Microbiology, Vol. 74, No. 2, 2005, pp. 128–135. Translated from Mikrobiologiya, Vol. 74, No. 2, 2005, pp. 157–165.
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EXPERIMENTAL ARTICLES

Effect of Alkylhydroxybenzenes, Microbial Anabiosis Inducers, on the Structural Organization of *Pseudomonas aurantiaca* DNA and on the Induction of Phenotypic Dissociation

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Received October 27, 2003

Abstract—We revealed a relationship between alkylhydroxybenzene (AHB)-induced changes in the structural organization of supramolecular complexes (SC) of the DNA of *Pseudomonas aurantiaca* and the phenotypic dissociation of this bacterium. The addition of 0.1–0.3 mM hexylresorcinol (C_6-AHB), a chemical analogue of microbial anabiosis autoinducers, caused the formation of cystlike refractile cells (CRC) in these gram-negative, nonsporulating bacteria. Inoculating pseudomonad CRC on solid nutrient media resulted in phenotypic dissociation of the microbial population that yielded several variants with different colony structure and morphology. This manifested itself in the conversion of the original S-colony-forming phenotype into the R form and in the formation of less pigmented colonies. These transitions were possibly linked to AHB-induced structural changes in the DNA. In vitro studies revealed that AHB could interact with DNA SC, resulting in their structural modification that manifested itself in changes in their viscoelasticity. DNA supramolecular complexes isolated from proliferating, stationary-phase, and anabiotic *P. aurantiaca* cells differed in their viscoelasticity and capacity to interact with AHB homologues with different hydrophobicity, such as hexylresorcinol and methylresorcinol (C_1-AHB). The DNA SC from actively proliferating cells were characterized by smaller viscoelasticity compared with those from stationary-phase and anabiotic cells, due to the difference in the DNA superspiralization degree and the physiological age of the bacteria involved. C_6-AHB produced a pronounced relaxing effect on the DNA SC from exponential-phase *P. aurantiaca* cells. The less hydrophobic C_1-AHB produced a similar relaxing effect on the DNA SC from stationary-phase cells. The curve of the dose-effect dependence of C_6-AHB had a breaking point within the submillimolar (10^{-4} M) concentration range. These concentrations induce the formation of cystlike anabiotic pseudomonad cells that are characterized by an unstable phenotype and dissociate into distinct variants upon inoculation on solid media.

Key words: anabiosis autoinducers, alkylhydroxybenzenes, refractile anabiotic cells, phenotypic dissociation, DNA supramolecular complexes, viscoelasticity of the DNA supramolecular complexes.

It has been demonstrated that low-molecular-weight anabiosis autoinducers (factors d_1) control the transition to the dormant state and its maintenance in a number of microorganisms including nonsporulating bacteria [1, 2]. In some microorganisms, factors d_1 are isomers and homologues of alkyl-substituted hydroxybenzenes (AHB), represented by alkylresorcinols [3]. An increase in their concentration in developing microbial cultures promotes the formation of dormant forms referred to as cystlike refractile cells (CRC). They retain their viability for a long time, their metabolic activity level is too low to be detected, and they are characterized by ultrastructural peculiarities [1, 2] and high phenotypic variability [4]. The germination of dormant cystlike forms and the initiation of a new developmental cycle of a culture are accompanied by a drastic increase in the dissociation index of the microbial population with the segregation of variants that differ in their morphology and colony structure. This was shown in studies with *Bacillus cereus* [4] and *Staphylococcus aureus* [5].

We investigated the mechanisms that are possibly involved in the AHB (factors d_1) effect on the process of phenotypic dissociation in bacteria. One mechanism is based on the AHB capacity to form complexes with proteins, changing the conformation and functional

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activity of the macromolecules [6] that may be involved in regulating the dissociation process at the transcriptional level. Alternatively, AHB may directly interact with DNA [7], affecting the cell's genetic material. The Ames test conducted with the histidine-auxotrophic mutant *Salmonella typhimurium* TA 100 revealed that AHB, depending on their hydrophobicity, produced either a weak mutagenic effect (if hexylresorcinol, C_6-AHB, was used) or a weak antimutagenic effect (if the system contained methylresorcinol C_1-AHB). These studies also demonstrated that AHB induced the conversion of the S variant of *S. typhimurium* into the R form [8].

