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EXPERIMENTAL ARTICLES

The Effect of Alkylhydroxybenzenes on Functional and Operational Stability of Antibodies

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Abstract—The influence of alkylhydroxybenzene (AHB) homologues of various hydrophobicity on antibody interaction with corresponding antigens under denaturing conditions (functional stability) and suboptimal conditions (operational stability) was revealed. AHBs were found to modulate the antibody sensitivity to heat denaturation and UV irradiation and expand the pH range of activity in different directions. These effects were found to depend upon the specific chemical nature and the AHB concentrations applied. Mechanisms of the AHB effect and possible perspectives of their use as antibody stabilizers are discussed.

Key words: antibodies, alkylhydroxybenzenes, alkylresorcinols, functional and operational stability, antibody stabilizers.

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Alkylhydroxybenzenes (AHBs) of microbial or plant origin exhibit an extremely wide range of biological activity and have been investigated in numerous interdisciplinary studies [1, 2]. The functions of alkylresorcinols, a class of AHBs, have been studied in detail by Polish authors concentrating on plant alkylresorcinols [1]. Initially, this group of substances provoked interest on the part of microbiologists due to their importance in membrane structure modification under conditions of developing hypometabolic and anabiotic states of bacteria accompanied by formation of dormant forms [2, 3], including *Azotobacter vinelandii* cysts [4], as well as cystlike dormant cells (CLCs) of nonspore-forming bacteria [2]. Today, researchers' attention focuses on AHB functions in stress response in bacteria and fungi [5]. AHBs are considered universal natural modifiers with adaptogenic functions that are active in biological objects of various levels of organization [2, 6]. Accordingly, such behavior is preconditioned by the ability of AHBs to form a number of weak hydrophobic and electrostatic interactions and hydrogen bonds with cellular membrane components [7], proteins [8], and nucleic acids [9], leading to changes in their structural organization and, consequently, to changes in the functional activity and stability of cellular biopolymers and supramolecular assemblies.

In our previous studies [10, 11], we have characterized the effects produced by AHBs on human and animal enzymes and immune proteins, as well as pronounced modulation of protein functional activity accompanied by alterations in their substrate and antigen specificity. The stabilizing effect of two AHB homologues defined as preservation of high enzyme activity of the modified enzyme after denaturing heat treatment (functional stability) or at suboptimal temperature of catalysis (operational stability) was demonstrated by the example of egg protein lysozyme [10].

The aim of the present work was to study the effect of chemical analogues of microbial AHB on the functional and operational stability of antibodies, which was assayed by their ability to bind to the corresponding antigens after thermal denaturing and UV irradiation and under suboptimal pH conditions.

MATERIALS AND METHODS

The chemical analogues of microbial alkylhydroxybenzenes were 99.9% pure homologues differing by alkyl chain length, which determined their hydrophobicity, that is, C7-AHB (M = 124) and C12-AHB (M = 194) (Sigma, United States) along with C9-AHB (M = 152) and C18-AHB (M = 278) (Enamine, Ukraine). Water solutions of 10^{-5}, 10^{-4}, and 10^{-3} M of the AHB homologues were used for antibody modification (ethanol solutions were used in case of C18-AHB).

AHBs effect on the functional and operational stability of the antibodies was assayed by their ability to bind the corresponding antigens. VektoTokso-IgG enzyme immunoassay kits (Vektor-Best, Novosibirsk, Russia) were used for quantitative determination of type G immunoglobulins against *Toxoplasma gondii*.

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Control samples from the kit with activity of 80 IU per 1 ml were used as antibody sources.

Quantitative antibody binding with the corresponding antigen was evaluated by solid-phase immunoassay using Uniplan and Proplan laboratory equipment sets (Pikon, Russia).

To modify the protein structure, prior to the experiment, the antibodies were mixed with AHB solutions in ratio of 1 : 1 and incubated at 37°C for 60 min. In the control variants, an equivalent amount of the solvent (5% ethanol in case of C18-AHB) was added instead of AHB solutions.

