THE USE OF DNase FOR PREPARATION OF HOST TISSUE DNA-FREE RICKETTSIAL SUSPENSIONS

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Summary. — Treatment of *Rickettsia prowazekii* (strain Breinl) grown in chick embryo (CE) yolk sacs with DNase resulted in preparation of host tissue DNA-free rickettsial suspensions suitable for isolation of rickettsial DNA. Of two DNA fractions obtained, the first and main one corresponded to rickettsial DNA (29% G+C) based on the physico-chemical characteristics, whereas the nature of second supplementary fraction (47.5% G+C) was not determined.

Key words: Rickettsia prowazekii; purification; DNA; DNase; chick embryo yolk sacs; tissue sediments

Introduction

Isolation of DNA from rickettsiae, obligatory intracellular parasites, is complicated by the necessity of its separation from host tissue DNA which is extracted usually together with rickettsial DNA. For this reason, to prepare rickettsial DNA, rickettsial suspensions devoid of host tissue components are used (Anacker *et al.*, 1980; Myers and Wisseman, 1980; Myers *et al.*, 1980). Besides that, there is possible to isolate DNA from partially purified rickettsial suspensions, from which host tissue DNA was separated by the use of DNase. This enzyme employed during purification steps of *Rickettsia typhi* grown in CE yolk sacs and *Rickettsia akari* as well as *R. typhi* grown in L cell cultures (Balayeva *et al.*, 1968; Weiss *et al.*, 1972) was used also in our study.

Material and Methods

R. prowazekii strain Breinl was grown in 6 to 7-day-old CE, which were inoculated into yolk sacs with 0.5 ml volumes containing about $10^{4.2}$ EID_{50} (egg infectious doses) of rickettsiae. To obtain a rich starting rickettsial suspension, the yolk sacs were selected which were harvested from dying or freshly died CE on days 6 – 7 post-infection (p.i.) and were found heavily infected with rickettsiae (based on microscopical evalution). Rickettsiae were stained by the method of Zdrodovsky (Zdrodovsky and Golinevitch, 1972).

Purification and DNase treatment of rickettsiae. The first purification steps comprised of differential centrifugation, centrifugation over bovine plasma albumin layer and trypsinization as descri-

bed elsewhere (Weiss *et al.*, 1975). The rickettsial pellet was resuspended in 9 vol of DNase solution in 0.05 mol/l Tris-HCl buffer, pH 7.4—7.5, containing 0.005 mol/l $MgCl_2$, and incubated for 30 min at room temperature under stirring. Treatment with DNase (technical DNase from the Leningrad abbatoir) was stopped by addition of EDTA to a final concentration of 0.01 mol/l. To remove remains of DNase, the rickettsial sediments were washed with K36 solution (Bovarnick and Snyder, 1949) in the presence of 0.01 mol/l EDTA. CE yolk sac sediments resulting from low-speed centrifugations were collected, pooled, trypsinized and subject to the DNase treatment as described above. The whole procedure starting from the harvest of CE yolk sacs lasted for 8—9 hr without any freezing of rickettsial material during the purification process.

Isolation of DNA. Suspensions of purified rickettsiae and sediments of infected host tissue, respectively, were lysed by combination of sodium dodecylsulphate (SDS) and pronase (Myers and Wisseman, 1980). DNA was isolated by the modified Marmur's method (Marmur, 1961) using chloroform and isoamyl alcohol extraction.

DNA analysis. The isolated DNA was ultracentrifuged in CsCl buoyant density gradient (Kaplan, 1969). The fractions obtained were analysed in Hitachi spectrotophometer at 260 nm. In some experiments, the same methods of ultracentrifugation, fractionation and gradient analyses were carried out with the lysates of purified rickettsiae and host tissue sediment, respectively, without a previous DNA isolation.

Determination of DNA base composition. Base composition was calculated from calibrated curve indicating dependence of proportional content of guanine and cytosine (%G + C) on the buoyant density of reference DNAs which were isolated from *Micrococcus luteus* (71% G + C, buoyant density 1.731 g/cm³) and *Vibrio cholerae* strain 2819 (47.5% G + C, buoyant density 1.707 g/cm³), respectively (kindly supplied by Department of Molecular Bases of Ontogenesis, Belozersky Interfaculty Research Laboratory of Molecular Biology and Bioorganic Chemistry, Moscow State University, Moscow).