These data raised the issue of whether the phenotypic dissociation of microbial populations is accompanied by microbial AHB-induced structural changes in the DNA. To detect such changes, we earlier employed a sensitive method of measuring viscoelasticity [9, 10]. Viscoelasticity is related to the DNA superspiralization degree, which varies depending on the cells' physiological age (the developmental stage of the microbial culture) [11] and is affected by hormones, antitumor preparations, and ionizing radiation [10, 11].

The goal of this work was to investigate the effect of microbial AHB (i) on the structural organization of DNA supramolecular complexes (DNA SC) of *Pseudomonas aurantiaca* cells (by measuring DNA SC viscoelasticity) and (ii) on the phenotypic dissociation of *Pseudomonas* cultures. *P. aurantiaca* was chosen as the subject of our research because AHB of the alkylresorcinol type are active components of anabiosis inducers in representatives of this genus [3].

MATERIALS AND METHODS

The studies were conducted with the gram-negative bacterium *Pseudomonas aurantiaca* B-1558 (from the All-Russia Collection of Microorganisms). *P. aurantiaca* cells were grown on 50% nutrient broth or on a synthetic medium of the following composition (g/l): glucose, 2; KH_2PO_4, 0.1; $(NH_4)_2SO_4$, 0.5; $K_2HPO_4 \cdot 3H_2O$, 1; $CaCl_2$, 0.2; $MgSO_4 \cdot 7H_2O$, 0.1; and yeast extract (Difco), 0.05. The medium also contained the following microelements (mg/l): $FeSO_4 \cdot 7H_2O$, 20; $MnCl_2 \cdot 4H_2O$, 20; $ZnSO_4 \cdot 7H_2O$, 0.4; $B(OH)_3$, 0.5; $CuSO_4 \cdot 5H_2O$, 0.05; and $Na_2MoO_4 \cdot 2H_2O$, 0.2; the pH value was 7.25 upon sterilization. The bacteria were cultivated at 28°C in 2-l flasks with 500 ml of medium or in 250-ml flasks with 50 ml of medium on a shaker (140–160 rpm). A stationary-phase culture grown on nutrient broth was used as inoculum. The inoculum amount provided an initial optical density of 0.1 (λ = 650 nm, l = 10 mm, measured with a Specord device, Carl Zeiss, Jena, Germany) of the culture.

Microbiological methods. Microscopic studies were conducted with a Reichert microscope (Austria) equipped with a phase-contrast device. The number of viable cells was determined from the colony-forming units (CFU) calculated upon inoculating diluted cell suspensions on Petri dishes with nutrient agar. CFU were counted for the dilutions that did not yield more than 80 colonies per dish. The Petri dishes were examined to detect the colonies morphologically distinct from the dominant type. Dry cell weight (DCW) was determined upon drying cells for 24 h at 105°C. Cell respiratory activity was recorded with an LP7E polarographer (Czechoslovakia) in a 1-ml Clark-type cell.

Ultramicroscopic studies were conducted with washed cells that were fixed in a 1.5% glutaraldehyde solution in 50 mM cacodylate buffer (pH 7.2) at 4°C for 1 h, followed by additional fixation in a 1% osmium tetraoxide solution in 50 mM cacodylate buffer at room temperature for 3 h. Upon dehydration with increasing ethanol concentrations, the cells were placed in Epon 812 epoxide resin. Ultrathin sections were prepared with an LKB ultramicrotome (Sweden) and contrasted with a 3% uranylacetate solution in 70% ethanol for 30 min and thereupon with lead citrate for 4–5 min. The sections were examined with a JEM-100B electron microscope (Japan) at an accelerating voltage of 60 kV.

Biochemical methods. Factors d_1 (microbial anabiosis autoinducers) were isolated from the culture liquid and cells of *P. aurantiaca* grown on a synthetic medium using methods described earlier [3]. Alkylhydroxybenzene (alkylresorcinol) contents in factor d_1 preparations were determined using the specific colorimetric reaction with a diazotized derivative of 3,3'-methoxybenzidine (Fast Blue salt BN-FBB, Sigma, D-3502) [12]. Supramolecular complexes were isolated from cells by the mild phenol method [9]. The DNA content in the isolated preparations was determined spectrophotometrically. The viscoelasticity of the DNA SC solutions (25 μg of DNA per 1 ml 0.14 M NaCl solution, pH 7.0) was measured in a Struchkov capillary high-gradient elastoviscosimeter [9] at 20°C. Viscoelasticity was expressed in $\eta_{specific}/C$, specific viscosity units (deciliter per gram, dl/g) calculated using the formula $\eta_{specific}/C = (t_p/t_0 - 1)/C_{DNA}$, where t_p is the elution time (s) of the DNA SC solution (10 ml) passing the elastoviscosimeter capillary, t_0 is the elution time of the control sample (without DNA SC) (s), and C is the DNA concentration in the tested sample [9].