The functional thermostability of antibodies was determined by the preservation of their residual activity after heating for 15 min in the temperature range from 50 to 80°C with a 3°C increment using a Termit solid-state thermostat (DNK-Tekhnologiya, Russia). Thermal inactivation of antibody–AHB complexes was assayed using the ET20 and ET50 values that correspond to a 20 and 50% decrease in binding of the antibodies to matching antigens sorbed in polystyrene plate wells.

The functional stability of intact and AHB-modified antibodies upon ultraviolet treatment was assayed after illumination of protein solutions with a broadband UV lamp (Osram, Germany). The energy exposure of the samples measured with a TKA-PKM UV radiometer (Russia) in the wavelength range from 200 to 280 nm was 24.85 W/m^2, and the time of exposure varied from 0 to 120 min with a 20-min increment. The UV radiation dose (in J) resulting in suppression of binding of the antibodies with the corresponding antigens by 50% of the initial value, ID50, was used as an integral characteristics of antibody sensitivity toward the factor.

To study the operational stability of the antibodies at various pH values, antibody solutions were mixed with equal volumes of phosphate buffer with a relevant pH value. Values of pH of the buffers varied from 3 to 11 with an increment of 1 unit, which was determined using an Ekspert-001 liquid analyzer (Ekoniks-Ekspert, Russia). The mixtures were introduced into the wells of polystyrene plates with sorbed antigens, and the binding efficiency was measured after 15-min incubation according to the conventional enzyme immunoassay procedure. The reaction result was estimated as the ratio between the binding values of intact and AHB-modified antibodies at each pH value.

All experiments were performed in five repeats at least. Statistical treatment of data obtained was carried out using the Statistica software package.

RESULTS AND DISCUSSION

The functional thermostability of native (without AHB) antibodies against *T. gondii* according to the data of antigen binding in polystyrene plate wells confirmed the characteristic S-shape curve of the resulting parameter against the intensity of the heat treatment [12]. The highest value of relative decrease in binding was registered in the range of 59 to 71°C, and the ET20 and ET50 values were detected at 61.5 ± 0.5 and 65.9 ± 0.4°C, respectively (Fig. 1).

In experimental samples, initial antibody modification with AHB resulted in changes in proteins' heat resistance depending on both the AHB homologue structure and the components' concentration. For example, C7-AHB in the control samples (without heating) did not significantly affect the binding ability of the modified antibodies and corresponding antigens. In contrast, after heating of the samples modified with C7-AHB at temperatures in the range of 50 to 65°C, significant dispersion of the resulting parameters was observed (Fig. 1a). One of the consequences of modification was an increase in ET20 values from 61.5 ± 0.5 to 62.9 ± 0.3°C ($P < 0.05$) in the antibody complex with 10^{-5} M AHB and decrease to 52.3 ± 0.4°C ($P < 0.001$) for 10^{-4} M C7-AHB. No reliable influence of antibody modification on the ET50 value was observed.

Antibody modification with a significantly more hydrophobic C12-AHB homologue initially led to a pronounced and concentration-dependent decrease in the functional activity which may be ascribed to protein structure modification due to the interactions of C12-AHB alkyl residues with the hydrophobic sites in the active antigen-binding site of the protein molecule giving rise to changes in affinity, avidity, and specificity as it has been previously shown [11]. In experimental samples, heat treatment of the modified antibodies resulted in further suppression of binding (Fig. 1b), which is evidenced not only by a significant decrease in ET20 values, but also by a decrease in ET50 values from 65.9 ± 0.4 to 64.5 ± 0.3°C with 10^{-4} M and to 63.7 ± 0.6°C with 10^{-3} M C12-AHB solution ($P < 0.05$).

The results obtained contradict the previously established heat-protective effects of AHB on enzyme proteins (functional and operational thermostability) [8, 10] and whole bacterial cells [2, 3]. While lysozyme modification with C7-AHB and C12-AHB resulted in a considerable retaining of its catalytic activity after heating (compared to the initial enzyme) [10], in the case of antibodies the same procedure, on the contrary, mostly increased their sensitivity to thermal denaturing.

