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EXPERIMENTAL ARTICLES

IR Spectroscopic Research on the Impact of Chemical Analogues of Autoregulatory d_1 Factors of Microorganisms on Structural Changes in DNA

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Abstract—Using IR spectroscopy, we investigated the impact of chemical analogues of autoregulatory d_1 factors of microorganisms (methylresorcinol, hexylresorcinol, and tyrosol) on the conformational changes in DNA in films upon altering (decreasing) the relative humidity. We analyzed the appearance/disappearance of characteristic absorption bands of A and B DNA forms and determined D_{1088}/D_{1224}, the ratio between the band intensities of symmetrical and asymmetrical oscillations in their phosphate groups. The data obtained suggest the slowing down of the B ⟶ A structural transition in DNA in the presence of methylresorcinol and its speeding up in the presence of tyrosol. We discuss the mechanisms of this phenomenon in relation to the chemical composition of d_1 factors and their biological function.

Key words: DNA conformational state, microbial autoregulatory d_1 factors, alkylresorcinols, IR spectroscopy.

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Autoregulatory d_1 factors of alkylresorcinol (AR) nature, which cause the transition of microorganisms to an anabiotic state [1], perform the functions of natural structural modifiers of cellular biopolymers and supramolecular structures due to their capacity to form complexes with membrane lipids [1, 2], enzyme proteins [1, 3, 4], and DNA [5]. The effects caused by AR–DNA interactions include mutagenesis and antimutagenesis [6, 7], induction of phenotypic dissociation [8], and changes in the elastoviscosity of DNA supramolecular complexes and in the nucleoid ultrastructure [9].

Several models have been developed to explain the mechanisms of interactions between d_1 factors (alkylresorcinols) and DNA. The formation of hydrogen bonds between the hydroxyl groups of AR aromatic rings and the DNA phosphate groups, and the participation of hydrophobic interactions in this process have been assumed, as well as the interactions between AR molecules, which result in a specific arrangement of the amphiphylic d_1 factor molecules around the DNA [5, 10].

However, the effects of d_1 factors on the conformational state of the DNA that are essential for its biological activity and resistance to deleterious environmental factors [11] have not been elucidated yet. Nevertheless, changes in the structural organization of the DNA molecule and its transition from the B form to the A form are involved in the formation of endospores in bacilli and their anabiotic state [9, 11].

Therefore, the goal of this work was to investigate the effects caused by the interactions between chemical analogues of autoregulatory d_1 factors and DNA using IR spectroscopy that provides information concerning the conformational state of the DNA.

MATERIALS AND METHODS

The Na^+ salt of highly polymeric DNA (Na^+ content 9.5% of dry weight) isolated from salmon milt roe (ISN) was used in the present work. The DNA was dissolved in distilled water at 4°C for one day in order to obtain a homogenous solution. The DNA concentration was determined spectrophotometrically at 260 nm using the molar extinction coefficient (ε_{260}) of 6600 cm^{-1} M^{-1} [12, 13]. A solution with a DNA concentration of 1.5×10^{-2} M was prepared. The solution was characterized by a D_{260}/D_{280} ratio of 1.8, which testified to a high purification degree of the DNA in terms of residual protein concentration [14]. According to the results of electrophoresis in 0.8% agarose gel, the tested DNA preparation contained a pool of irregular fragments with sizes of 200 to 25000 bp.

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We used the following alkylresorcinols (AR) as chemical analogues of bacterial autoregulatory d_1 factors: methylresorcinol (MR, MM 124), hexylresorcinol (HR, MM 194), and tyrosol (2-(4-hydroxyphenyl)ethane-1-ol, T, MM 138). The purification degree of these substances was 99.9% (Sigma), and their chemical structure is depicted in Fig. 1. The substances were initially dissolved in aqueous (5%) ethanol and subsequently added to the DNA solutions at a molar ratio of 1 : 10. The final AR concentration was, therefore, 1.5×10^{-3} M. We selected the AR concentration at which the AR absorption bands in the IR range made no contribution to the total DNA–AR spectrum.