High-grade (99.9%) preparations of 4-*n*-hexylresorcinol (C_6-AHB, molecular weight 194, applied as an aqueous solution of the potassium salt) and methylresorcinol (C_1-AHB, molecular weight 124, aqueous solution) were used as chemical analogues of microbial anabiosis autoinducers.

Statistical treatment of the data obtained was carried out using standard mathematical methods such as the Student t test and Microsoft Excel software. The difference between the experimental and the control data sets was regarded as significant if the probability index (p) was below 0.05.

Table 1. Dose-dependent effect of C_6-AHB, a chemical analogue of anabiosis autoinducers, on the viability of cystlike refractile *Pseudomonas aurantiaca* cells and on the phenotypic dissociation of the pseudomonads

C_6-AHB concentration in the culture, mM	Number of viable cells in the culture		Dissociant spectrum	
	CFU number/ml	% of the control	Colony structure and morphology	Percentage of the total colony number, %
0 (control)*	$(1.4 \pm 0.1) \times 10^8$	100	Rounded pink colonies, S type, oily, edges even	100
0.1	$(8.0 \pm 0.5) \times 10^7$	57	Rounded weakly pigmented pinkish colonies, S type, relatively dense	10–20
0.3	$(5.0 \pm 0.4) \times 10^6$	4	Bright pink colonies, R type, edges uneven, the surface rough	80–90
0.5	$(6.8 \pm 0.5) \times 10^3$	0.005	R-type colonies, smaller in size	90
1.0	0	0	No colonies	–

* Onset of the stationary phase without C_6-AHB.

RESULTS

We earlier established that a gram-negative bacterium of the genus *Pseudomonas*—*P. carboxydoflava*—synthesizes AHB (C_{19}- and C_{21}-alkylresorcinols) that possess factor d_1 (anabiosis autoinducer) activity [1, 3, 13]. In this work, we report that such autoregulators also occur in another pseudomonad species (*P. aurantiaca*). Factor d_1 preparations were isolated from the culture liquid and cells of *P. aurantiaca* at the early stationary phase, when factor d_1 concentrations reach the highest levels [13]. Using the specific colorimetric test for alkylresorcinols, we demonstrated that their content in *P. aurantiaca* cells and in the culture liquid was 0.13 mg/g of DCW and 0.1 mg/l (0.11 mg/g if calculated on the DCW basis), respectively. The results obtained concerning the contents of intracellular alkylresorcinols are consistent with the data for other *Pseudomonas* species [12, 13]. The AHB preparations obtained exhibited factor d_1 activity. Their addition at concentrations of 10–20 μg/ml (calculated on the AHB basis) to an early stationary-phase *P. aurantiaca* culture resulted in the formation of dormant refractile cells, in analogy to the systems investigated earlier [1, 3]. Taking into account the results obtained, we conducted studies with C_6-AHB and C_1-AHB, chemical analogues of d_1 factors that differ in their hydrophobicity degree.

The first series of studies dealt with their influence on the formation of cystlike refractile cells (CRC) in *P. aurantiaca*. The addition of 0.1–1 mM C_6-AHB to cell suspensions resulted in a rapid conversion of 80–90% of *P. aurantiaca* cells into refractile forms that occurred within 15 min after introducing the analogue into the culture, based on the results of microscopic studies. However, the retention of the capacity to form colonies upon inoculation on MPA was conditional in the refractile cells on the C_6-AHB concentration within a comparatively narrow concentration range (Table 1). The number of viable cells in the suspensions that contained 0.1 mM C_6-AHB and were stored at room temperature for 7 days was 57% of the CFU number in an early stationary-phase (24-h) culture without AHB. The CFU number in CRC suspensions with 0.3 mM C_6-AHB was 4%. Increasing the C_6-AHB dose to 0.5–1 mM lowered the CFU number to 0.005% or below. Hence, the formation of viable refractile pseudomonad cells was induced by C_6-AHB only within a narrow concentration range (0.1 to 0.3 mM). The less hydrophobic d_1 analogue C_1-AHB failed to induce CRC formation. Refractile *P. aurantiaca* cells were characterized by (i) retained viability (the CFU number was 4–57%) under autolysis-promoting conditions (prolonged storage at 18–24°C in the growth medium); (ii) a lack of energy-supplying processes (respiration was inhibited, based on polarographic data); (iii) a specific ultrastructure characterized by an enlarged periplasmic space, a fine-grained cytoplasmic structure, and a compact nucleoid (data not shown). Taken together, these CRC peculiarities of tested pseudomonads are analogous to those in the anabiotic forms of *P. carboxydoflava* [1] and *Bacillus cereus* [2] that were investigated by us earlier.

