ISSN 0026-2617, Microbiology, 2006, Vol. 75, No. 5, pp. 575–581. © Pleiades Publishing, Inc., 2006.
Original Russian Text © O.K. Davydova, D.G. Deryabin, G. I. El'-Registan, 2006, published in Mikrobiologiya, 2006, Vol. 75, No. 5, pp. 662–669.

EXPERIMENTAL ARTICLES

Long-Term Preservation of DNA in Aqueous Solutions in the Presence of the Chemical Analogues of Microbial Autoregulators

O. K. Davydova[a], D. G. Deryabin[a, 1], and G. I. El'-Registan[b]

[a] *Orenburg State University, pr. Pobedy 13, Orenburg, 460018 Russia*
[b] *Vinogradskii Institute of Microbiology, Russian Academy of Sciences, pr. 60-letiya Oktyabrya 7, k. 2, Moscow, 117811 Russia*

Received December 12, 2005; in final form, April 23, 2006

Abstract—he fact of long-term preservation of the physicochemical properties of DNA molecules in aqueous solutions in complexes with methylresorcinol, hexylresorcinol, and tyrosol, the chemical analogues of microbial autoregulators (d_1 factors) from the group of alkylhydroxybenzenes (AOB), was established. Compared to the control variants of storage of aqueous DNA solutions, the AOB influence consisted in the sum of correlating effects: the prevention of DNA degradation (according to spectrophotometric parameters) and the preservation of its viscous characteristics and electrophoretic mobility. The initial DNA properties were preserved to the greatest degree in the presence of hexylresorcinol, the compound with the longest alkyl radical. Possible mechanisms of the protective action of alkylhydroxybenzenes in relation to DNA are discussed, namely, the prevention of its hydrolysis due to isolation from the aqueous environment and maintaining DNA stability in the dormant forms of microorganisms.

DOI: 10.1134/S0026261706050079

Key words: DNA, physicochemical properties of DNA, DNA stabilization, alkylhydroxybenzenes, d_1 factors of microorganisms.

The autoregulatory d_1 factors of microorganisms are present in a number of bacteria and yeasts by alkylhydroxybenzenes (AOB); these compounds show a capacity for physicochemical interactions with a wide range of (macro)molecules, thus producing a whole spectrum of biological effects. For instance, the interaction of AOB with membrane lipids results in an increase in membrane microviscosity to the point of lipid stroma polycrystallization [1, 2]. The formation of complexes of AOB with proteins results in an increase in their stability (the chaperon effect); in the case of the enzyme proteins, this is connected with a nonspecific decrease or increase in their enzymatic activity, depending on the hydrophobicity of AOB as ligands and their concentration [2–5]. The previously demonstrated capacity of AOB for interaction with DNA [2, 6] manifests itself in the development of both mutagenic [7] and antimutagenic effects [8], also depending on the structure of AOB.

At the cellular level, the most general and universal effect of AOB is an increase in the resistance of the subcellular structures interacting with them and of the cell as a whole to damaging environmental factors [9–11]. This effect is important and ecologically significant for the development of resistance of microorganisms to stress and for the transition of cells to the state of anabiosis during the formation of resting forms. One of the well-documented aspects of this effect of AOB is an increase in the stability of proteins and nucleic acids against high temperatures [5, 6, 11]; this finding agrees well with the notions of the high level of thermal resistance of microbial cystlike resting forms whose formation is induced by the high level of AOB in microbial cultures [12–15]. On the other hand, the stabilization of biopolymers and, especially, of nucleic acids must also result in the long-term preservation of their physicochemical properties under denaturing conditions and will, therefore, predetermine the retention of their functional activity. Earlier, we revealed the phenomenon of DNA stabilization in complexes with AOB [6].

The aim of this investigation was to study the duration of the "preserving" effect of alkylhydroxybenzenes on high-polymer DNA, which was assayed by the conservation of the physicochemical properties of this biopolymer in aqueous solutions.

[1] Corresponding author; e-mail: dgderyabin@yandex.ru

MATERIALS AND METHODS

Methylresorcinol (MR, molecular weight = 124) and hexylresorcinol (HR, molecular weight = 194) of 99.9% purity, as well as the structurally similar tyrosol (2-(4-hydroxyphenyl)ethane-1-ol, T, molecular weight = 138), which is an autoregulatory d_1 factor of the yeast *Saccharomyces cerevisiae* [17], were used as the chemical analogues of the d_1 factors of bacteria [2, 16]. The working concentrations of these substances in the solutions studied were 10^{-3}, 10^{-4}, and 10^{-5} M.

