DNA Base Composition of *Rickettsia tsutsugamushi* Determined by Reversed-Phase High-Performance Liquid Chromatography

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The DNA base composition of *Rickettsia tsutsugamushi* was determined by reversed-phase high-performance liquid chromatography and compared with that of *Rickettsia rickettsii*. The G+C contents were 28.1 to 30.5 mol% for *R. tsutsugamushi* and 32.1 mol% for *R. rickettsii*.

Classification of rickettsiae has been based primarily on phenotypic characteristics. These characteristics include the ecological, biological, and immunological properties of these organisms and the diseases which they produce. The genus *Rickettsia* has been divided into the typhus, spotted fever, and scrub typhus groups (9).

The G+C contents of rickettsiae have been utilized as an objective index of taxonomical relatedness among rickettsiae (3, 8). These values have been determined by the buoyant density method (8) and the thermal denaturation method (3, 8) and have clearly shown that the organisms are taxonomically related. However, *Rickettsia tsutsugamushi*, a rickettsia of the scrub typhus group, was not included in the previous studies, and the genetic relatedness of this organism to other rickettsiae remains to be determined.

In this study, the DNA base composition of *R. tsutsugamushi* was determined by reversed-phase high-performance liquid chromatography (HPLC).

MATERIALS AND METHODS

*Rickettsiae*. *R. tsutsugamushi* strains Gilliam, Karp, Kato, Kawasaki, Kuroki, and Shimokoshi were propagated by using L929 or Vero cells. The standard strains (strains Gilliam, Karp, and Kato) were repeatedly plaque cloned before propagation (4). *Rickettsia rickettsii* strain Bitterroot was propagated by using L929 cells. These rickettsiae were purified by density gradient centrifugation with Percoll (Pharmacia LKB Biotechnology AB, Uppsala, Sweden) (7).

Preparation of rickettsial and cellular DNAs. A suspension of purified rickettsial cells was treated with 0.1 mg of DNase I (Sigma Chemical Co., St. Louis, Mo.) per ml for 1 h at 37°C. After the DNase was inactivated, the rickettsial cells were collected and treated for 1 h at 56°C with 0.1 mg of proteinase K (Merck & Co., Inc., Rahway, N.J.) per ml in a solution containing 10 mM Tris-HCl (pH 8.0), 10 mM EDTA, and 0.6% sodium dodecyl sulfate. Uninfected L929 and Vero cells were collected and treated with proteinase K as described above. The DNA was extracted with phenol and precipitated with ethanol. The resulting DNA pellet was dissolved in a 10 mM Tris-HCl (pH 7.5)-5 mM EDTA solution and treated with a mixture of RNase A type III (0.1 mg/ml; Sigma) and RNase T1 (2 µg/ml; Sigma) for 1 h at 37°C. The DNA was subjected to another cycle of phenol extraction and ethanol precipitation and then redissolved in distilled water at a concentration of 1 mg/ml.

Determination of molar ratio by HPLC. The HPLC analysis was performed by using the method of Tamazaka and Komagata (6), with slight modifications. A DNA solution was boiled for 5 min and chilled. The resulting denatured DNA preparation received an equal volume of a nuclease P1 (Sigma) solution (0.1 mg/ml of nuclease P1 in 40 mM sodium acetate-2 mM ZnSO4 [pH 5.3]) and was incubated for 1 h at 50°C. The DNA digest was dephosphorylated with alkaline phosphatase (100 U of calf intestine alkaline phosphatase [Takara Shuzo, Kyoto, Japan] per ml in 0.17 mM MgCl2-0.33 M Tris-HCl [pH 8.1]) for 1 h at 37°C. The resulting hydrolysate was analyzed by HPLC by using a model 655 system (Hitachi, Ltd., Tokyo, Japan); an aliquot of the hydrolysate was applied to a COSMOSIL 5 C18 column (Nacalai Tesque, Kyoto, Japan) and eluted with a mixture of 0.2 M NH4H2PO4 (pH 4.5) and acetonitrile (20:1, vol/vol) at a flow rate of 1 ml/min. The A270 was monitored. The relative amount of each nucleoside was calculated on the basis of the peak area in the HPLC elution profile. Bacteriophage λ DNA (G+C content, 49.8 mol%; Takara Shuzo) was used as a standard to determine the base ratios of rickettsial DNAs.

RESULTS

DNA base composition. Authentic deoxyribonucleotides eluted from a reversed-phase column in the following order: deoxyxycytidine, deoxyguanosine, deoxythymidine, and deoxyadenosine (6). An elution profile of a hydrolysate of highly purified λ DNA contained four peaks corresponding to these deoxyribonucleotides (Fig. 1a). When monitored at 270 nm, the areas of the peaks paralleled the molar ratio of deoxyribonucleotides.

The deoxyribonucleotides from cellular and rickettsial DNAs were clearly separated by their elution profiles as was the case with the highly purified λ DNA (Fig. 1b through f). Pretreatment of the DNAs with a combination of RNases A and T1 was indispensable for eliminating the ribonucleosides from RNA that contaminated the original DNA preparations. Thus, the amount of each deoxyribonucleoside was accurately determined on the basis of the peak area. The values obtained are shown in Table 1. The G+C content of *R. rickettsii* Bitterroot was 32.1 mol%. This value is very similar to the values of 32.4 and 32.0 mol% determined by the buoyant density method and the thermal denaturation method, respectively (8). Under the same conditions, the G+C content of *R. tsutsugamushi* ranged from 28.1 to 30.5 mol%. There was a small but significant difference in G+C content between *R. rickettsii* and *R. tsutsugamushi*.

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**DISCUSSION**

The DNA base ratios of microorganisms are specific to individual species and have been used to identify and classify organisms. The DNA base ratio approach has also been applied to the classification of rickettsiae (3, 8). However, previous studies did not include *R. tsutsugamushi* strains, probably because of the technical difficulty in purifying them.

Determining the DNA base ratio of *R. tsutsugamushi* with an accurate method should be very important, and the HPLC technique (2) can do this. In fact, this technique has been used in taxonomical studies of bacteria (5). In these studies, the base ratios were calibrated on the basis of the absorption of known concentrations of standard deoxyribonucleosides. In our study, we used λ DNA as a standard to calculate the base ratios.

The G+C content of *R. rickettsii* determined by reversed-phase HPLC was identical to the G+C contents determined by the classical methods (3, 8). This proves the usefulness of the HPLC technique for determining the G+C contents of rickettsiae. Under our conditions, the G+C contents of *R. tsutsugamushi* ranged from 28.1 to 30.5 mol%. These values do not overlap the value obtained for *R. rickettsii* (a member of the spotted fever group) but coincide with the values obtained for rickettsiae belonging to the typhus group (3, 8). However, rickettsiae with the same G+C contents are not necessarily identical. Further taxonomical study of the species or strains will require DNA homology tests.

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