When inoculated on nutrient agar, CRC obtained with 0.1–0.5 mM C_6-AHB and stored for 7 days yielded colonies that were morphologically different from those of the original (dominant) S-type variant (Table 1). CRC obtained with 0.1 mM C_6-AHB gave rise to populations that included S-type colonies; however, they were weakly pigmented, in contrast to those of the original variant. Their number reached 20% of the total colony number. A higher C_6-AHB concentration (0.3 mM) caused the conversion of original S phenotype cells into R forms (Fig. 1), and the percentage of dissociants that yielded R-type colonies was 80–90% (Table 1). The data obtained attest to an increase in the phenotypic dissociation level in bacterial populations that formed from CRC, subject to regulation by the C_6-AHB level, as was earlier shown with *Bacillus cereus* [4], *Staphylococcus aureus* [5], and *Salmonella typhimurium* [8].

The relationship between the phenotypic dissociation of bacterial cultures and the AHB (C_6-AHB) level

can be due to direct interactions between AHB and DNA. They result in structural changes in the DNA that account for intrapopulation variability, according to a modern concept [14]. Structural changes in the DNA manifest themselves in the DNA SC viscoelasticity [9–11]. DNA supramolecular complexes were isolated from *P. aurantiaca* cells that varied in their physiological age and metabolic activities. They included exponentially growing and stationary-phase cells and CRC obtained using 0.1 mM C_6-AHB. The average DNA content in the preparations obtained was 25 mg/g of DCW, which is consistent with the data available in the literature concerning the DNA quantity in bacterial cells.

DNA SC solutions (25 μg of DNA per 1 ml) that were isolated from physiologically different pseudomonad cells differed in their viscoelasticity, a physicochemical property that is related to the DNA superspiralization degree. The viscoelasticity of DNA SC solutions from actively growing *P. aurantiaca* cells was 29–33% lower than that of DNA SC solutions from stationary-phase cells and anabiotic forms resulting from the effect of 0.1 mM C_6-AHB (Table 2). The differences revealed by us confirm our earlier data on the dependence of DNA SC viscoelasticity in *E. coli* and *B. subtilis* on their physiological age [9].

The high viscoelasticity values of DNA SC preparations from stationary-phase and anabiotic cells reflect their higher DNA superspiralization degree and, accordingly, a more compact arrangement of the nucleoid. This conclusion is corroborated by the results of a comparative analysis of ultrathin sections of *P. aurantiaca* cells. In contrast to actively growing exponential-phase cells whose nucleoid is evenly distributed in the cytoplasm (Fig. 2a), the nucleoid of stationary-phase cells is more compact and contains thickened electron-dense DNA fibrils (Fig. 2b). Nucleoid compaction was also revealed in electron micrographs of ultrathin sections of refractile pseudomonad cells that were obtained by adding C_6-AHB (0.1 mM) and of CRC (data not shown). Analogous changes (a dense arrangement and compaction of the nucleus) were also found in electron microscopic studies on sections of starving *E. coli* cells [15].

However, the decreased viscoelasticity value of actively growing *P. aurantiaca* cells can also be due to the fact that the replicating DNA of proliferating cells is partly relaxed and its double strands are unwound, while the DNA of stationary-phase cells is superspiralized and its replication is arrested.

DNA binding to proteins and lipids can also contribute to the increase in the DNA SC viscoelasticity in stationary-phase and anabiotic pseudomonad cells, apart from DNA superspiralization and nucleoid compaction [11, 16]. Starving *E. coli* cells contain several types of DNA-bound proteins that account for the compact arrangement of the nucleoid and provide for the DNA resistance to stress factors. The expression of some of these proteins, e.g., Dps, is associated with the stationary phase [17]. Small acid-soluble DNA-binding proteins (SAPs) were revealed in endospores of bacilli; these proteins are encoded by the spore genome and account for the stability of the nucleoids in these dormant forms [18].