A commercial preparation of high-polymer linear DNA isolated from the bovine spleen and purified to the homogeneous state was used in the experiments. The DNA concentration in the solutions studied was 10^{-4} M (in terms of nucleotides). Its molar ratios with AOB in different samples were 1 : 10, 1 : 1, and 10 : 1. The incubation of DNA with AOB was carried out for eight weeks at 4°C. Individual DNA or AOB solutions at equimolar concentrations incubated under the same conditions were used as controls.

The spectral absorption characteristics of DNA, AOB, and the DNA + AOB mixtures were determined using a Fluorat-02 Panorama spectrofluorimeter (NPO Lumeks, Russia) in the range of 210–300 nm. For the quantitative assessment of the degree of DNA degradation in the time course of the experiment, the ratio of its absorption at 260 (maximum) and 240 nm was calculated using the formula (D_{260}/D_{240}). For the DNA + AOB mixtures, the formula $(D_{\text{mix}260} - D_{\text{AOB}260})/(D_{\text{mix}240} - D_{\text{AOB}240})$ was used, where $D_{\text{mix}260}$ and $D_{\text{mix}240}$ are the optical densities of the DNA + AOB mixture at 260 and 240 nm, and $D_{\text{AOB}260}$ and $D_{\text{AOB}240}$ are the optical densities of AOB at 260 and 240 nm.

The viscosity of intact DNA solutions, as well as of DNA in the presence of AOB, was measured with an Ostwald viscosimeter with a working capillary diameter of 0.86 mm by determining the relative viscosity value (η) from the t/t_0 ratio, where t is the solution efflux time; t_0 is the efflux time of distilled water.

Electrophoresis of the samples was carried out in 0.8% agarose gel in the presence of 0.5 μg/ml of ethidium bromide at the electrode voltage 150 V and the current 100 mA, so that 1 cm of gel accounted for 5 V/cm. DNA migration after 120 min of electrophoresis was assessed using a Vilber Lourmat transluminator (France) and the digital images acquired were processed using the Image J software package. The *Hin*dIII DNA restricts of phage λ (Sibenzim, Russia) were used as molecular mass markers, and the DNA samples studied were subdivided into relatively "long-stranded" and "short-stranded" fragments according to the results of comparison of their electrophoretic mobility with that of The 4361 bp marker (the fourth band from the start).

Scanning electron microscopy of the DNA + AOB samples on the silicon substrate was carried out using a JSM-20 electron microscope (JEOL, Japan) by scanning the same local area of the sample at different magnifications.

The experiments were performed in at least three repetitions and three series of experiments were staged. Statistical analysis was performed using the standard mathematical methods in the SPSS for Windows software package. The procedure of multiple correlation analysis was used for the integrated assessment of the characteristics describing the preservation of the DNA physicochemical properties in the presence of AOB.

RESULTS AND DISCUSSION

The concepts of DNA degradation in aqueous solutions developing in time, usually linked to spontaneously proceeding energy-independent hydrolysis of phosphodiester bonds in the sugar–phosphate framework of this polymer, formed the basis of this research [18]. The gradual accumulation of such single-stranded breaks at a distance of several nucleotide pairs from each other results in the degradation of high-polymer DNA into more short-stranded fragments. The pattern of a change in the DNA physicochemical properties in this case consists of the progressive loss of its inherent optical parameters, a decrease in viscosity, and an increase in electrophoretic mobility.

Thus, when we analyzed the optical properties of individual DNA solutions (control), calculating the ratios of absorption at the maximum (260 nm) and in the short-wave arm of the spectrum (240 nm), the D_{260}/D_{240} value was 1.85 in the first week of storage. In the process of DNA incubation in aqueous solutions, the values of this ratio decreased to 1.68 at four weeks and subsequently decreased progressively to less than 1.0 at eight weeks (Fig. 1a).

The analysis of the results of capillary viscosimetry also gave evidence of the degradation of individual DNA progressing over time, indicated by the decrease in the relative viscosity values of its aqueous solutions from 10.13 in the first week of incubation to 6.0 at four weeks and to 3.1 at eight weeks of incubation (Fig. 1b). The conversion of the results of viscosimetry at the time indicated above to the molecular mass values [19] showed a decrease in the average length of the fragments of this polymer from 12011 to 5446 nucleotide pairs (bp) and subsequently to 2017 bp, which is fully consistent with the notions of the mechanisms of its degradation described above.