To determine the melting temperature (T_m) of DNA samples, preparations obtained by preparative ultracentrifugation were used. The fractions containing rickettsial DNA were pooled, dialysed against citrate buffered saline (Marmur, 1961) and heated in the spectrophotometer P-800 with thermostatic quartz windows at 260 nm. The base composition of DNA was determined from its T_m measured in triplicates using the formula proposed by Marmur and Doty (1962).

Results

In the process of rickettsial purification, different concentrations of DNase, namely 100, 50 and 25 µg/ml were employed. These of higher DNase concentrations (100 and 50 µg/ml) resulted in a visual clearing of rickettsial suspensions and in a marked increase in their density. In the preparations obtained, the microscopic examination of stained smears revealed only single rickettsiae. Higher amounts of well-stained morphologically typical rickettsiae were seen, however, when DNase concentration was decreased to 25 µg/ml, so that in further experiments only this lower DNase concentration was used.

Infectivity of DNase-treated rickettsiae was tested on guinea pigs inoculated intraperitoneally with 10^{-3} dilution of rickettsial suspension (based on the weight of crude sediment) in 1.5 ml volumes. All animals developed febrile illness typical of experimental typhus infection. Febrile reaction begining after 2—3 days incubation period lasted for 8 days reaching the values of 39.8—40.2 °C. In guinea pig sera harvested on day 22 p.i., titres of 160 of complement-fixing antibodies to *R. prowazekii* antigen were demonstrated. The results obtained suggest the preservation of *R. prowazekii* virulence for guinea pigs after its DNase treatment.

DNase-treated partially purified rickettsiae as well as the CE yolk sac sediments obtained by low speed centrifugation during the purification
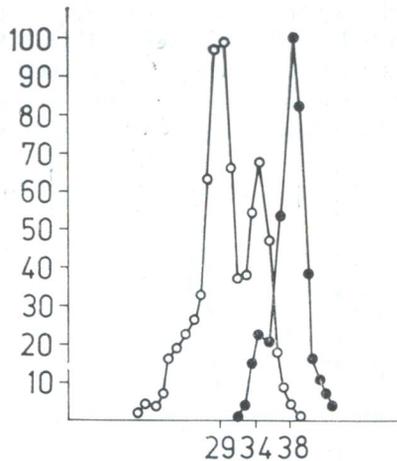
Fig. 1.

Absorbance spectra of DNA solutions isolated by preparative ultracentrifugation in CsCl density gradient

Rickettsial suspension (●), standard DNA (○)
Abscissa: fraction number; ordinate: absorbance at 260 nm (%)

were immediately lysed as soon as DNase had been washed out. The lysates were kept at −20 °C. From 35—50 yolk sacs about 5 ml of purified rickettsial lysate and about 2 ml of host tissue sediment lysate were obtained. From these 500—900 and 150—200 μg DNA, respectively, were isolated and further analysed by preparative ultracentrifugation in CsCl density gradient. As controls DNA preparations were used prepared by the same method from purified, but DNase untreated rickettsial suspensions or from DNase untreated suspensions of ncn-infected CE yolk sacs harvested at the same time and subjected the same purification steps as the infected ones.

DNA preparations isolated from purified rickettsial suspensions differed whether they were cr were not subjected to the DNase treatment. The main DNA fraction isolated from the lysates of rickettsial suspensions prior to the treatment with DNase had the base composition of 42—43% G+C corresponding according to our own data to the characteristic of egg DNA (42.5% G+C) and confirming the previous data of others (Smith and Stoker, 1951). Only traces of the preparation had lower values of this index.