The samples for IR spectroscopy (300 μl) were placed on ZnSe supports and thereupon dehydrated stepwise at the relative humidity (r.h.) levels generated over the following saturated salt solutions: KNO_3 (r.h. 95%), KCl (r.h. 85%), NaCl (r.h. 75%), $NaNO_2$ (r.h. 66%), and $Mg(NO_3)_2$ (r.h. 56%). After the required r.h. value was established in the tested films (by incubating them for two weeks), the samples were placed in sealed polyethylene sacks that prevented r.h. changes during the measurements [12].

IR spectra were recorded by means of an Infra-LUM FT-02 IR Furier spectrometer (Lumex R & D Company, Russia) within the 800–1800 cm^{-1} range with a resolution of 1 cm^{-1}. The data were normalized using the 1220–1240 cm^{-1} internal standard band [12, 15].

RESULTS

The intensity changes of the individual absorption maximums and shifts in their frequencies [14–19] were analyzed in the IR spectra of control and experimental (AR-treated) samples of the DNA films. The interpretation of these data is presented in the table.

The IR spectrum of the sodium salt of salmon milt roe DNA (the control sample) at 95% relative humidity displayed the pattern corresponding to the B conformation of the DNA molecule (Fig. 2). This conclusion was based on (i) the characteristic bands at 836, 895, and 936 cm^{-1} that are qualitative markers of the B form of DNA (Table), and (ii) the ratio of the intensities of the 1224 and 1088 cm^{-1} bands that vary depending on the antisymmetrical and symmetrical oscillations in the phosphate groups (PO_2^-) involved. Interestingly, the latter parameter allows quantitative determination of the hydration degree of the sugar–phosphate backbone of the tested biopolymer and is directly influenced by the interconversion of the B and A DNA forms [16, 17]. For instance, at 95% relative humidity, the tested control DNA samples were characterized by a D_{1088}/D_{1224} ratio of 1.52, the typical value of the B form of DNA [20]. A stepwise decrease in relative humidity from 95% to 56% resulted in characteristic changes in the IR spectra of the control sample, including elimination of the B form-specific absorption bands (Fig. 2) with concomitant formation of new bands at 864 and 1185 cm^{-1} that started at 75% relative humidity (Fig. 2). These bands are indicative of the A form of DNA. The changes in the relative intensity of the A and B forms of DNA marker bonds depending on relative humidity are presented on Fig. 3. These changes were accompanied by changes in the ratio between the intensity of the bands caused by symmetrical and antisymmetrical oscillations in the phosphate groups. A decrease in relative humidity resulted in a significant shift in band position (from 1224 to 1242 cm^{-1}), and the intensity ratio increased to 1.74 (Fig. 4), which also indicated the transition from the B to the A DNA form [19, 20]. Upon a considerable decline in relative humidity, the IR DNA spectra displayed a 899–878–864 cm^{-1} triplet with a characteristic shape and a manifest 808 cm^{-1} band, features that are typical of the A form of DNA (table).

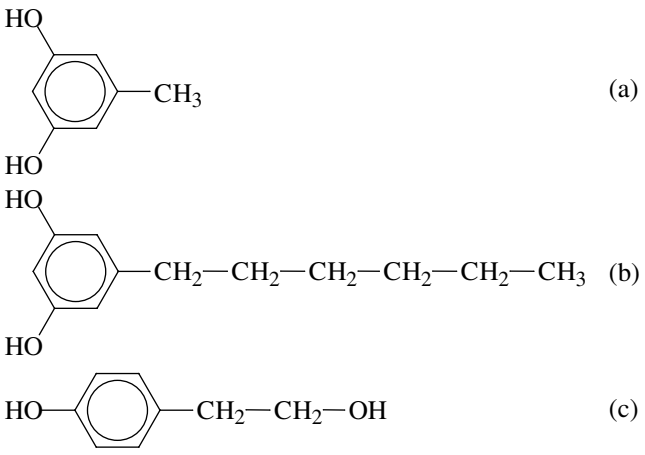

Fig. 1. Chemical formulas of the tested autoregulatory d_1 factors: (a) methylresorcinol; (b) hexylresorcinol; (c) tyrosol.