The results of electrophoretic behavior of DNA in agarose gel correlated with the optical and viscosimetric manifestations of its degradation. An increase in the electrophoretic DNA mobility (U_{eph}) was observed in the electrophoregrams, which indicated a decrease in the relative content of the long-stranded fragments in the sample with a simultaneous increase in the short-stranded ones (Fig. 2). The average calculated length of the DNA fragments decreased progressively from 11370 bp at the beginning of the experiment to 9300 bp at four weeks and to 8123 bp at eight weeks of incubation, which is further proof of this mechanism of

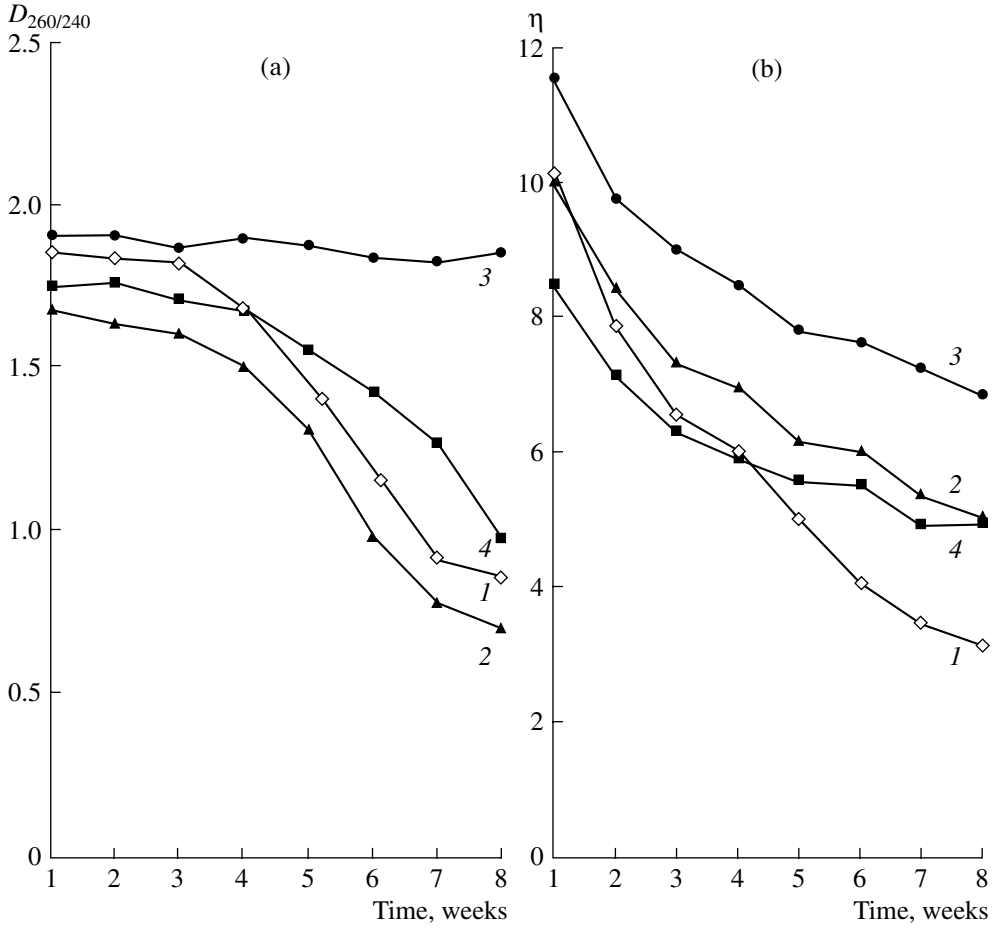

Fig. 1. Dynamics of changes in the (a) optical and (b) viscous properties of (*1*) DNA, (*2*) DNA + MR, (*3*) DNA + HR, and (*4*) DNA + T at a molar ratio of DNA : AOB = 1 : 10.

biopolymer degradation due to the appearance of single- and double-stranded breaks in its molecule.

Against this background, the conservation of the physicochemical DNA properties in the presence of alkylhydroxybenzenes, which was characterized by us as a "preserving" effect of AOB, is fundamentally important. Both the distinctions in the efficacy of the action of MR, HR, and T (resulting from the differences in their chemical structure), and the concentration dependencies of their effects were established. HR and T revealed the most marked stabilizing effect when their DNA : AOB molar ratio was 1 : 10, whereas MR was the most efficacious in the DNA : MR = 1 : 1 variants (table, Fig. 1a).

When studying the optical properties of the DNA + HR/T solutions (at the 1 : 10 molar ratio), we found that the calculated $(D_{mix260} - D_{AOB260})/(D_{mix240} - D_{AOB240})$ value in the variant with HR virtually did not change (at the beginning of the experiment, 1.9; after eight weeks of storage, 1.85). In the variant with tyrosol, this parameter, although it did change, displayed a less pronounced tendency to decrease (at the beginning of the experiment, 1.75; after eight weeks of storage, 0.97) compared to the optical characteristics of individual DNA solutions in the control variants (at the beginning of the experiment, 1.85; after eight weeks, 0.85). With a decrease in the concentration of HR/T (the DNA : AOB molar ratio = 1 : 1), the calculated $(D_{mix260} - D_{AOB260})/(D_{mix240} - D_{AOB240})$ value in the DNA + HR variant decreased from 2.05 to 1.62 after eight weeks of incubation. However, the stabilizing effect of HR was retained, whereas in the variants with T, this parameter changed in a way similar to the control.