Analysis of the reason for such behavior is a matter of special study. One issue that needs to be confirmed is the specific molecular organization of enzyme and immune proteins—in particular, the differences in potential structural targets of AHBs, which are yet to be determined. For example, one of the proposed mechanisms of the protective effect of hydrophobic long-chain AHBs is their interaction with the hydrophobic sites of the protein globule core preventing its denaturing and preserving the structural organization of the most conservative region i.e. the enzyme active

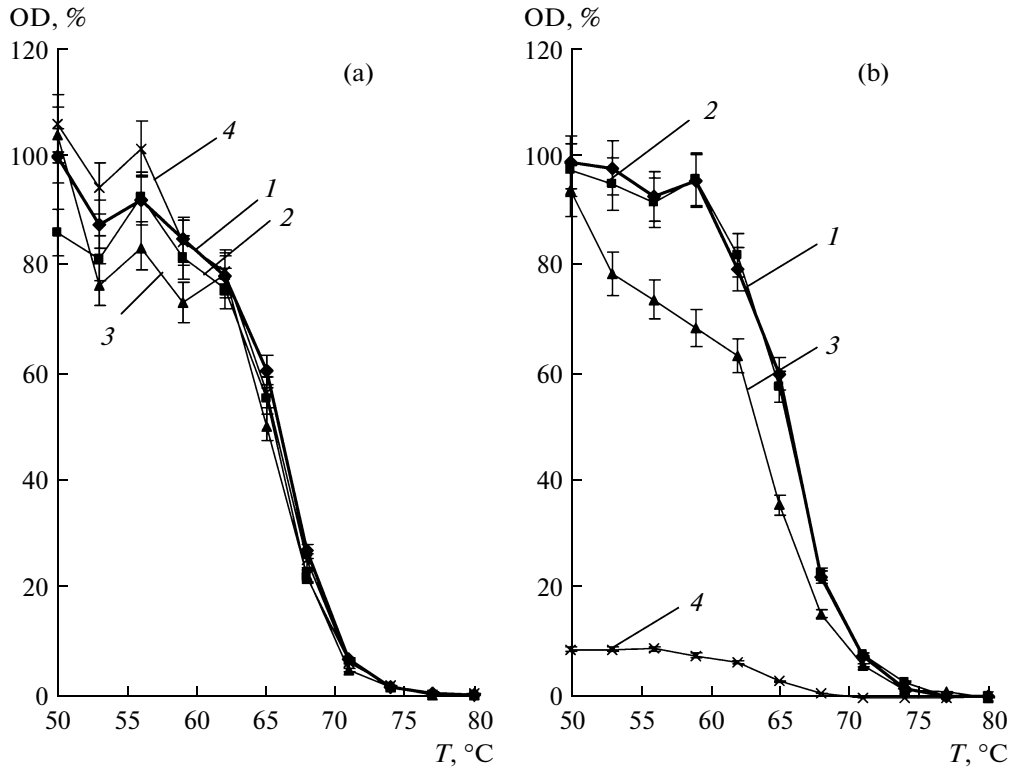

Fig. 1. Binding affinity values of intact antibodies against *Toxoplasma gondii* and their complexes with C7-AHB (a) and C12-AHB (b) after heating. Temperature, °C, is plotted against the *x* axis; relative values of antibody binding to the corresponding antigens, %, against the *y* axis. Control (without AHB) (*1*), in complex with 10^{-5} (*2*), 10^{-4} (*3*), and 10^{-3} M AHB (*4*).

center [13]. In contrast, the active antigen-binding sites of immunoglobulins exposed to the surface of the molecule are the most variable parts of the protein molecule with a high content of hydrophobic amino acids, which are potentially affine with the nonpolar radicals of AHB. From this point of view, the data obtained may indirectly suggest direct interaction between AHBs and the functional groups in the proximity of antibody active sites.

Another denaturing factor AHB protection against which has been previously demonstrated is UV irradiation [14]. The mechanism of protein damage is associated with absorption of photons in the UV range by aromatic amino acid residues resulting in photolysis and protein denaturing. In the control samples, UV irradiation of antibodies against *T. gondii* led to a dose- and time-dependent decrease in binding with the corresponding antigens. The UV radiation dose inducing 50% inactivation (ID50) upon irradiation of intact antibodies was calculated to be in the range of 1017 ± 50 to 1048 ± 39 J and increased to 2401 ± 127 J in the presence of ethanol (5%).

