Supramolecular systems based on alkylated *p*-sulfonatocalix[*n*]arenes: aggregation and catalytic and biological activity

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Aggregation, catalytic activity, and the influence of alkylated *p*-sulfonatocalix[*n*]arenes (SCA; $n = 4, 6, 8$) on the energy exchange in cells of wheat roots were studied. In water SCA are surfactants, and their efficiency changes with an increase in the number of aromatic rings (n) and the length of hydrocarbon substituents (R) in the molecule. In the range of critical micelle concentration, SCA form micelles with an effective hydrodynamic diameter of ~200 nm. The catalytic activity of micellar solutions of SCA ($n = 4, 8$; $R = C_{12}H_{25}$) in hydrolysis of ethyl 4-nitrophenyl chloromethylphosphonate (ENCP) decreases with an increase in n, whereas the binding constant of ENCP increases. The modifying effect of SCA on the membranes and the energy exchange in cells of wheat roots was revealed: the membranotropic activity of SCA increases with an increase in the hydrophobicity of substituents R and the macrocycle size.

Key words: alkylated *p*-sulfonatocalix[*n*]arenes, surfactants, micelle formation, nanoparticles, catalytic activity, cells of wheat roots, energy exchange, ethyl 4-nitrophenyl chloromethylphosphonate, hydrolysis.

Considerable attention is recently given to the study of methods for synthesis, properties, and the scope of application of *p*-sulfonatocalix[*n*]arenes (SCA) and their derivatives.[1—16] One of the most promising and interesting directions of using SCA is the medical biological area,[17] which is caused by high solubility of SCA in water, low toxicity,[17] and accessibility of functionalization, which makes it possible to control physicochemical properties of these compounds by changing the nature of substituents and the number of aromatic rings. For instance, the introduction of alkyl radicals into the SCA molecule favors the change in its hydrophilic-lipophilic balance and the appearance of surfactant properties.[1,4] A consequence of an increase in the number of aromatic rings is the conformational mobility of the macrocycle,[5,6,9—11,17] resulting in the appearance of the adaptive ability to substrate binding (flexible "tuning").[18] Although many publications are devoted to *p*-sulfonatocalix[*n*]arenes, the aggregation behavior of SCA modified by alkyl radicals was not systematically studied.

The mechanism of interaction of biologically active compounds with the cell membrane surface depends, to a great extent, on hydrophobicity of these compounds and their aggregation state.[19—22] Many micelle-forming surfactants possess antimicrobial activity and can enhance the effect of sulfamide drugs and solubilize hydrophobic drugs by transforming them into the soluble form.[19,23] It is known that the energy processes in cells are tightly related to the phosphorylation-dephosphorylation reactions of membrane proteins.[24] The study of biological aspects of application of nonmodified *p*-sulfonatocalix[*n*]arenes showed that unsubstituted SCA ($n = 4$, R = H) manifest supramolecular catalytic activity in hydrolysis of ATP,[17] which can change substantially in the case of alkylated SCA. Therefore, the complex study of the aggregation properties of alkylated SCA, their catalytic activity in the model reaction of hydrolysis of phosphorus acid esters, and the influence on biosystems is an urgent problem, whose solution will help to establish the relationship "chemical structure—aggregation behavior—biological activity" and to extend the range of using the SCA derivatives in new technologies.

The purpose of this work is the study of the aggregation behavior of *p*-sulfonatocalix[*n*]arenes containing

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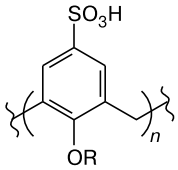
different numbers of alkylated phenol rings (n = 4, 6, 8; R = Bu^n, C_8H_{17}, $C_{12}H_{25}$), the catalytic activity of the SCA in the model reaction of hydrolysis of ethyl 4-nitrophenyl chloromethylphosphonate, and their modifying effect on the membranes and the energy exchange in wheat roots.



