PROBING THE STRUCTURE OF BACTERIAL DEOXYRIBONUCLEOPROTEINS BY EXOGENOUS AND ENDOGENOUS NUCLEASES.

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UDC 576.8.098.396.332

Digestion of deoxyribonucleoproteins (DNP) of Gram-negative bacteria by micrococcal nuclease and by Ca^{++}- and Mg^{++}-activated bacterial endonuclease followed by electrophoresis revealed a group of DNP-fragments of discrete size in each species, as well as large nuclease-resistant DNP fractions. The approximate molecular weight of the smallest bacterial DNP fragment was estimated to be 120-140 base pairs. At least two cationic proteins, with electrophoretic mobility close to that of eukaryote histone H4 were discovered in the DNP of each species of bacteria. The presence of a universal principle of regular subunitary DNP structure in bacteria is postulated.

KEY WORDS: bacterial deoxyribonucleoprotein; subunitary structure; endonuclease.

Bacterial deoxyribonucleoprotein (DNP), on examination in the electron microscope, appears to be structurally composed of nucleosome-like particles [6]. However, probing the structure of bacterial DNP by fragmentation of isolated nucleotides by micrococcal nuclease [11] did not reveal a set of fragments of bacterial DNP whose size was a multiple of that of its elementary structural unit, nor has biochemical evidence of the presence of a regular principle of organization of DNP in bacteria been obtained. This failure is evidently due to the particular features of organization of bacterial DNP and, in particular, to the unprotected nature of the spacer regions in it between the nucleosome-like structures.

The authors have attempted to find regularity in the structural organization of bacterial DNP by the same method as in eukaryotes, i.e., by treating DNP with exogenous and endogenous nucleases contained within bacterial spheroplasts, on the assumption that some spacer regions in bacterial DNP are protected *in situ* against nuclease attack, so that it would be possible to detect fragments of bacterial DNP whose size is a multiple of the elementary structural unit. In addition, to examine the question of whether DNP in bacteria of different taxa is organized on a universal principle, a comparative analysis was undertaken of its structure in various representatives of the Gram-negative bacteria.

EXPERIMENTAL METHOD

Cultures of *Escherichia coli*, *Pseudomonas fluorescens* strain No. 561, and *Serratia marcescens* were grown (up to 10^{10} cells) for 24 h at 30°C in 250-ml conical flasks, each containing 25 ml of standard nutrient broth, each flask being seeded with 0.1 ml of a 24-h culture.

Bacterial spheroplasts were obtained by treatment with lysozyme [12] in a concentration of 12.5-25 μg/ml of a solution containing 0.25 M sucrose, 1 mM $CaCl_2$, and 0.01 M Tris-HCl, pH 8.0, with micrococcal nuclease (from Sigma) in a concentration of 5 μg/ml. Nucleolysis was stopped by the addition of a solution of 0.1 M EDTA-Na_2, pH 8.0, to a final concentration of 2 mM and by cooling to 0-4°C, after which the spheroplasts were again sedimented by centrifugation at 13,000g for 10 min. The lysed spheroplasts were homogenized in a solution of the following composition: 0.012 M Tris-HCl, 0.01 M NaH_2PO_4, 0.003 M EDTA-Na_2, pH 7.7 (solution A). The supernatant obtained after centrifugation at 13,000g for 10 min was used as the source of bacterial DNP.

Laboratory of Neurochemistry, I. M. Sechenov Institute of Evolutionary Physiology and Biochemistry, Academy of Sciences of the USSR, Leningrad. Department of Microbiology, A. A. Zhdanov Leningrad University. (Presented by Academician of the Academy of Medical Sciences of the USSR A. A. Smorodintsev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 3, pp. 307-309, March, 1980. Original article submitted December 28, 1978.

 0007-4888/80/8903-0304$07.50

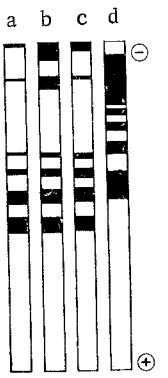

Fig. 1

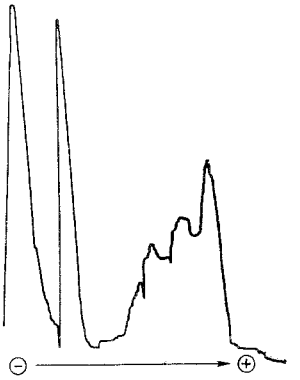

Fig. 2

Fig. 1. Electrophoresis of DNP fragments of *Escherichia coli* (a), *Pseudomonas fluorescens* (b), and *Serratia marcescens* (c), obtained by cleavage by micrococcal nuclease, and of DNA from rat liver chromatin fragmented with Ca,Mg-dependent endonuclease (d).

Fig. 2. Densitogram of spectrum of *Serratia marcescens* DNP fragments obtained by cleavage by micrococcal nuclease.

The bacterial DNP also was fragmented after incubation of the spheroplasts in 2 ml of a solution activating eukaryote Ca, Mg-dependent endonuclease [3], of the following composition: 0.25 M sucrose, 0.05 M Tris-HCl, pH 7.5, 0.025 M KCl, 0.005 M $MgCl_2$, and 0.002 M $CaCl_2$. Incubation was continued for 10 min at 20°C. Nucleolysis was stopped by the addition of 10 volumes of a solution containing 0.25 M sucrose, 0.005 M Tris-HCl, pH 7.5, 0.025 M KCl, and 0.005 M $MgCl_2$, and the sample was centrifuged at 13,000g for 10 min. Bacterial DNP was extracted in the same way as after treatment with micrococcal nuclease.