In the experimental samples, while preliminary incubation of the antibodies with short-chain C7-AHB and C9-AHB didn't result in any significant changes in their functional activity, it was accompanied by a reliable increase in UV resistance of the complexes (table). ID50 values calculated for 10^{-3} M homologue solutions reliably increased up to 1423 ± 75 J ($P < 0.01$) in case of C7-AHB and to 1223 ± 54 J ($P < 0.05$) for C9-AHB.

Against this background, interaction of the antibodies with long-chain hydrophobic C12-AHB and C18-AHB resulted in a pronounced nonlinear dose-dependent change in their capacity of binding the corresponding antigens. Use of concentrated 10^{-3} M solutions of the homologues was accompanied by a negative effect on UV resistance, which was observed to decrease by 30–40%. However, at lower concentrations, long-chain AHBs acted as UV protectors which was registered by an increase of ID50 values to 1472 ± 74 J (by 45%) and 2996 ± 183 J (by 25%) for 10^{-4} M solutions of C12-AHB and C18-AHB, respectively.

This block of experimental results of the block is in accordance with the stabilizing effect of AHBs previously demonstrated for proteins [14, 15] and nucleic acids [16], which has conditioned some AHBs to be used as radioprotectors [15, 17]. When discussing mechanisms of protective action, one should keep in mind that absorbance maxima of C7-AHB (277 nm) and C12-AHB (280 nm) are close to those of the tyrosine and tryptophan aromatic residues in protein structure (approximately 280 nm). Therefore, partial overlapping of the spectra in the UV range creates a possibility for an "optical screen" made of AHB molecules that would absorb photons on their way to the

UV radiation doses (J) resulting in 50% inactivation (ID50) of intact (control) and AHB-modified antibodies

Homologues used	Control (without AHB)	AHB concentration		
		10^{-5} M	10^{-4} M	10^{-3} M
C7-AHB	1021 ± 41 100%	986 ± 46 97%	1194 ± 48 117%	1423 ± 75** 139%
C9-AHB	1048 ± 39 100%	966 ± 49 92%	927 ± 44 88%	1223 ± 54* 117%
C12-AHB	1017 ± 50 100%	1169 ± 81 115%	1472 ± 74** 145%	709 ± 40** 70%
C18-AHB	2401 ± 127 100%	2737 ± 134 114%	2996 ± 183* 125%	1445 ± 72** 60%

Note: The radiation doses (J) are presented in upper rows, relative values (% to control), in lower rows.
* $P < 0.05$, ** $P < 0.01$.

protein molecule, dissipating the damaging energy. Another component of the protective activity of AHBs is probably due to their pronounced antioxidant activity as scavengers for reactive oxygen species arising in the process of water photolysis [5, 18]. However, these hypotheses cannot explain the dependence of the UV-protective effect on the chemical structure of homologues, as well as nonlinear concentration dependencies of AHB effects on protein UV-resistance observed in the experiments. Therefore, one should surmise direct interaction in the antibody–AHB system; this assumption is in agreement with the previously demonstrated ability of long-chain AHBs to increase ribonuclease (*Bacillus intermedias* binase) resistance to UV irradiation [14].

Study of pH influence on the affinity of the antibodies toward the corresponding antigens a revealed nonlinear dependency. In the control samples, the highest binding affinity of intact antibodies was registered in the pH range from 6 to 8; it halved at pH 4 and 9 and progressively approached zero at pH reaching the extreme values of the studied range (Fig. 2).