1—8

n = 4: R = Bu^n (**1**), C_8H_{17} (**2**), $C_{12}H_{25}$ (**3**), H (**6**);
n = 6: R = $C_{12}H_{25}$ (**4**), H (**7**); n = 8: R = $C_{12}H_{25}$ (**5**), H (**8**)

Experimental

The SCA under study were synthesized according to procedures described previously.[4,7,11,15] The IR and ^{1}H NMR spectra of compounds **1—8** correspond to the published ones. The surface activity of the SCA was studied recording the surface tension (σ) isotherms in water—air systems by the du Nouy ring detachment method (Sigma 702ET high-precision tensimeter), and the relative measurement error by results of three independent experiments did not exceed 1%. The association of the SCA in water was studied by the conductometric method, determining the electroconductivity (χ) of solutions at 25 °C (inoLab Cond Level 1 conductometer, relative measurement error 0.5%). The temperature was maintained constant by a thermal sensor with an accuracy of ±0.1 °C, and the pH values of solutions was measured with an I-160 ionometer. The particle size (effective hydrodynamic diameter, D) was determined by the light scattering method on a FotoCor Complex photon correlation spectrometer of dynamic and static light scattering. An He—Ne gas laser with a power of 10 mW and a wavelength of 633 nm served as a radiation source. Signals were analyzed with a one-plate multichannel correlator, and the data were processed using programs from the set of the instrument. The function obtained was processed using the Alango Dynals v2.0 universal program for processing of dynamic light scattering data. The relative measurement error of effective hydrodynamic diameters of associates did not exceed 10%. The electrokinetic potential (ζ-potential) of particles in SCA solutions was measured on a Malvern Instruments Zetasizer Nano ZS high-sensitive analyzer. The procedure of sample preparation for studies of sizes of the associates and ζ-potential (the use of disposable pipettes, cells with caps or immersible electrodes from the set of the instrument, and disposable filters 0.45 μm, Millex HN) provided the necessary "dust-removal" of solutions. Nanoassociate sizes in solutions were measured after the control study of freshly bidistilled water, which was used for the preparation of solutions only if the analyzer detected the full absence of any particles. The measurements were carried out under the conditions of temperature maintenance at 25 °C. The kinetics of hydrolysis of the substrate, *viz.*, ethyl 4-nitrophenyl chloromethylphosphonate (ENCP), was studied by spectrophotometry under the conditions of the pseudo-first-order reaction that occurs with 4-nitrophenolate formation (λ = 400 nm) on a Perkin—Elmer Lambda 35 spectrometer at 25 °C, pH 9.2. The observed rate constants (k_{obs}) were determined by the first-order equation. The binding constants of the substrates (K_b) by micelles, the critical micelle concentrations (CMC), and the rate constants of the reaction in the micellar pseudo-phase (k_m) were determined by the data of the concentration dependences of k_{obs}. For this purpose, the equation for the calculation of the kinetic curves reaching a plateau, which takes into account the substrate distribution between the volume and micellar phase, was used[25—28]

$$k_H = (k_{H_2O} + k_m K_b C)/(1 + K_b C), \quad (1)$$

where k_{H_2O}/s^{-1} is the reaction rate constant in the aqueous phase, and C/mol L^{-1} is the SCA concentration corrected to the CMC. The object of studies in biological experiments was the spring wheat "Lyuba" (*Triticum aestivum* L.), whose acrospires were grown on distilled water for 4 days.[22] Immediately after cutting off, roots of the wheat acrospires were placed into the working solutions and incubated in the Warburg apparatus.[22] The formation and consumption of K^+ ions by the root cells were determined by the change in the potassium content in the incubation medium. Measurements were carried out on a PFM flame photometer. Preliminarily the roots were incubated in the Warburg vessels, which made it possible to detect simultaneously the oxygen consumption by the cells.

Results and Discussion

The surface tension isotherms of the alkylated derivatives of *p*-sulfonatocalix[4]arene with the substituents R = Bu^n (**1**), C_8H_{17} (**2**), and $C_{12}H_{25}$ (**3**) are shown in Fig. 1. The surface tension isotherm of the micelle-forming surfactant sodium dodecyl sulfate (SDS) is presented for comparison. In the case of compound **3**, micelle formation is stepwise, which can be due to the formation and rearrangement of micelles (Table 1, see Fig. 1). The inflection points in the plot $\sigma = \log C$ correspond to these processes. The critical micelle concentration of compounds **1—3** is approximately an order of magnitude lower than the CMC of SDS,[23] which is equal to $8 \cdot 10^{-3}$ mol L^{-1}. Probably, this is due to the pre-organized character of four polar head groups and nonpolar hydrocarbon radicals in the same SCA molecule, which facilitates micelle formation similarly to the processes of dimeric surfactants.[23] The data in Table 1 show that the CMC of compounds **1—3** change slightly with the elongation of the hydrocarbon radical, whereas for surfactants of the SDS type the CMC decreases strongly, as a rule, with the elongation of the alkyl chain of the substituent.[23] The surface tension σ in the CMC point of solutions of SDS **1—3** is considerably higher than that in the case of SDS. The concentration dependence of the electroconductivity of solutions of SCA **3** typical of these compounds is shown in Fig. 2. The concentration, at which the inflection in

the $\chi = f(C)$ plot is observed, is close to the CMC obtained by the tensimetric method (see Fig. 2, Table 1).