Fig. 1 demonstrates the absorbtion spectra of DNA isolated from purified and DNase-treated rickettsial suspensions and from standard DNAs of the known nucleotide composition. The peak of absorption of DNA under study was moved in comparison with that of standard DNA. Moreover, the investigated DNA was divided into 2 fractions in CsCl gradient: the main fraction (29% G+C) and smaller supplementary one (47.5% G+C). The main fraction appeared to be rickettsial, its characteristics corresponding to data of other authors (Tyeryae et al., 1973; Myers and Wisseman, 1980), the nature of supplementary fraction is unknown so far.

Fig. 2 shows the absorption spectra of 3 solutions: 1. DNA purified according to Marmur (1961) from the lysate of the sediment of DNase-treated yolk sac fragments from R. prowazekii-infected CE; 2. the lysate of R. prowazekii purified with the use of DNase; 3. the lysate of the sediment of DNase-trea-

ted yolk sac fragments from *R. prowazekii*-infected CE. In all 3 solutions investigated, both rickettsial DNA (29% G+C) and small amount of DNA of other nature (47.5% G+C) were determined. Besides that, the results indicate that DNase treatment led to comparable successful destruction of egg DNA irrespective of whether fragments of tissue sediments or partially
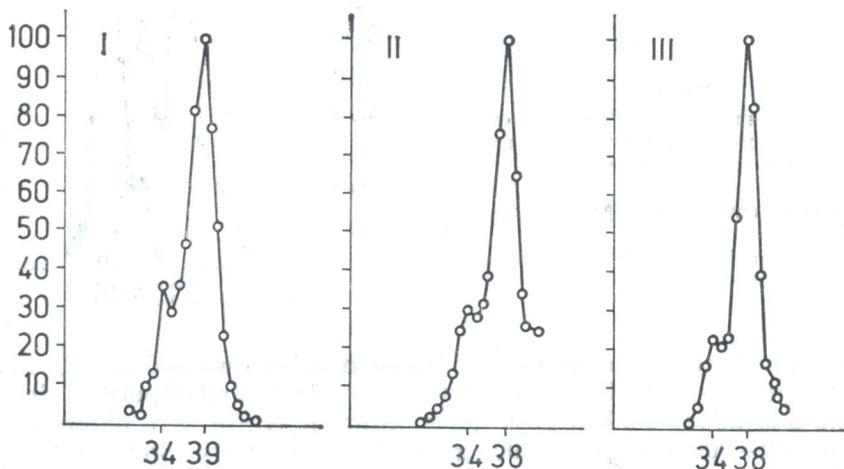

Fig. 2.

Absorbance spectra of DNA solutions isolated after DNase treatment in CsCl density gradient
I — purified DNA; II — DNA from the rickettsial lysate; III — DNA from the CE yolk sac sediment
Abscissa: fraction number; ordinate: absorbance at 260 nm (%).

purified rickettsial suspensions were treated. To confirm the data on DNA base composition achieved by preparative ultracentrifugation, in each experimental variant 3—4 fractions containing the highest amount of DNA were collected, pooled and proceeded as described in Materials and Methods. As control, DNA preparation from *V. cholerae* was used.

T_m of control and investigated DNA samples was $88.8 \pm 0.2\,°C$ and $81.4 \pm 0.1\,°C$, which corresponded to 47.6 ± 0.4 and $29.5 \pm 0.25\%$ G+C content, respectively. The data obtained were in accord with those determined by the previous method.

Discussion

Our results suggest that DNase treatment can be used for isolation of DNA from *R. prowazekii* (strain Breinl) grown in CE yolk sacs by separating egg DNA. Such treatment (DNase in given concentration) of partially purified rickettsial suspension made it possible to achieve DNA preparations corresponding in their physico-chemical characteristics completely to rickettsial DNA (29% G+C, $T_m = 81.4\,°C$). In contrast, the main DNA

fraction isolated from DNase untreated rickettsial suspensions had a different base composition (42—43% G+C).

The results also confirm the efficacy of DNase treatment for preparation of host tissue DNA-free rickettsial suspensions. Furthermore, rickettsial DNA could also be isolated from DNase-treated tissue sediments of CE, which are discarded usually during the purification of rickettsiae. It increases the economy of proposed purification method, because of the possibility to utilize practically all rickettsial biomass from CE yolk sacs.

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