In the experimental samples, preliminary incubation of the antibodies with C7- (Fig. 2a) and C12-AHB (Fig. 2b) resulted in differently directed changes in their binding affinity, depending on pH values, AHB hydrophobicity, and active concentrations. The effect of the amphiphilic C7-AHB was different of that of the hydrophobic C12-AHB and consisted of a relative change in the binding values of modified antibodies. The amphiphilic homologue at a high concentration of 10^{-3} M decreased the antibody binding affinity at all pH values studied. On the contrary, antibody interaction with lower concentrations of C7-AHB caused an increase in antigen binding both within the neutral pH range from 6 to 8 and at pH decrease to 4 (Fig. 2a). Antibody modification with C12-AHB, while inhibiting antigen binding at neutral pH, significantly increased the antibody operational stability at suboptimal pH values of 10–11. The most pronounced decrease of the relative binding values was observed at low pH values and within the C12-AHB concentration range of 10^{-4}–10^{-3} M (Fig. 2b). At 10^{-4} M concentration of the homologue, a doubling of the relative antibody–antigen binding values was observed at pH 10.

When discussing the results, one should mention the data on increasing of the operational stability of AHB-modified enzyme proteins [8, 10] along with the fact that one of the techniques of antibody stabilization at low pH values involves addition of cereal grain extracts or hydrolysates [19] providing natural AHBs of plant origin [1]. Both plant and microbial AHBs are represented by a mixture of isomers and homologues [20]. In the present study, we used individual homologues that, depending on their structure, were capable of either hydrophobic or hydrogen–hydrogen interactions with proteins altering the pH range of the functional activity of the latter. At low (acidic), pH protonated AHBs acted as antibody destabilizers, while the effect of widening of the operational pH range was demonstrated at high (alkaline) pH when deprotonated AHB molecules affected proteins mainly by means of hydrophobic interactions.

Therefore, the data obtained demonstrated a number of reliable effects of alkylhydroxybenzenes on the functional and operational stability of the antibodies, which may be considered another series of arguments in favor of direct interactions between AHB molecules and immune proteins [11]. In contrast to the previously known examples of increase of the functional and operational stability of AHB-modified enzyme proteins [10, 14, 15], the effects registered upon AHB interaction with immune proteins were differently directed, depending on the AHB structure and concentration, as well as on the nature of the damaging factor. Long-chain AHBs increased the antibody sensitivity to thermal denaturing, while reliably (by 20–45%) decreasing their sensitivity to UV irradiation, and ambiguously altered the functional activity pH