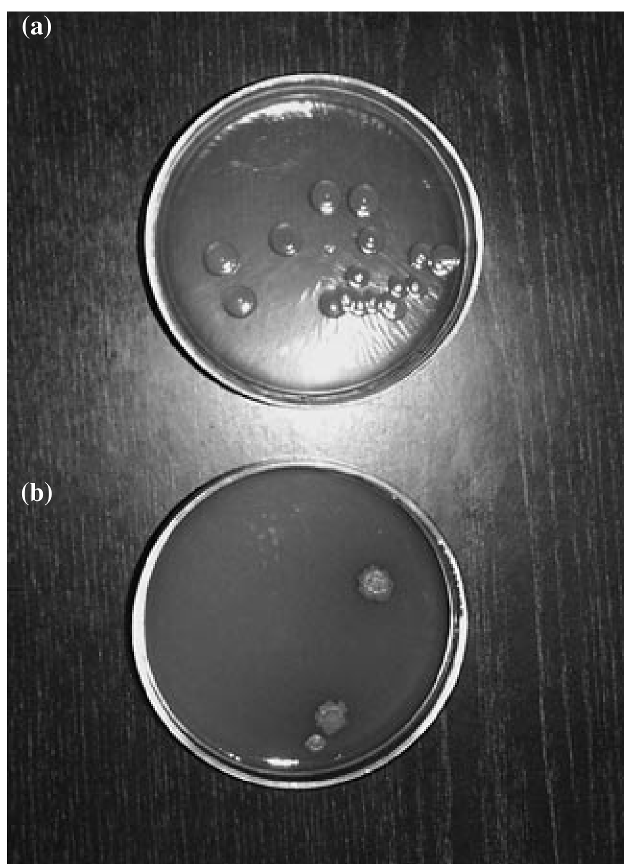

Fig. 1. Appearance of *P. aurantiaca* colonies forming after inoculating (a) the control variant and (b) refractile anabiotic cells obtained with 3×10^{-4} M C_6-AHB.

Nonenzymatic processes including DNA phase transitions exert a significant influence on the changes in the DNA structural organization and nucleoid compaction in stationary-phase, starving, and dormant cells [15]. It was established that DNA exists in the A, not the B, form in *Bacillus* spores. The A form is more resistant

Table 2. Viscoelasticity values of DNA SC isolated from *P. aurantiaca* cells in different physiological states

Source of DNA SC	viscoelasticity value, dl/g
Exponential culture	602 ± 18
Stationary-phase culture	903 ± 27
Anabiotic cells	851 ± 24

Note: The table gives the average data of five repeats; standard deviation values did not exceed 3%.

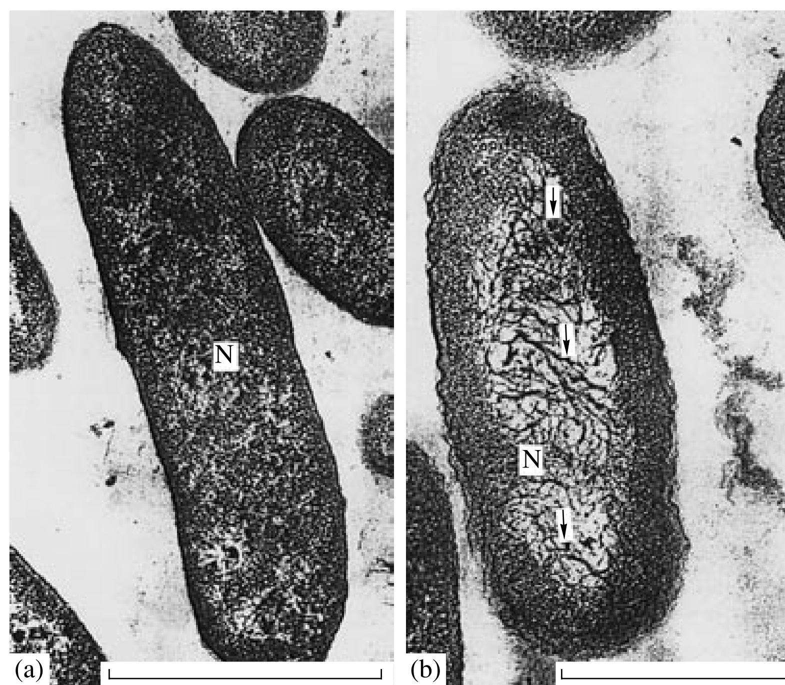

Fig. 2. Micrograph of ultrathin sections of *P. aurantiaca* cells from (a) exponential-phase and (b) stationary-phase cultures. N is nucleoid; arrows show electron-dense DNA fibrils. Bar represents 0.5 μm.

to deleterious factors [18]. The involvement of physical and chemical processes in the modification of the DNA structural organization can implicate the operation of several mechanisms including interactions with various ligands.