The bacterial DNP obtained after nuclease treatment were analyzed by electrophoresis in 5% acrylamide gel: a ratio of acrylamide to bisacrylamide 48:1, 10 cm high. The bacterial DNP were applied to the gel in a dose of 4 optical absorption units at 260 nm. Solution A was used as the electrode buffer. Electrophoresis was carried out with a current of 5 mA to the gel for 1 h at 4°C. To determine the approximate molecular weight of the bacterial DNP fragments, a parallel electrophoretic study was made of rat liver chromatin, digested with Ca, Mg-dependent endonuclease [2], and of DNA isolated from chromatin by the method described previously [9]. The gels were stained for DNA with a 1% solution of toluidine blue in 1% acetic acid and with ethidium bromide (20 μg/ml), decolorizing with water. The gels were scanned on the Chromoscan-200 instrument (Joyce-Loeble, England).

To isolate cationic proteins of bacterial DNP the spheroplasts were lysed in solution A, sonicated on the UZDN-1 ultrasonic disperser with a current of 0.3 A, frequency 22 Hz, for 20 sec, and centrifuged at 13,000g for 10 min. The supernatant of the bacterial DNP was purified in an "Elphor-VaP-5" continuous flow electrophoresis apparatus (West Germany) with a strength of 44 mA and a voltage of 1100 V. After electrophoresis, cationic proteins were isolated from the fractions containing bacterial DNP with 0.4 N H_2SO_4 by the method described in [5]. Electrophoresis of cationic proteins of bacterial DNP and of calf thymus histones was carried out by the method described in [10]. The gels were stained with a 0.3% solution of Amido Black in 20% ethanol and 7% acetic acid, decolorizing with 0.9 N acetic acid.

EXPERIMENTAL RESULTS

The results of electrophoresis of fragments of bacterial DNP of all three species studied, obtained by treatment with micrococcal nuclease, are illustrated in Fig. 1 (a-c). Two groups of bands can be seen on the gels from each species. One is located near the start zone of the polyacrylamide gel and evidently consists of large nuclease-resistant DNP fragments which migrate slowly during electrophoresis. The other group consists of four frag-

ments which are distributed by relative electrophoretic mobility as multiples of the size of the smallest of them. A densitogram of the spectrum of fragments excised by micrococcal nuclease in *Serratia marcescens* spheroplasts is illustrated in Fig. 2 and shows that the most rapidly migrating DNP fraction also has the greatest density, whereas the proportions of the other fractions diminish successively. Comparison of the results of electrophoresis of DNP fragments of the three species of Gram-negative bacteria, susceptible to micrococcal nuclease attack, shows their surprising similarity, although species such as *Pseudomonas fluorescens* and *Escherichia coli* are taxonomically remote.

The discovery of a group of fragments whose size was a multiple of that of the smallest, among fragments of bacterial DNP obtained by cleavage with micrococcal nuclease, can be regarded as biochemical evidence of the regular structural organization of bacterial DNP, and the similarity of the electrophoretic mobility of these groups of fragments in different species of Gram-negative bacteria can be explained by the universality of the regular principle of DNP organization among bacteria.

The attempt to probe bacterial DNP by activation of endobacterial nucleases thus proved highly successful. All three species of bacteria were found to contain endonuclease activity, activated by Ca^{++} and Mg^{++}, causing cleavage of bacterial DNP in a similar way to micrococcal nuclease, judging from the spectra of the NDP fragments discovered by electrophoresis in acrylamide gel. Characteristically the proportions of bacterial DNP isolated by extraction from lysed spheroplasts, treated both with micrococcal nuclease and with bacterial Ca,Mg-endonuclease activity were very close, and they accounted for up to one-third of the DNP content of the intact cells, evidence of the restricted accessibility of DNP *in situ* to nuclease attack because of their association, evidently, with intracellular membranes.

For a rough estimate of the size of the smallest elementary structural subunit of bacterial DNP thus discovered, parallel with electrophoresis of the bacterial DNP fragments obtained by treatment with nucleases, DNA fragments from rat liver chromatin, digested with Ca,Mg-dependent endonuclease, which in size are multiples of a nucleosome [2, 7], also were subject to electrophoresis (Fig. 1d). The size of DNA of rat liver nucleosomes, according to different workers, is about 200 base pairs [8]. Compared with the most rapidly migrating fragment of liver chromatin DNA, corresponding to nucleosomal DNA, the most rapidly migrating fragment of bacterial DNP moves faster during electrophoresis (Fig. 1). However, the position of this last fragment in the gel was above the band corresponding to a DNA fragment measuring 100 base pairs. Considering the structural organization of DNA in the smallest bacterial DNP fragment, on account of binding with cationic proteins, the size of the DNA in this fragment can be estimated to be roughly 120-140 base pairs, which is close to estimates obtained by other workers [11].

Analysis of the composition of cationic proteins in bacterial DNP purified by continuous-flow electrophoresis showed that at least two protein fractions, located in the gel at the level of calf thymus histone H4, were present in all three species.

Probing bacterial DNP by its cleavage *in situ* with exogenous (micrococcal) and endogenous (endobacterial, activated by Ca^{++} and Mg^{++}) nucleases, in conjunction with biochemical methods, thus showed the presence of regular organization of DNP in prokaryotes.

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