On the subject of the DNA structural changes caused by the changes in relative humidity, we should also point out that the DNA remained double-stranded. This conclusion is supported by the complete retention of the band with the highest frequency (1712 cm^{-1}) within the range corresponding to the double bonds of nitrogen bases resulting from the oscillations in the guanine carbonyl group and in the thymine C=O group in stacked complementary base pairs [21]. Accordingly, we failed to detect the band at 1692 cm^{-1}, caused by DNA unwinding, that results in separation of the stacked bases and their being embedded in the aqueous microenvironment [22] (data not shown).

In experimental samples, we dehydrated DNA–alkylresorcinol complexes. The changes in the IR spectra of the complexes with methylresorcinol were analogous to those in the spectra of control samples, although less pronounced (Fig. 2). First, the disappearance of characteristic B form bands with maxima at 836, 895, and 936 cm^{-1} from the DNA–MR sample

Main Bands in the DNA IR Spectrum (800–1350 cm^{-1})

IR Maximum, cm^{-1}	Interpretation	Source
808	Characteristic of the A form of DNA	[17]
836	Oscillations in the deoxyribose sugar ring. Characteristic of the B form of DNA	[18, 19]
865–878–899	Characteristic of the A form of DNA	[17, 15]
895	Oscillations in the deoxyribose sugar ring. Characteristic of the B form of DNA	[18, 19], [15]
936	Oscillations in the deoxyribose sugar ring. Characteristic of the B form of DNA	[18]
958–970–978	Characteristic of the A form of DNA	[17]
968	C–C oscillations in deoxyribose	[18, 19]
1021	Oscillations in the sugar ring	[18]
1053	Valence-related oscillations in the deoxyribose C–O bond	[18, 19]
1088	Symmetrical oscillations in the phosphate group	[18, 19]
1185	Oscillations in deoxyribose. Characteristic of the A form of DNA	[15]
1224	Antisymmetrical oscillations in the phosphate group	[19, 15]
1328	Oscillations in the thymine. Characteristic of the B form of DNA	[15]

occurred at 56% relative humidity, whereas analogous changes in the IR spectra of the DNA of the control samples were observed already at 75% relative humidity. Second, the data presented in Fig. 3 demonstrate that decreasing r.h. results in a decrease in the intensity of the D_{936} maximum of the B form in the control DNA spectrum, and it disappears at a humidity level of 66% ($D_{936}/D_{926} < 1$). Complexation of DNA with methylresorcinol significantly retarded the elimination of this maximum, and the D_{936}/D_{926} ratio exceeded unity at all tested r.h. levels.

With methylresorcinol, changes in the ratios between the intensities of symmetrical and antisymmetrical oscillations in DNA phosphate groups are less significant at all tested r.h. values. These ratios remain within the 1.38–1.57 range that is characteristic of the B conformation of this biopolymer (Fig. 4). A considerably decreased shift in the antisymmetrical oscillations of phosphate groups (from 1226 to 1233 cm^{-1}), in contrast to the shift in native control DNA (from 1224 to 1242 cm^{-1}), is additional evidence that the DNA hydration degree remains high (Fig. 2).

The IR spectra of DNA–tyrosol complexes yielded substantially different results. Decreasing the r.h. level caused a rapid disappearance of the characteristic bands of the B form with concomitant formation of the bands with maxima at 864 and 1185 cm^{-1} (Fig. 2). The process of the formation of the 1185 cm^{-1} band that is indicative of the A form and is quantitatively characterized by the D_{1185}/D_{1195} ratio values [20] was analyzed. It was revealed that this value exceeds unity only at an r.h. level of 56% in native DNA; however, this occurs at an r.h. level of 75% in the presence of tyrosol (Fig. 3b). Additional changes in the IR spectra of the DNA–tyrosol complex manifested themselves in a marked decrease in the intensity of the 968 cm^{-1}, 1021 cm^{-1}, and 1053 cm^{-1} bands of the oscillating deoxyribose ring and in a significant shift of the band reflecting antisymmetrical phosphate group oscillations (from 1229 to 1242 cm^{-1}, Fig. 2). The ratios of the intensity of symmetrical and antisymmetrical oscillations in DNA phosphate groups with tyrosol already exceeded those in native DNA samples at an r.h. value of 95% (Fig. 4). The difference was statistically significant, and it gradually increased in the course of film dehydration, reaching the 1.8 level (typical of the DNA A form [23]) at an r.h. of 66%.