When DNA was treated with methylresorcinol, we observed the reverse picture. In the DNA : MR = 1 : 1 variants, the stabilization effect was evident: while at the beginning of the experiment the $(D_{mix260} - D_{AOB260})/(D_{mix240} - D_{AOB240})$ value was 1.89, after eight weeks of incubation it was close to the initial value, 1.73. However, in the DNA solutions with the DNA : MR = 1 : 10, i.e., when the AOB concentration increased, the effect was opposite to that observed in solutions with HR, i.e., the $(D_{mix260} - D_{AOB260})/(D_{mix240} - D_{AOB240})$ value sharply decreased to 0.7.

Thus, the "preserving" effect of AOB not only depended on the structure of AOB, but different AOB

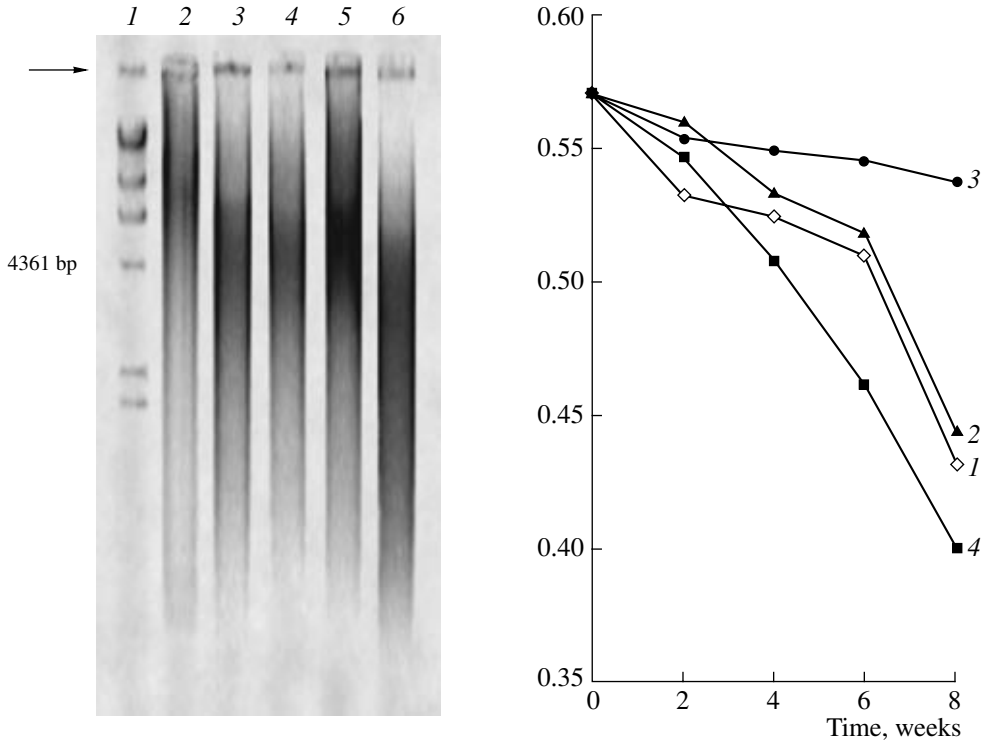

Fig. 2. Electrophoretic mobility of individual DNA and DNA in complexes with MR, HR, and T (AOB : DNA = 1 : 1). Left—the results of electrophoresis; (*1*) DNA molecular mass markers (λ/*Hin*dIII); (*2*) DNA at one week of incubation; (*3–6*) samples after eight weeks of incubation; (*3*) DNA; (*4*) DNA + MR; (*5*) DNA + HR; (*6*) DNA + T. The arrow marks the start. Right: the dynamics of a decrease in the share (from 1.0) of long-stranded fragments (4362 bp) depending on the incubation time of the DNA + AOB complexes: (*1*) DNA; (*2*) DNA + MR; (*3*) DNA + HR; (*4*) DNA + T.

exhibited specific concentration dependencies. In the variants with the most efficacious analogue hexylresorcinol, the stabilizing effect increased with an increase in the AOB : DNA molar ratios (table).

