the CN-1 fraction

Sugars	Relative retention time ^{a)}			Glycolipid of		CN-1 fraction
	Ut-IV	CV (CT3)-1	CN-1 fraction			
Unknown sugar III	0.35, 0.38	23 ^{b)}	— ^{c)}	18		
Rhamnose	0.44	8	9	—		
Mannose	0.82, 0.87	8	10	—		
Unknown sugar I	0.88	35	22	—		
Galactose	0.86, 0.92, 0.96	9	14	—		
Glucose	1.00, 1.04	9	26	—		
Unknown sugar IV	1.04	17	15	13		
Unknown sugar II	1.53, 1.58, 1.62	100	100	100		

a) Relative retention time is based on the retention time of the first peak of glucose as unity.

b) Relative molar ratios are based on unknown sugar II as unity.

c) Not detected.

mannose, galactose, glucose, or unknown sugar I (Table 1), suggesting that unknown sugars II, III, and IV were associated with the serovar *canicola*-specific antigenic determinant.

The glycolipid antigen of CV (CT3)-1 lacked unknown sugar III, which was commonly found in the glycolipid and the CN-1 fraction of Ut-IV, suggesting that unknown sugar III is responsible for the serovar-specificity of the antigenic determinant recognized by antibody CT3.

DISCUSSION

Monoclonal antibody CT3 recognized the serovar-specific antigenic determinant of *Leptospira interrogans* serovar *canicola*¹⁰⁾. The antigenic variant CV (CT3)-1, which did not react with the CT3 antibody, was selected from Ut-IV¹¹⁾. We therefore compared the neutral sugar compositions of the glycolipid of Ut-IV with that of CV (CT3)-1 to elucidate the serovar-specific antigenic determinant of *Leptospira interrogans* serovar *canicola*.

The glycolipid of CV (CT3)-1 did not react with antibody CT3, indicating that this glycolipid lacked the serovar-specific antigenic determinant recognized by the antibody (Fig. 1). Some differences were observed in the neutral sugar compositions of these two glycolipids. Briefly, the glycolipid of CV (CT3)-1 lacked unknown sugar III and the molar ratio of glucose was much higher than that in Ut-IV (Table 1). Unknown sugar III was detected in the CN-1 fraction, which reacted with monoclonal antibody CT3, and glucose was not detected (Table 1). These findings suggest that unknown sugar III is responsible for the serovar-specific antigenic determinant of *Leptospira*

interrogans serovar *canicola*. Ono *et al.*¹⁰⁾ suggested that unknown sugar II was associated with the antigenic determinant of *Leptospira interrogans* serovar *canicola*, because this sugar was commonly detected in the glycolipid antigen and the CN-1 fraction. In the present study, unknown sugar II was detected as a major component not only in the glycolipid and the CN-1 fraction of Ut-IV but also in the glycolipid of CV (CT3)-1, which did not react with the CT3 antibody. Unknown sugar II, therefore, is considered to be a major component of sugar chains of *Leptospira interrogans* serovar *canicola*, but not to be involved in serovar-specificity.

From its retention time and mass spectrum (Fig. 2), unknown sugar III appeared to be different from any of the neutral sugars that have been detected in various leptospire, such as rhamnose, fucose, ribose, arabinose, xylose, 4-O-methylmannose, mannose, galactose and glucose¹⁴⁾. The structure of unknown sugar III, however, could not be defined in the present study, because of its small yield.

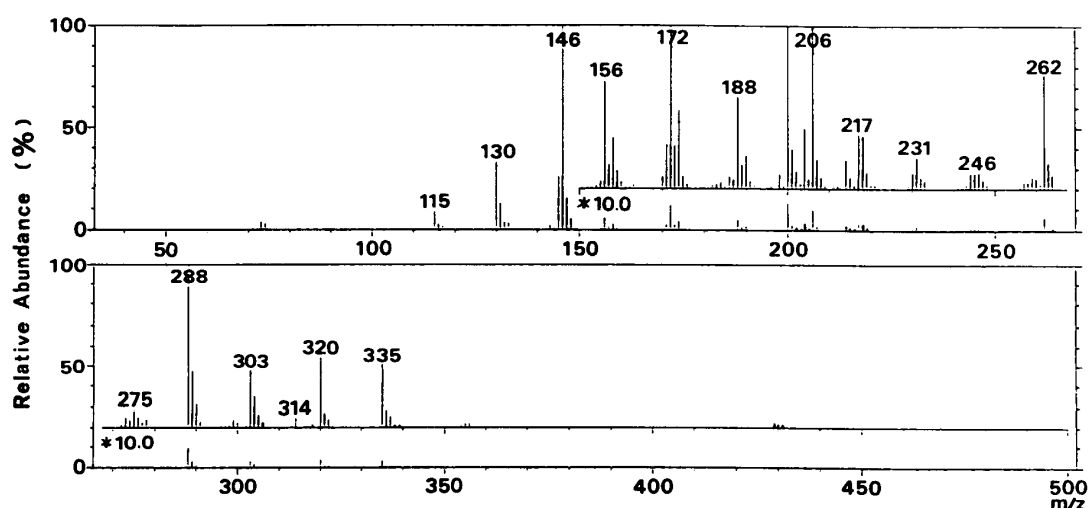


Fig. 2. Mass spectrum of the trimethylsilylated unknown sugar III obtained from the glycolipid of Ut-IV. The spectrum was recorded at an ionization potential of 23 eV. The m/z 73 fragment was a standard molecular-ion peak, and the m/z 117, 204, 217, and 305 fragments are characteristic of the mass spectra of trimethylsilylated neutral sugars.

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