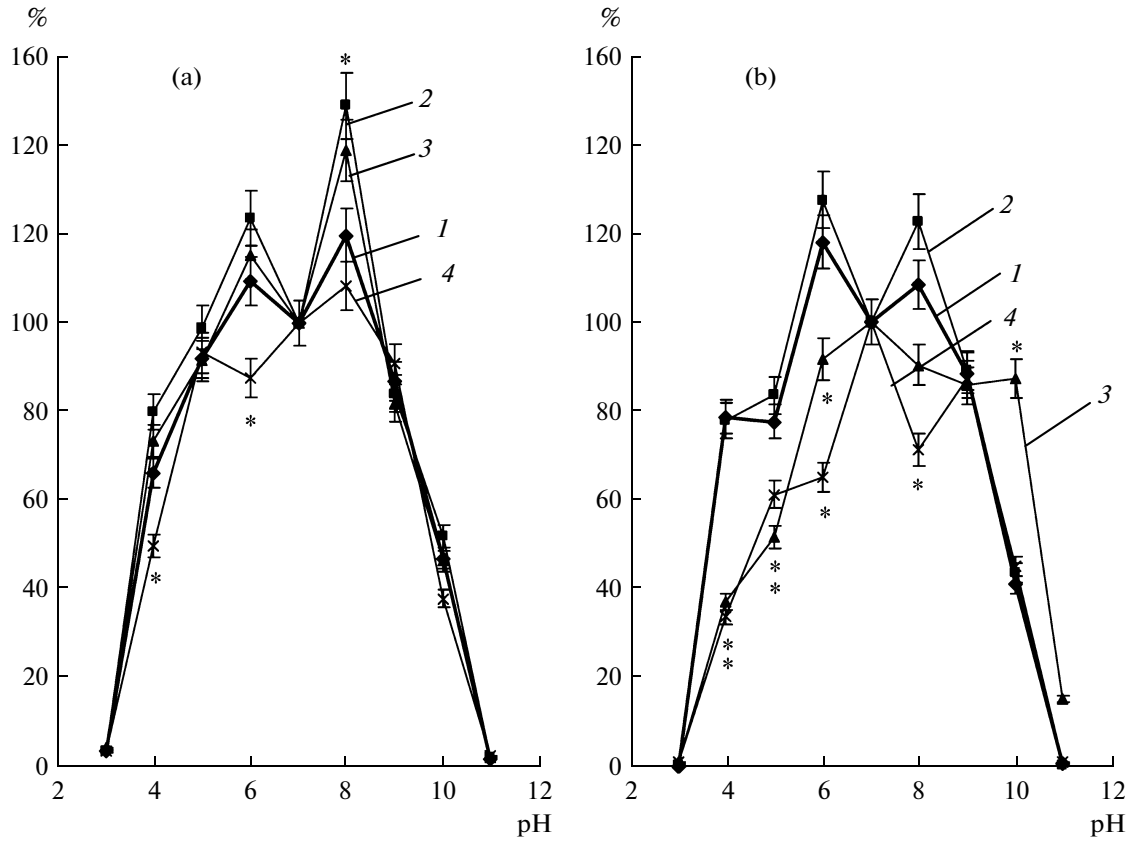

Fig. 2. Binding parameters of intact antibodies against *T. gondii* and their complexes with C7-AHB (a) and C12-AHB (b) at various pH values. pH units are plotted against the *x* axis; relative values of antibody binding corresponding antigens, % to the 100% binding of intact antibodies under optimal pH conditions, against the *y* axis. Control (without AHB) (*1*), in complex with 10^{-5} (*2*), 10^{-4} (*3*), and 10^{-3} M AHB (*4*); $*P < 0.05$.

range, which was narrowed in acidic and widened in alkali media. Taking into account the decrease in the binding affinity of AHB-modified immune proteins with the corresponding antigens in conjunction with widening of the antigen specificity spectrum [11], one has to admit that the AHB–antibody interaction allows for fine tuning of immune proteins' activity. A more in-depth study is desirable for possible regulation of immune reactions. The effect of alkylhydroxybenzene on the dynamics of the antibody functional activity at long incubation in aqueous solutions is also of interest and may be studied on the basis of our earlier data on increased hydrophobicity of AHB-modified protein molecules [10] and formation of hydrophobicity effects on other biopolymers [21]. These problems will be the subject of our further studies.

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REFERENCES

1. 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.
2. 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. 309–314].
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. Reuch, R.N. and Sadoff, H.L., Novel Lipid Components of the *Azotobacter vinelandii* Cyst Membrane, *Nature*, 1983, vol. 302, pp. 268–270.
5. El-Registan, G.I., Mulyukin, A.L., Nikolaev, Yu.A., Stepanenro, I.Yu., Martirosova, E.I., Syanenko, E.F., Strakhovskya, M.G., and Revina, A.A., The Role of Low-Molecular-Weight Autoregulatory Factor (Alkylhydroxylbenzenes) in Resistance to Radiation and Heat

Shock, *Advances Space Res*,, 2005, vol. 36, pp. 1718–1728.