To understand principles of self-organization of micelles of alkylated SCA, one needs information about the influence of the concentration on the nanoparticle size. The surface tension isotherms and the concentration dependence of the particle size of SCA **3** are shown in Fig. 3. A tendency of the nanoparticle to enlarge with an increase in the SCA concentration and reaching a plateau after the CMC confirm micelle formation in the solution. As can be seen from the data in Table 1, micelles of SCA **1** (R = Bu^n) are larger than micelles of **3** (R = $C_{12}H_{25}$). Probably, an efficient micelle formation of SCA requires the length of the hydrocarbon substituent in the molecule to be short (R = Bu^n). The introduction of a longer radical into the SCA molecule worsens the surfactant properties of the compound and decreases the sizes of the nanoparticles formed (see Table 1). According to modern data,[23] a sharp increase in the micelle size at low surfactant concentrations is characteristic of rod-like (vermiform) micelles with the circular transversal cross-section. The linear length of rod-like micelles can vary in wide range: from 10 nm to many hundreds of nanometers.[23]

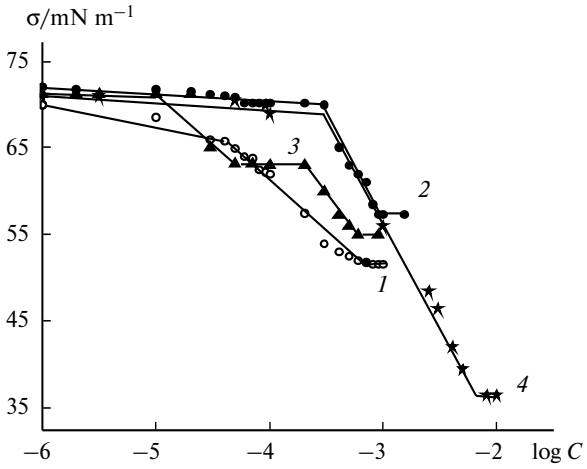


Fig. 1. Surface tension (σ) isotherms of aqueous solutions of SCA **1** (*1*), **2** (*2*), and **3** (*3*) and SDS (*4*) at 25 °C.

Table 1. Critical micelle concentrations (CMC) of alkylated *p*-sulfonatocalix[*n*]arenes **1—5** and the effective hydrodynamic diameter (*D*) of micelles **1—5** at the concentrations higher than the CMC, 25 °C

Compound	CMC · 10^4/mol L^{-1}		*D*/nm
	tensimetry	conductometry	
1	8.0	8.0	200
2	9.0	10.0	200
3	6.0	4.0	120
4	6.0	4.0	250
5	8.0	5.0	200

According to the conductometric data (see Fig. 2, Table 1), SCA are electrolytes and, hence, it was interesting to study the electrokinetic characteristics of dispersed SCA particles. The concentration dependences of the electrokinetic potential (ζ-potential) of nanoparticles in solutions of SCA **3** are shown in inset in Fig. 2. The dependences show that the both parameters, *viz.*, χ and ζ-potential, increase simultaneously with an increase in the SCA concentration. The inflection in the dependence $\chi = f(C)$ in the CMC point is accompanied by reaching a plateau by the ζ-potential. The shape of the concentration dependences of the ζ-potential of the nanoparticles and the electroconductivity of solutions (see Fig. 2) and of the nanoparticle size and surface tension of solutions (see Fig. 3) indicate that such properties of SCA solutions as the electroconductivity and surface tension correlate with the size of micelles and their surface charge.