The results of capillary viscosimetry indicating a less marked decrease in the viscosity of DNA solutions in the presence of AOB agreed to a significant degree with the data shown above (Fig. 1b). In these experiments, the extent of the "preserving" effect also depended on the dose and the specific features of the chemical structure of alkylhydroxybenzenes; in this respect HR also displayed the highest activity. Thus, after eight weeks of incubation of the DNA + HR solutions, their relative viscosity value in the DNA : AOB =

A change in the optical, viscous, and electrophoretic DNA properties during long-term incubation with AOB

Experimental variant	DNA : AOB ratio, M : M	D_{260}/D_{240}			η			U_{eph}		
		week 1	week 4	week 8	week 1	week 4	week 8	week 1	week 4	week 8
DNA		1.85	1.68	0.85	10.13	6.00	3.10	57	52	43
DNA + MR	10 : 1	1.80	1.18	1.33	10.10	6.07	3.21	57	52	43
	1 : 1	1.89	1.64	1.73	9.99	6.92	4.91	57	53	44
	1 : 10	1.67	1.50	0.70	10.20	6.87	5.01	56	53	46
DNA + HR	10 : 1	1.83	1.75	1.48	10.27	6.30	3.22	57	52	44
	1 : 1	2.05	1.88	1.62	11.52	8.48	6.81	57	55	54
	1 : 10	1.90	1.89	1.85	11.61	8.88	7.03	58	56	56
DNA + T	10 : 1	2.18	1.73	0.61	10.10	5.90	3.00	58	52	42
	1 : 1	1.88	1.76	0.85	8.46	5.88	4.91	57	51	40
	1 : 10	1.75	1.67	0.97	9.51	6.11	4.80	58	50	39

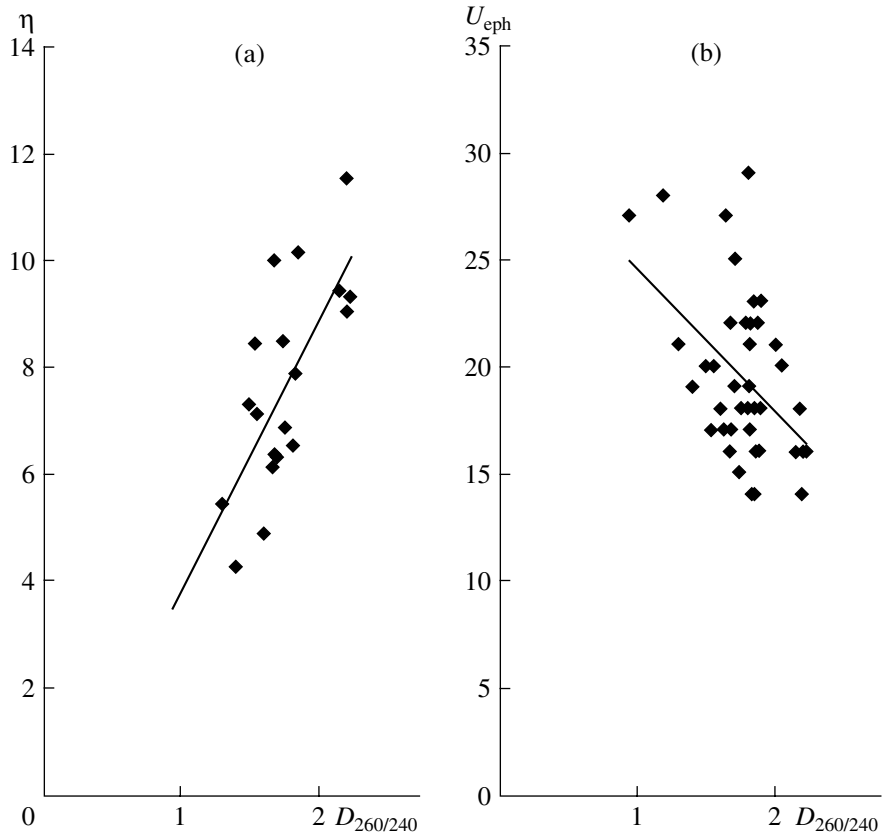

Fig. 3. Correlation between (a) the optical and viscous properties and (b) the optical properties and electrophoretic mobility of DNA + HR in aqueous solutions.

1 : 1 and DNA : AOB = 1 : 10 variants was 6.81 and 7.03, respectively. This corresponded to a 6588 bp average length of the DNA fragments in solutions and exceeded more than threefold the 2017 bp value determined for the control DNA solutions. In the presence of MR and T, this effect was less pronounced and manifested itself only when their high concentrations were used (table). The results obtained agree with the data on the influence of different AOB homologues (HR and MR) on changes in the elasticity and viscosity of the supramolecular DNA complexes isolated from the cells of *Pseudomonas aurantiaca* of different physiological age [20].