The influence of hexylresorcinol on the conformational changes in the DNA molecule was relatively insignificant against this background. These changes generally resembled those occurring in a native DNA molecule (control samples) during a gradual r.h. decrease. The differences documented by us suggested that the B ⟶ A transition of DNA conformers is to some extent stimulated in DNA–HR complexes if the r.h. level exceeds 66% (Fig. 4).

In conclusion, it should be noted that no significant shift of the 1712 cm^{-1} band was detected with all the above chemical analogues of microbial d_1 factors and at all tested r.h. values. This suggests that the DNA remains a double-stranded molecule despite the impact of factors that predominantly affect the hydration degree of its sugar–phosphate backbone.

DISCUSSION

The DNA conformation determines the biological activity of the macromolecule in vivo. As a rule, the DNA is in the B form in vegetative cells. It converts into the A conformation in the endospores of bacilli during the development of their dormant state. The A conformation is characterized by an enhanced resistance to a number of deleterious factors [11]. One strategy that apparently can enable us to control the functional state of bacterial cells is to search for substances that can regulate the intensity of B⟶ A transitions.

The main factor determining the DNA conformation (the choice between the A and the B form) is believed

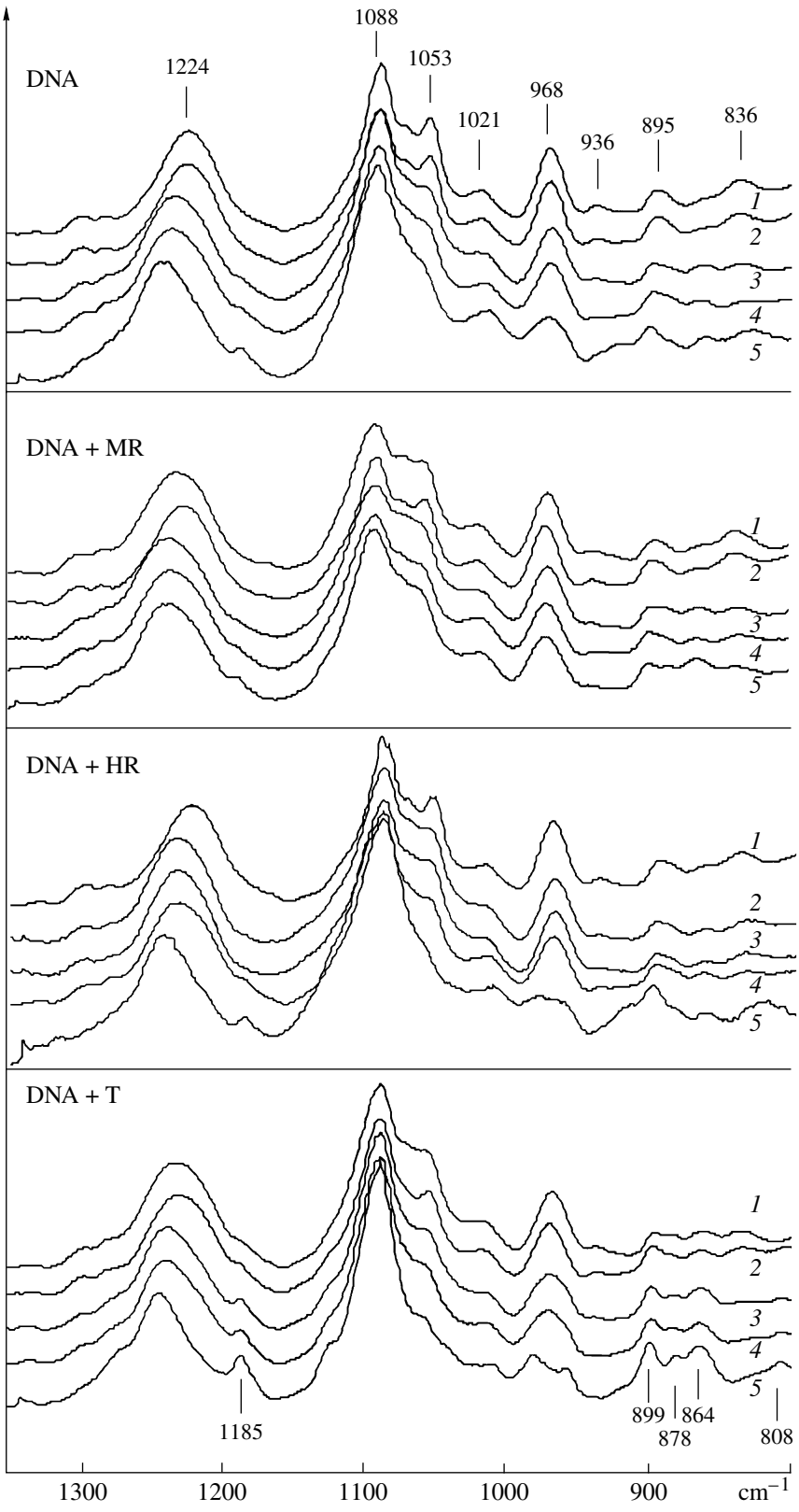

Fig. 2. IR Spectra of native DNA films and DNA films with d_1 factors at various r.h. values: *1*, 95%; *2*, 85%; *3*, 75%; *4*, 66%, and *5*, 56%. Horizontal axis, reciprocal wavelength, cm^{-1}; vertical axis, optical density, relative units.

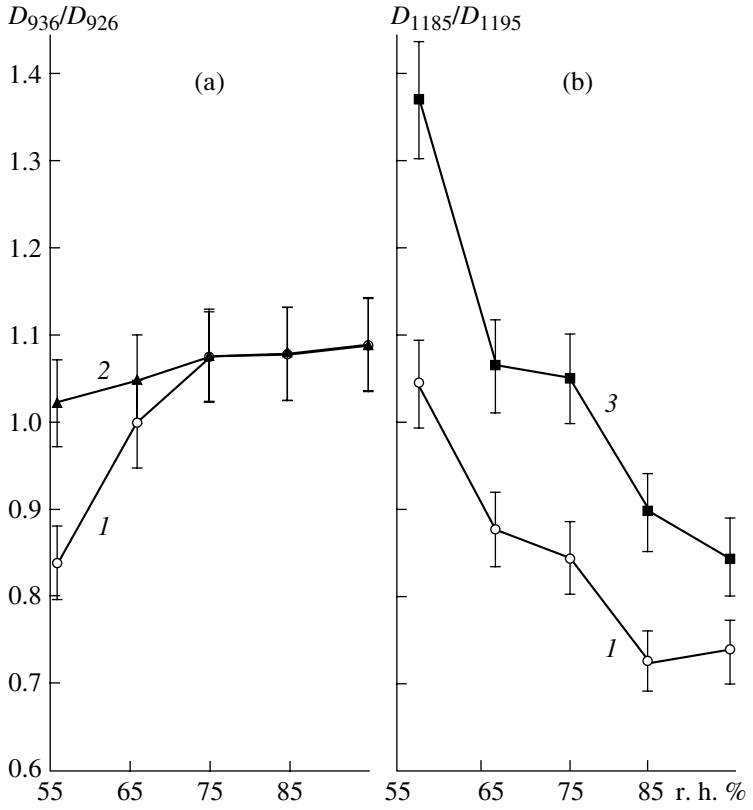

Fig. 3. Changes in the relative intensity of A and B form-characteristic bands for DNA per se (*1*), DNA + MR (*2*), and DNA + T (*3*). Horizontal axis, relative humidity, %; vertical axis, D_{936}/D_{926} (a) and D_{1185}/D_{1195} (b) values.