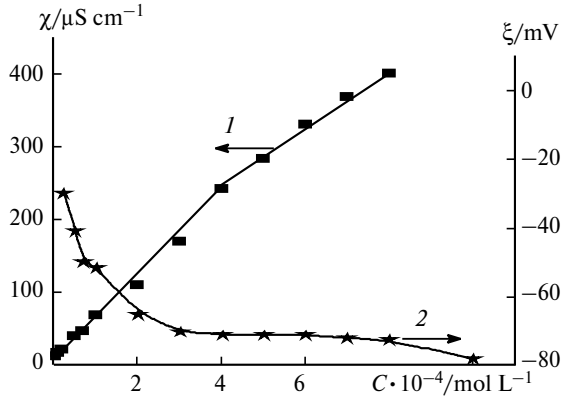


Fig. 2. Electroconductivity (χ) of an aqueous solution of SCA **3** (*1*) and the ζ-potential of nanoparticles of SCA **3** (*2*) *vs* concentration (*C*) at 25 °C.

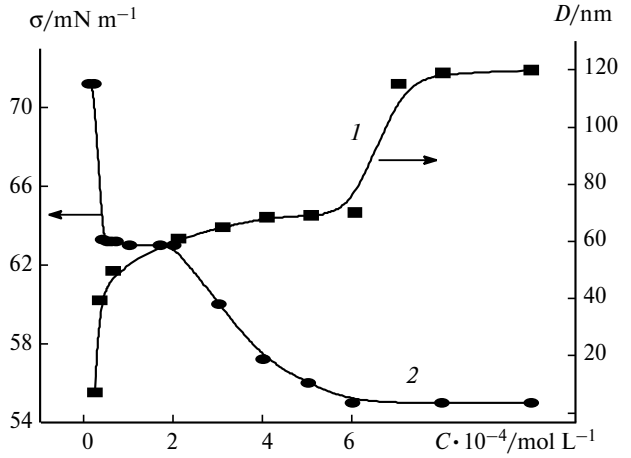


Fig. 3. Surface tension (σ) isotherm of an aqueous solution of SCA **3** (*1*) and the change in the effective hydrodynamic diameter (*D*) of nanoparticles of SCA **3** (*2*) *vs* concentration (*C*) at 25 °C.

It is reasonable to assume that the formation of micelles of SCA **3** characterized by the negative value of the ζ-potential (see Fig. 2) is associated with the dissociation of protons of sulfonate groups, which should decrease the pH of the solution. However, solutions of SCA **3** in the studied concentration range have neutral pH values pH (7.0—7.5), which indirectly indicates that the sulfonate groups are either nonionized, or weakly ionized. In this case, the negative ζ-potentials of the nanoparticles, possibly, imply that the external electric field applied on the solution during electrophoretic experiment favors the proton dissociation of the sulfonate groups and the formation of negatively charged particles, which are uncharged or weakly ionized under normal conditions.

To study the influence of the macrocycle on the self-association of alkylated SCA, we chose compounds **3—5**, whose molecules contain different numbers of aromatic rings: $n = 4$ (**3**), 6 (**4**), and 8 (**5**) at the same length of the hydrocarbon substituent ($R = C_{12}H_{25}$). The surface tension isotherms of these compounds are shown in Fig. 4. They show that with an increase in the macrocycle size the surface activity of the SCA decreases, whereas the CMC value remains almost unchanged (see Table 1). In all cases, the micelle formation process is stepwise, which is related to the rearrangement of nanoparticles, as can be judged from the dynamic light scattering data (see Fig. 3). The ζ-potential values of nanoparticles of SCA **3** and **4** at the concentration $2 \cdot 10^{-4}$ mol L^{-1} are rather high (approximately −70 mV) but lower than those of nanoparticles of SCA **5** (−80 mV) at the same concentration. Solutions of SCA **5** in the concentration ranges below and above the CMC are nearly neutral (pH 6), *i.e.*, the micelle formation of SCA **5** does not acidify the medium as in the case of SCA **3**. This indirectly indicates that the sulfonate groups of micelles of SCA **5** are weakly ionized under normal conditions, whereas the negative values of the ζ-potential are related, as for SCA **3**, to the behavior of the particles during electrophoresis. Unlike compounds **3** and **5**, the micelle formation of SCA **4** is accompanied by the strong acidification of the medium (pH 2.5) similarly to the micelle formation of some organic acids in a surfactant medium.[29] Unlike anionic micelles of **4**, micelles of SCA **3** and **5** have no surface charge or ionized weakly, which was confirmed by us when studying the reactivity of the supramolecular systems based on these compounds.