We conclude that the dependence of the DNA SC viscoelasticity on the physiological age of the cells indicates that the aging of a microbial culture and the transition to proliferative (stationary-phase cells) and metabolic (anabiotic cells) dormancy result in changes in the DNA conformation. The peculiarities of the structural state of the DNA of actively dividing, stationary-phase, and anabiotic cells apparently determine the specific pattern of its interactions with ligands. Alkylhydroxybenzenes (AHB), chemical analogues of anabiosis autoinducers, can perform the ligand function, because their capacity to interact with DNA was demonstrated using the Ames test [8]. The intra- and intercellular AHB (factor d_1) quantities change during the development of microbial cultures, reaching the maxima in stationary-phase and dormant cells. This was earlier demonstrated with microorganisms belonging to various taxa, including bacteria of the genus *Pseudomonas* [13]. Therefore, the subsequent experiments dealt with the effects of interactions between DNA SC (from *P. aurantiaca* cells characterized by different physiological age) and chemical analogues of microbial anabiosis inducers (C_6-AHB and the less hydrophobic C_7-AHB). They were added at concentrations of 10^{-8}–10^{-3} M to DNA SC solutions that contained 25 μg/ml DNA.

The results of our measurements of the viscoelasticity of DNA SC solutions 15 min after the effects of C_6-AHB and C_1-AHB showed that both AHB homologues changed viscoelasticity values (Figs. 3a, 3b). However, the DNA SC isolated from exponentially growing and stationary-phase pseudomonad cells differed in their sensitivity to C_6-AHB and C_1-AHB. The more hydrophobic C_6-AHB produced a pronounced effect on the DNA SC from exponentially growing *P. aurantiaca* cells (Fig. 3a), whereas the less hydrophobic analogue C_1-AHB was more efficient with the DNA SC of stationary-phase cells (Fig. 3b). The concentration dependence of the effects of both homologues on DNA SC followed a similar pattern, but the sensitive developmental stages were different. The viscoelasticity of the DNA SC from exponentially growing cells decreased in a linear fashion with an increase in the C_6-AHB concentration within the range 10^{-6}–10^{-4} M, whereas an increase in the C_1-AHB concentration produced virtually no effect on it (Fig. 3a). The DNA SC viscoelasticity in stationary-phase cells decreased in a linear fashion with an increase in the C_1-AHB, not C_6-AHB, concentration (Fig. 3b). Importantly, the concentration dependence curves of the C_6-AHB effect on “exponential-phase” DNA SC (Fig. 3a) and of the C_1-AHB effect on “stationary-phase” DNA SC (Fig. 3b) both had a breaking point at a ligand concentration of 10^{-4} M. At this point, the alkylhydroxybenzene effect changed its direction: the viscoelasticity of both DNA SC preparations increased with a further increase in the AHB concentration. The breaking point corresponded to a maxi-