The results of electrophoresis of the DNA + AOB mixtures confirmed the existence of the "preserving" effect of alkylhydroxybenzenes, but only for hexylresorcinol. Its action resulted in preventing the high-polymer DNA molecules from disintegrating into more short-stranded fragments with a higher electrophoretic mobility (Fig. 2). Thus, while in the control DNA solution, the share of long-stranded fragments with a molecular mass of more than 4361 bp progressively decreased from 57 to 43% over eight weeks of incubation, in the presence of HR (DNA : MR = 1 : 1), significant changes were not observed; in this variant, long-stranded fragments predominated. When MR or T were used, electrophoretic revealed no significant evidence of prevention of DNA degradation.

Thus, the sum of the previously obtained results allows us to confirm the existence of a unique "preserving" effect of AOB on DNA stored for prolonged time in aqueous solutions. This effect is revealed when the process of degradation of DNA, existing in aqueous solutions in complexes with AOB, is studied, during prolonged storage, in terms of optical characteristics, the retention of viscous properties, and electrophoretic mobility. It manifests itself in the relative stability of these parameters compared to their change in the control solutions of individual DNA. Among the AOB studied, the stabilizing effects are most pronounced for HR, which agrees with the previously reported activity of HR as an anabiosis autoinducer [12–15].

Analyzing the results of the study of the optical, viscous, and electrophoretic properties of DNA in complexes with AOB, it is necessary to point out that we observed not a number of independent structural changes affecting the macromolecular properties mentioned, but a unified process of DNA stabilization (prevention of degradation) in aqueous solutions characterized by the three parameters used. The high level of correlation between them proves this assumption. Figure 3 illustrates the direct relationship between the optical

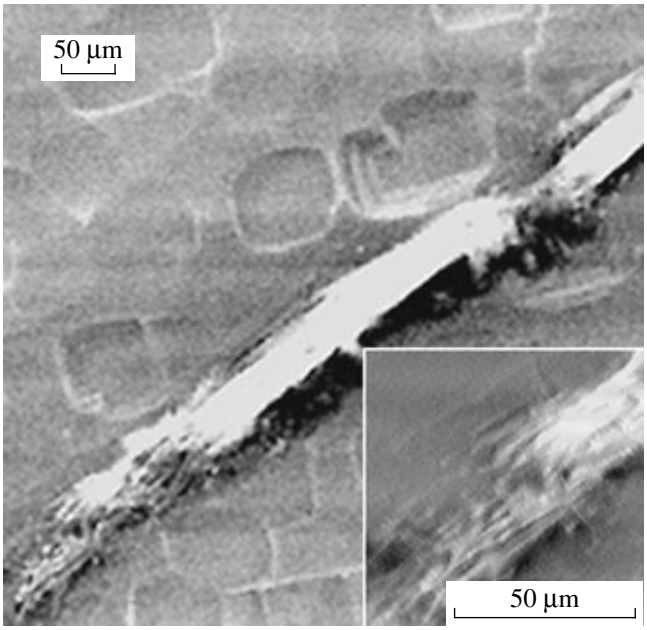

Fig. 4. Results of the scanning electron microscopy of the DNA + HR complex. Insert: the egress of the DNA strands from the common "cable" structure.

and viscous properties of the DNA + HR solutions (r = 0.463; $P < 0.001$), as well as the inverse correlation between the conservation of the optical properties of DNA and the characteristics of the electrophoretic mobility of its constituent fragments (r = –0.435; $P <$ 0.01).

Discussing the possible mechanisms of the formation of the AOB "preserving" effect, we should draw attention to its proportionality to the alkyl radical length in different AOB (this radical forms the hydrophobic pole of these molecules), and to the marked concentration dependence of the action of AOB. In this context, there is reason to suggest that in the development of these effects, not only direct contacts between AOB and DNA but also the surface -AOB-AOB-AOB- interactions leading to the formation of micelle-like structures on this polymer play an important part. During long-term incubation (eight weeks), they aggregate and merge to form alkylhydroxybenzene envelopes around the orderly arranged DNA molecules. Their study by scanning electron microscopy revealed extended "cable" structures several dozens to hundreds of μm thick, consisting of a number of in parallel-oriented DNA strands united by a common alkylresorcinol "sheath." Microscopy of the terminal ends of these structures revealed the patterns of untwisting DNA strands, each of which retained its individuality (Fig. 4). The isolation thus achieved of the DNA molecule from the aqueous surroundings may be one of the causes (possibly the main one) preventing the hydrolysis of the sugar–phosphate framework of this biopolymer during its long-term storage in aqueous solutions.