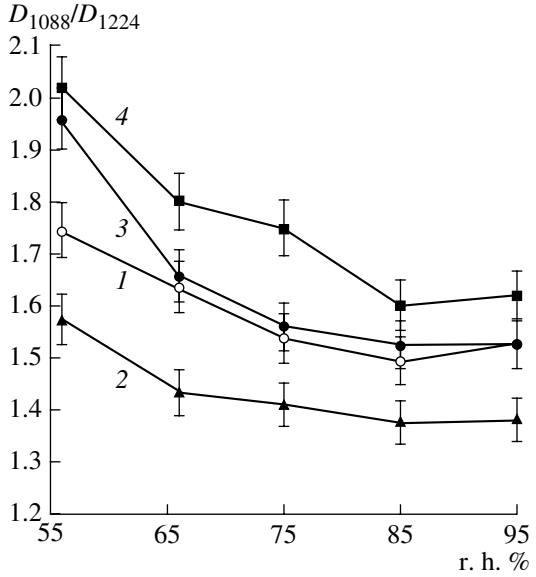

Fig. 4. Changes in D_{1083}/D_{1224}, the ratio between the frequency intensities of symmetrical and antisymmetrical oscillations in the phosphate groups of DNA per se (*1*) and DNA with MR (*2*), HR (*3*), and T(*4*), in relation to the r.h. values. Horizontal axis, relative humidity, %; vertical axis, D_{1088}/D_{1224}.

to be water activity [24]. Its values in DNA films coincide with those of relative humidity. However, in the presence of some nonelectrolytes the B conformation can exist at the water activity values below the critical threshold required for the B ⟶ A transition. Molecules of these substances, such as inositol and ethylene glycol [25, 26], contain several densely arranged polar OH groups. Presumably, the stabilization of the DNA B form can be ensured, apart from high water activity, by the presence of a significant number of hydroxy groups in the vicinity of the DNA molecule. Therefore, the impact of water activity on the conformational state of DNA molecules is apparently due to the fact that water behaves as a polar solvent [27].

These considerations should be taken into account while discussing the effects produced by methylresorcinol. Like the other molecules mentioned above, methylresorcinol is a polyol containing two closely located OH groups (Fig. 1a). It can, therefore, replace water molecules in the DNA microenvironment, preventing the B ⟶ A transition upon a decrease in relative humidity. Of special importance in terms of the mechanism of action of methylresorcinol is the fact that the CH_3 radical this molecule contains is not sufficiently hydrophobic. Hexylresorcinol, another alkylresorcinol homologue with a similar structure, but with a longer alkyl radical (Fig. 1b), cannot prevent the B ⟶ A tran-

sition in the DNA molecule. Presumably, the differences in the mode of action of MR and HR on the DNA are due to (i) different distribution patterns of electron density in their molecules, which accounts for different polarity degrees of their rings with substituents (OH groups) and (ii) the effects produced by the HR hydrophobic alkyl radical per se.

The stimulatory effect of hexylresorcinol on the B ⟶ A transition revealed by us occurs at low r.h. values only. Presumably, its mechanism of action on the conformational changes in the DNA is significantly different from that of MR. The stimulation of the B ⟶ A transition can result from the formation of micella-like nanostructures around DNA macromolecules by the interacting HR molecules, as was demonstrated by us earlier. A significant hexylresorcinol concentration is a prerequisite for this process. This accounts for the fact that the facilitation of the B ⟶ A transition occurs in our system only upon significant r.h. decrease, accompanied by an increase in the concentration of this d_1 analogue.

As for tyrosol, which efficiently stimulates the B ⟶ A transition in DNA, we should take into account the fact that this polyol contains only one hydroxy group in its benzene ring. It has an ethane-1-ol radical in the *p* position (Fig. 1c). Based on these structural peculiarities, we can consider tyrosol a phenetyl alcohol. Alcohols are known to decrease the r.h. level and to convert DNA into the A form; this property is used to isolate DNA from aqueous solutions [27].

The results obtained open up new potentialities for the goal-directed synthesis of modified chemical analogs of d_1 factors that influence the intensity of B ⟶ A transitions in DNA molecules.

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