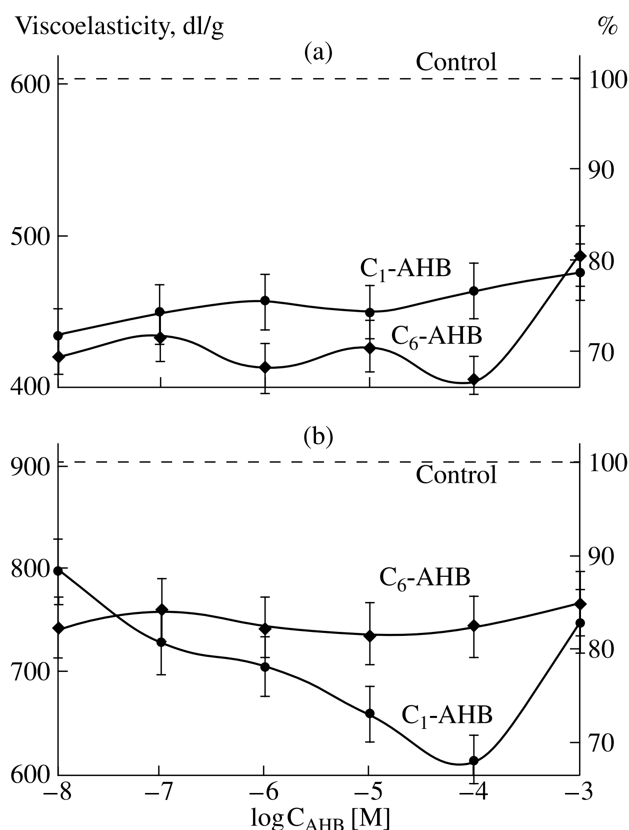

Fig. 3. Dependence of the viscoelasticity of DNA SC preparations from (a) exponential-phase and (b) stationary-phase cultures on C_6-AHB and C_1-AHB concentrations.

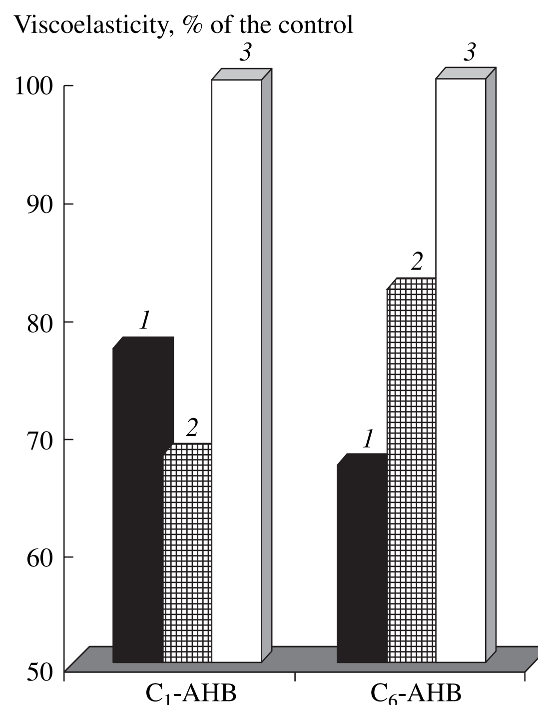

Fig. 4. Effects of 10^{-4} M C_1-AHB and C_6-AHB on the viscoelasticity of the DNA SC preparations isolated from (*1*) exponential-phase and (*2*) stationary-phase cells. 100% correspond to the viscoelasticity of DNA SC preparations (*3*) in control variants without AHB.

mum viscoelasticity decrease that was 33 and 32% in the DNA SC isolated from exponential-phase cells (with C_6-AHB) and stationary-phase cells (with C_1-AHB), respectively (Fig. 4). Hence, it was at an AHB concentration of 10^{-4} M that the contrasting patterns of the effects of the tested homologues on DNA SC were especially manifested in *in vitro* experiments. It was at this submillimolar concentration that C_6-AHB induced the formation of refractile anabiotic *P. aurantiacus* cells characterized by an unstable phenotype. Their inoculation yielded a population with a high dissociation degree, giving rise to variants that differed in terms of their morphology and colony structure.

DISCUSSION

The fact that analogues of microbial AHB with different hydrophobicity degrees exhibit different ligand efficiency when interacting with the DNA SC from exponential- or stationary-phase cells lends support to the above data on the changes in the DNA structural organization accompanying transitions from one physiological state to another. These changes can account for an increased or decreased DNA affinity for ligands characterized by different hydrophobicity (AHB or other chemical substances) and influence their association and reassociation, affecting the functional activity of the DNA in metabolically active or dormant cells. An important area of research concerning dormancy mechanisms deals with the peculiarities of the DNA structural organization in the cells of developing cultures assuming the dormant state or reversion to the active state. In vegetative bacterial cells, the DNA exists as a supramolecular complex and forms 12–80 topologically closed, superspiralized membrane-attached loops [19]. The DNA topology is an important factor involved in the regulation of transcription, translation, and recombination in bacteria [19]. High-molecular-weight DNA SC (3×10^8 Da) were isolated from *E. coli* and *B. subtilis* and found to contain minor amounts of tightly bound acidic histon-free proteins ($1 \pm 0.1\%$) and lipids ($0.9 \pm 0.08\%$). They are involved in the organization of superspiralized DNA loops and in their attachment to the membrane [11, 16]. It was shown that *E. coli* contains DNA-bound proteins that produce their effect on the nucleoid structure. The qualitative and quantitative composition of these proteins is conditional on the growth and developmental stage of the tested culture. Some of them are involved in replication, recombination, and transcription regulation, while other proteins (e.g., the nonspecific protein Dps) occur in the nucleoids of starving late-stationary-phase cells and enhance the DNA resistance to stress [17].