6. Il'inskaya, O.N., Kolpakov, A.I., Mulyukin, A.L., Dreier, F., and El'-Registan, G.I., Effect of Membrane-Active Microbial Autoregulators on the Growth of Cultured ras-Transformed Fibroblasts, *Prikl. Biokhim. Mikrobiol.*, 2000, vol. 36, no. 5, pp. 549–554 [*Appl. Biochem. Microbiol.* (Engl. Transl.), vol. 36, no. 5, pp. 473–477].
7. Vostroknutova, G.N., Kaprel'yants, A.S., Svetlichnyi, V.A., El'-Registan, G.I., Shevtsov, V.V., and Ostrovskii, D.N., Membrane-Active Characteristics of the Preparation from Bacterial Culture Liquid Possessing an Autoregulatory Effect, *Prikl. Biokhim. Mikrobiol.*, 1983, vol. 19, no. 4, pp. 547–551.
8. 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].
9. 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. 616–625 [*Microbiology* (Engl. Transl.), vol. 74, no. 5, pp. 533–541].
10. Petrovskii, A.S., Deryabin, D.G., Loiko, N.G., Mikhailenko, N.A., Kobzeva, T.G, Kanaev, P.A., Nikolaev, Yu.A., Krupyanskii, Yu.F., Kozlova, A.N., and El'-Registan, G.I., Regulation of the Functional Activity of Lysozyme by Alkylhydroxybenzenes, *Mikrobiologiya*, 2009, vol. 78, no. 2, pp. 176–185 [*Microbiology* (Engl. Transl.), vol. 78, no. 2, pp. 144–153].
11. Deryabin, D.G., Mikhailenko (Romanenko), N.A., and El'-Regitan, G.I., The Effect of Alkylhydrozybenzenes on the Antigen-Binding Capacity of Antibodies, *Mikrobiologiya*, 2009, vol. 78, no. 5, pp. 629–635 [*Microbiology* (Engl. Transl.), vol. 78, no. 5, pp. 569–574].
12. Li, S-Q., Zhang, Q.H., Lee, Y-Z., and Pham, T.-V., Effects of Pulsed Electric Fields and Thermal Processing on the Stability of Bovine Immunoglobulin G (IgG) in Enriched Soymilk, *J. Food Sci.*, 2003, vol. 68, no. 4, pp. 1201–1207.
13. Krupyanskii, Yu.F., Esin, S.V., Korotina, O.A., Usanov, M.A., Dembo, K.A., Volkov, V.V., Loiko, N.G., Nikolaev, Yu.A., and El'-Registan, G.I., Effects of the Interaction between the Chemical Chaperon Hexylresorcinol with Lysozyme: Changes in Structure, Dynamics, and Functional Activity, *Biofizika*, 2010, vol. 55 (in press).
14. 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. 224–330 [*Microbiology* (Engl. Transl.), vol. 69, no. 2, pp. 180–185].
15. Stepanenko, I.Yu., Strakhovskaya, M.G., Belenikina, N.S., Nikolaev, Yu.A., Mulyukin, A.L., Kozlova, A.N., Revina, A.A., and El'-Registan, G.I., Protection of *Saccharomyces cerevisiae* against Oxidative and Radiation-Caused Damage by Alkylhydroxybenzenes, *Mikrobiologiya*, 2004, vol. 73, no. 2, pp. 204–210 [*Microbiology* (Engl. Transl.), vol. 74, no. 2, pp. 163–169].
16. Davydova, O.K., Deryabin, D.G., and El'-Registan, G.I., Influence of Chemical Analogues of Microbial Autoregulators on the Sensitivity of DNA to UV Radiation, *Mikrobiologiya*, 2006, vol. 75, no. 5, pp. 654–661 [*Microbiology* (Engl. Transl.), vol. 75, no. 5, pp. 568–574].
17. Vagel, A. and Roo, E., Alkylresorcinols—Rare Chemicals Available in Bulk, *Innov. Pharm. Tech.,* 2004, pp. 94–95.
18. Revina, A.A., Larionov, O.G., Kochetova, M.V., Lutsik, T.K., and El'-Registan, G.I., Spectrophotometric and Chromatographic Investigation of the Radiolysis Products in Aerated Aqueous Alkylresorcinol Solutions, *Izv. RAN. Ser. Khim.*, 2003, no. 11, pp. 2257–2263.
19. Stabilization of Immunoglobulins at Low pH, United States Patent 7220409. Filing Date: 08.29.2001.
20. El'-Registan, G.I., Mulyukin, A.L., Nikolaev, Yu.A., Suzina, N.E., Gal'chenko, V.F., and Duda, V.I., Adaptogenic Functions of Extracellular Autoregulators of Microorganisms, *Mikrobiologiya*, 2006, vol. 75, no. 4, pp. 446–456 [*Microbiology* (Engl. Transl.), vol. 75, no. 4, pp. 380–389].
21. Davydova, O.K., Deryabin, D.G., and El'-Registan, G.I., Long-Term Preservation of DNA in Aqueous Solutions in the Presence of the Chemical Analogues of Microbial Autoregulators, *Mikrobiologiya*, 2006, vol. 75, no. 5, pp. 662–669 [*Microbiology* (Engl. Transl.), vol. 75, no. 4, pp. 575–581].