The study of the hydrolysis of ethyl 4-nitrophenyl chloromethylphosphonate in the supramolecular systems based on SCA **3—5** showed that the observed reaction rate constants (k_{obs}) depend on the concentration of compounds **3** and **5**: they increase with an increase in the concentrations and reach a plateau, which is typical of the micelle-catalyzed reactions[25] (Fig. 5, inset). The reaction parameters calculated by Eq. (1) are listed in Table 2. The reaction rate constants in the micellar phase of SCA **3** and **5** slightly exceed those for an aqueous medium (k_{H_2O}). The catalytic activity of the supramolecular systems of SCA **3** and **5** is comparable with that of the systems based on neutral surfactants, such as Triton-X-100 (see Ref. 27) or amphiphilic calix[4]-resorcinarenes with nonionogenic moieties.[26] The high constants of binding of the ENCP substrate by the SCA micelles confirm the earlier established regularity of efficient binding of the substrates by calixarene micelles.[26—28] The catalytic activity of micelles of **3** and **5** ($n = 4$, 8; $R = C_{12}H_{25}$) in the hydrolysis of ENCP decreases by an order of magnitude with an increase in n, whereas the binding constant of the substrate increases approaching the K_b value of enzymatic reactions for SCA **5**.[30] The kinetic curve of the concentration dependence of SCA **4** differs noticeably from the corresponding curves for other calixarenes (Fig. 5): the k_{obs} values decrease beginning

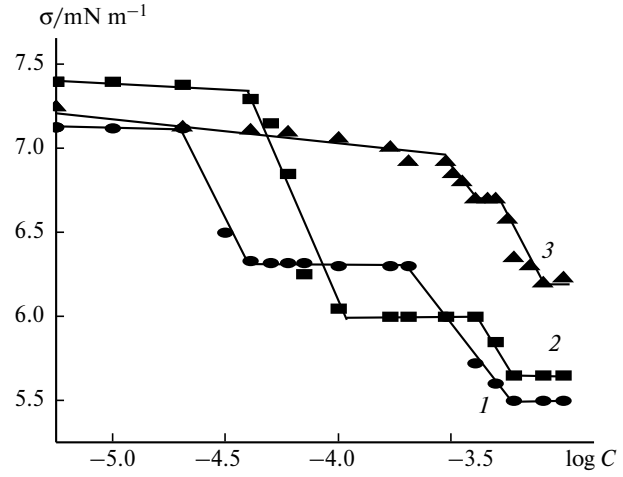


Fig. 4. Surface tension (σ) isotherms of aqueous solutions of SCA **3** (*1*), **4** (*2*), and **5** (*3*) at 25 °C.

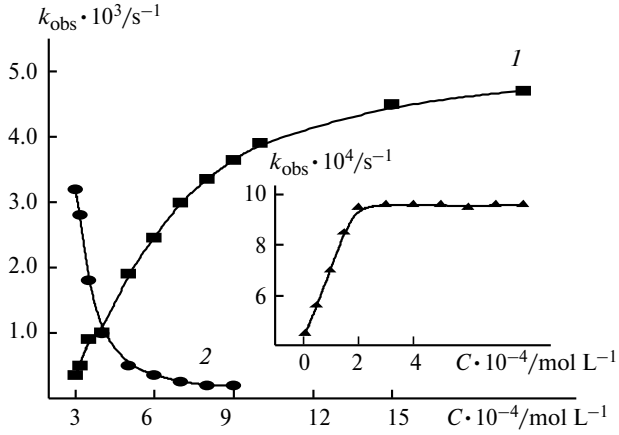


Fig. 5. Observed rate constant (k_{obs}) of hydrolysis of ENCP *vs* concentration (C) of aqueous solutions of SCA **3** (*1*) and **4** (*2*) (pH 9.2, 25 °C). Inset: the dependence of k_{obs} of hydrolysis of ENCP on the concentration of aqueous solutions of SCA **5** (pH 9.2, 25 °C).

Table 2. Kinetic parameters of hydrolysis of ethyl 4-nitrophenyl chloromethylphosphonate in supramolecular systems based on SCA **3—5**

Compound	K_b /L mol^{-1}	k_m /s^{-1}	CMC /mol L^{-1}	k_m/k_{H_2O}
3	1100	$9.2 \cdot 10^{-3}$	$3.0 \cdot 10^{-4}$	230
4	12000	$3.4 \cdot 10^{-4}$	$3.0 \cdot 10^{-4}$	8
5	11000	$1.0 \cdot 10^{-3}$	$5.9 \cdot 10^{-6}$	25

from the concentration $3 \cdot 10^{-4}$ mol L^{-1} close to the CMC of this compound (see Table 1). This behavior of the micellar system in hydrolysis of 4-nitrophenyl esters of phosphorus acids are usually related to the inhibition effect of anionic micelles of the surfactant,[25] which mainly occurs due to the electrostatic repulsion of the nucleophiles (hydroxyl ions) from the negatively charged surface of the micelles that solubilize the substrate. Thus, the assumption that SCA **4**, unlike compounds **3** and **5**, forming, most probably, weakly ionized micelles, form micelles of the anionic type is confirmed by the kinetic data.