The differences in the structural organization of the nucleoid in metabolically active and dormant bacterial cells account for the different efficiency of the DNA SC interactions with ligands that differ in their hydrophobicity. A more challenging task is to explain the nonlinear pattern of the dependence of DNA SC viscoelasticity on AHB concentration. Increasing the C_6- or C_1-AHB concentration (with "exponential-" or "stationary-phase" DNA SC, respectively) resulted in a decrease in viscoelasticity, followed by an increase in it. A similar but more pronounced pattern of viscoelasticity changes was revealed in DNA preparations isolated from *E. coli* cells under the influence of ethidium bromide, an intercalating agent. At low concentrations, it produced a relaxing effect (an viscoelasticity decrease) that reached its maximum level. A further concentration increase lowered the retardation time (τ_0) value, i.e., increased the viscoelasticity, due to the winding of some DNA loops in the reverse direction [10]. As for our studies, the declining part of the plot (with a minimum at $C_{AHB} = 10^{-4}$ M) representing the dependence of "exponential-" and "stationary-phase" DNA SC viscoelasticity on C_6-AHB (Fig. 3a) and C_1-AHB (Fig. 3b) concentrations seemed to reflect a decrease in the DNA superspiralization degree, whereas the ascending part of the plot, conversely, corresponded to an increase in it. However, alkylresorcinols do not function as intercalating agents like ethidium bromide that is inserted between DNA bases, pushing them apart and modifying the local chain structure. C_6-AHB and C_1-AHB are hardly capable of electrostatic interactions of the kind that is peculiar to intercalating cations in their reaction with DNA, a polyanion. Possibly, alkylhydroxybenzenes (C_6-AHB and C_1-AHB) are located in DNA striae, interacting with phosphate groups and atoms of the bases by virtue of the hydrogen bonds formed by the hydroxy groups of their aromatic rings. This suggestion is consistent with the fact that the patterns of the concentration dependence of DNA SC viscoelasticity are similar in both tested homologues. The impact of the AHB hydrophobicity degree on its selective binding to the DNA SC from cells of various age groups was discussed above. As for long-chain alkylresorcinols, it was shown that the effects resulting from their binding to DNA vary depending on the alkyl radical length [8, 20]. It is a tempting idea to test the influence of microbial anabiosis autoinducers (AHB) on the DNA transition from the B form to the A form. This transition was revealed during the dehydration of DNA preparation in model studies. It also occurs in dormant bacterial forms (*Bacillus* endospores) [18].

It should be stressed that the DNA SC viscoelasticity changes caused by microbial anabiosis autoinducers (AHB) at concentrations inducing the transition of *P. aurantiaca* vegetative cells to the anabiotic state suggest the involvement of these autoregulators in the structural reorganization of the bacterial genome. This results in the phenotypic dissociation of bacteria that produce variants differing in their morphology and colony organization. The structural modification of the genome that influences its stability and the gene expression involved in the transition of bacteria to the dormant state and its maintenance can be due, at least in part, to DNA complexation with alkylhydroxybenzenes, whose concentration increases upon the formation of dormant forms and decreases upon their germination [1, 13]. Such DNA SC components as lipids and proteins can also exert their influence on these physical and chemical interactions. Therefore, comparative studies of DNA-bound lipids and proteins in microbial cells existing in various physiological states represent a promising area of future research that would significantly contribute to our understanding of the mechanisms involved in the interconversion of microbial dormant forms.

ACKNOWLEDGMENTS

We wish to thank G.K. Gerasimova from the Blokhin Oncology Research Center (Russian Academy of Medical Sciences) for her assistance in our studies. We are grateful the late Prof. S.G. Batrakov for valuable discussion. This work was supported by the Russian Foundation for Basic Research, project nos. 02-04-49150, 03-04-48403, and 04-04-49710; the Moscow Region Government, project no. 01-04-97013; and the A. von Humboldt-Stiftung, Bonn, Germany, project no. 1032332.

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