This conclusion is consistent with the data on the substitution of membrane phospholipids with long-stranded alkylhydroxybenzenes (alkylresorcinols) in azotobacter cysts. They form the alkylresorcinol membrane, which surrounds the protoplast of a resting cell, thus contributing to the maintenance of the ametabolism (anabiosis) state and to an increase in resistance to damaging effects in the resting forms of this type [21].

On the whole, the results obtained agree well with the notions of the integral results of the biological action of AOB on bacterial and yeast cells resulting in the formation of cystlike resting forms, which are metabolically inert, resistant to the effects of external factors, and long-lived [12–15]. In the process, the conservation of DNA with the involvement of microbial AOB seems to be one of the indispensable conditions for the transition of a cell to a resting state.

ACKNOWLEDGMENTS

This work was supported the Russian Ministry of Education, project no. A03-2.12-437 for research works of postgraduate students of higher educational institutions, and the Russian Foundation for Basic Research, project no. 04-04-49710.

REFERENCES

1. Kaprel'yants, A.S., Suleimenov, M.K., Sorokina, A.D., Deborin, G.A., El'-Registan, G.I., Stoyanovich, F.M., Lille, Yu.E., and Ostrovskii, D.N., Structural and Functional Changes in Bacterial and Model Membranes Caused by Phenolic Lipids, *Biol. Membr.,* 1987, vol. 4, pp. 254–267.
2. Kozubek, A. and Tyman, J.H.P., Resorcinolic Lipids, the Natural Non-Isoprenoid Phenolic Amphiphiles and Their Biological Activity, *Chem. Rev.,* 1999, vol. 99, no. 1, pp. 1–31.
3. Bespalov, M.M., Kolpakov, A.I., Loiko, N.G., Doroshenko, E.V., Mulyukin, A.L., Kozlova, A.N., Varlamova, E.A., Kurganov, B.I., and El'-Registan, G.I., The Role of Microbial Dormancy Autoinducers in Metabolism Blockade, *Mikrobiologiya,* 2000, vol. 69, no. 2, pp. 217–223 [*Microbiology* (Engl. Transl.), vol. 69, no. 2, pp.174–179].
4. Kolpakov, A.I., Il'inskaya, O.N., Bespalov, M.M., Kupriyanova-Ashina, F.G., Gal'chenko, V.F., Kurganov, B.I., and El'-Registan, G.I., Stabilization of Enzymes by Dormancy Autoinducers as a Possible Mechanism of Resistance of Resting Microbial Forms, *Mikrobiologiya,* 2000, vol. 69, no. 2, pp. 224–230 [*Microbiology* (Engl. Transl.), vol. 69, no. 2, pp.180–185].
5. Martirosova, E.I., Karpekina, T.A., and El'-Registan, G.I., Enzyme Modification by Natural Chemical Chaperons of Microorganisms, *Mikrobiologiya,* 2004, vol. 73, no. 5, pp. 708–715 [*Microbiology* (Engl. Transl.), vol. 73, no. 5, pp.609–615].

6. Davydova, O.K., Deryabin, D.G., Nikiyan, A.N., and El'-Registan, G.I., Mechanisms of Interaction between DNA and Chemical Analogues of Microbial Anabiosis Autoinducers, *Mikrobiologiya,* 2005, vol. 74, no. 5, pp. 614–625 [*Microbiology* (Engl. Transl.), vol. 74, no. 5, pp. 533–541].

7. Il'inskaya, O.N., Kolpakov, A.I., Zelenikhin, P.V., Kruglova, Z.F., Choidash, B., Doroshenko, E.V., Mulyukin, A.L., and El'-Registan, G.I., The Effect of Anabiosis Autoinducers on the Bacterial Genome, *Mikrobiologiya,* 2002, vol. 71, no. 2, pp. 194–199 [*Microbiology* (Engl. Transl.), vol. 71, no. 2, pp.164–168].

8. Gasiorowski, K., Szyba, K., Brokos, B., and Kozubek, A., Antimutagenic Activity of Alkylresorcinols from Cereal Grains, *Cancer Letters,* 1996, vol. 106, pp. 109–115.

9. Il'inskaya, O.N., Kolpakov, A.I., Shmidt, M.A., Doroshenko, E.V., Mulyukin, A.L., and El'-Registan, G.I., The Role of Bacterial Growth Autoregulators (Alkyl Hydroxybenzenes) in the Response of Staphylococci to Stresses, *Mikrobiologiya,* 2002, vol. 71, no. 1, pp. 23–29 [*Microbiology* (Engl. Transl.), vol. 71, no. 1, pp.18–24].

10. Stepanenko, I.Yu., Mulyukin, A.L., Kozlova, A.N., Nikolaev, Yu.A., and El'-Registan, G.I., The Role of Alkylhydroxybenzenes in the Adaptation of *Micrococcus luteus to Heat Shock*, 2005, vol. 74, no. 1, pp. 26–33 [*Microbiology* (Engl. Transl.), vol. 74, no. 1, pp. 20–26].

11. El-Registan, G.I., Mulyukin, A.L., Nikolaev, Yu.A., Stepaneno, I.Yu., Kozlova, A.N., Martirosova, E.I., Shanenko, E.F., Strakhovskaya, M.G., and Revina, A.A., The Role of Microbial Low-Molecular-Weight Autoregulatory Factors (Alkylhydroxybenzenes) in Resistance of Microorganisms To Radiation and Heat Shock, *J.Adv. Space Res,* 2005, vol. 36, no. 9, pp. 1718–1728.

12. Mulyukin, A.L., Lusta, K.A., Gryaznova, M.N., Kozlova, A.N., Duzha, M.V., Duda, V.I., and El'-Registan, G.I., Formation of Resting Cells by *Bacillus cereus and Micrococcus luteus, Mikrobiologiya*, 1996, vol. 65, no. 6, pp. 782–789 [*Microbiology* (Engl. Transl.), vol. 65, no. 6, pp. 683–689].

13. Demkina, E.V., Soina, V.S., El'-Registan, G.I., and Zvyagintsev, D.G., Reproductive Resting Forms of *Arthrobacter globiformis, Mikrobiologiya*, 2000, vol. 69, no. 3, pp. 377–382, [*Microbiology* (Engl. Transl.) vol. 69, no. 3, pp. 309–313].

14. Loiko, N.G., Soina, V.S., Sorokin, D.Yu., Mityushina, L.L., and El'-Registan, G.I., Production of Resting Forms by the Gram-Negative Chemolithoautotrophic Bacteria *Thioalkalivibrio versutus and Thioalkalimicrobium aerophilum, Mikrobiologiya*, 2003, vol. 72, no. 3, pp. 328–337 [*Microbiology* (Engl. Transl.) vol. 72, no. 3, pp. 285–294].

15. Mulyukin, A.L, Soina, V.S, Demkina, E.V, Kozlova, A.N., Suzina, N.E, Dmitriev, V.V, Duda, V.I, and El-Registan, G.I, Formation of Resting Cells by Non-Spore-Froming Microorganisms as a Strategy of Long-Term Survival in the Environment, Hoover, R.B., Rozanov, A.Yu., and Lipps, J.H., Eds., *Proceedings of SPIE. V. 4939. Instruments, Methods, and Missions for Astrobiology VI*, Bellingham: SPIE, 2003, pp. 208–218.

16. Osipov, G.A., El'-Registan, G.I., Svetlichnyi, V.A., Kozlova, A.N., Duda, V.I, Kaprel'yants, A.S., and Pomazanov, V.V., On the Chemical Nature of the d Regulatory Factor of *Pseudomonas carboxydoflava, Mikrobiologiya*, 1985, vol. 54, no. 2, pp. 186–190.

17. Batrakov, S.G., El'-Registan, G.I., Pridachina, N.N., Nenashev, V.A., Kozlova, A.N., Gryaznova, M.N., and Zolotareva, I.N., Tyrosol, the d_1 Autoregulatory Factor of *Saccharomyces cerevisiae* yeasts, *Mikrobiologiya,* 1993, vol. 62, no. 4, pp. 633–638.

18. Shishniashvili, D.M., Lystsov, V.N., and Moshkovskii, Yu.Sh., Changes in the DNA Secondary Structure Caused by Palladium Ions, in *Konformatsionnye izmeneniya biopolimerov v rastvorakh* (Conformational Changes of Biopolymers in Solutions), Moscow: Nauka, 1973, p. 207.

19. Ashmarin, I.P., *Molekulyarnaya biologiya, izbrannye razdely* (Molecular Biology, Selected Topics), Moscow: Meditsina, 1974.

20. Mulyukin, A.L., Vakhrushev, M.A., Strazhevskaya, N.B., Shmyrina, A.S., Zhdanov, R.I., Suzina, N.E., Duda, V.I., Kozlova, A.N., and El'-Registan, G.I., Effect of Alkylhydroxybenzenes, Microbial Anabiosis Inducers, on the Structural Organization of *Pseudomonas aurantiaca* DNA and on the Induction of Phenotypic Dissociation, *Mikrobiologiya,* 2005, vol. 74, no. 2, pp. 157–165 [*Microbiology* (Engl. Transl.) vol. 74, no. 2, pp. 128–135].

21. Reusch, R.N. and Sadoff, H.L., Novel Lipid Components of the *Azotobacter vinelandii* Cyst Membrane, *Nature,* 1983, vol. 302, no. 5905, pp. 268–270.