We have earlier found that the amphiphilic derivatives of calix[4]resorcinarenes are membranotropic compounds capable of exerting different effects on the energy exchange in plant cells depending on the structure of the head groups and the size of nanoparticles formed by these groups.[22] In the present work, we revealed the most significant structural differences in the series of SCA (macrocycle size, n; the presence of hydrophobic substituents, R) affecting the characteristics of the nanoparticles, probably, respective for the modifying action of SCA solutions on the energy exchange in plant cells. The study of the physiological action on the plant cells of solutions of unmodified **6—8** and alkylated SCA **1—5** showed that calixarenes can exert different effects on the energy exchange of plant cells in the presence of the same head sulfonate groups: they can stimulate, suppress, or exert no effect. For instance, tetrameric (n = 4) SCA **1—3** and **6** in a concentration of $5 \cdot 10^{-5}$ mol L^{-1} exerted almost no effect on the energy exchange in wheat roots. Based on the results obtained, we may assume that this behavior of the tetrameric SCA is related to an insufficient hydrophobicity and low surface charges of the nanoparticles: this affects the ζ-potential values (changing from −20 to −40 mV).

More hydrophobic conformationally mobile SCA **7** and **4** (n = 6; R = OH, $C_{12}H_{25}$, respectively) in a concentration of $5 \cdot 10^{-5}$ mol L^{-1} exert a noticeable but opposite effect on the oxygen consumption by the roots, the pH of the incubation medium, and the escape of potassium ions from the cells: SCA **7** suppresses the energy exchange of cut-off wheat roots, and SCA **4** stimulates it (Fig. 6, *a*). The reason for different directions of the bioeffects of compounds **4** and **7** can be both their different lipophilicities and, hence, the ability to penetrate into the cell membrane,[31] as well as different surface charges of nanoparticles, which reflects the ζ-potential value, which is much lower for nanoparticles of SCA **7** (−30 mV) compared to that of nanoparticles of SCA **4** (−70 mV).

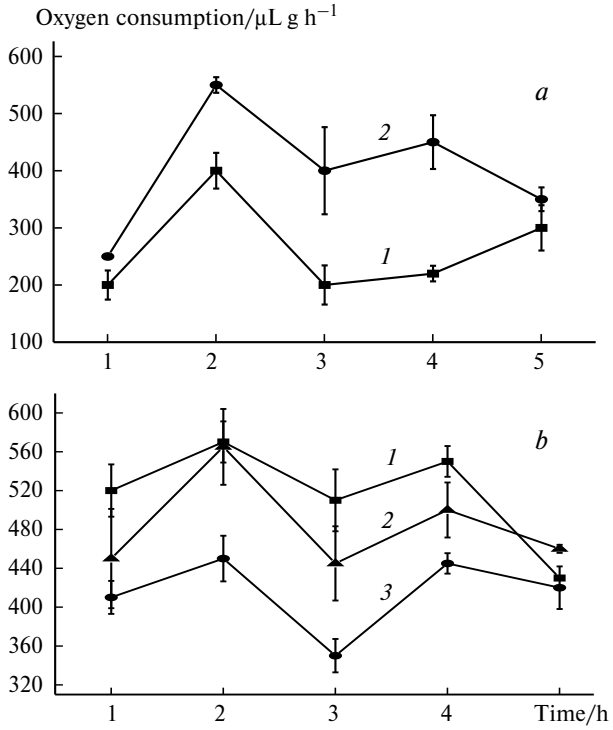


Fig. 6. Change in the rate of oxygen consumption by cut wheat roots under the action of SCA solutions: *a*, reference sample (*1*) and SCA **4** (*2*); *b*, reference sample (*1*), SCA **8** (*2*), and SCA **5** (*3*).

The further increase in the macrocycle size results in the situation when octameric SCA **5** (n = 8, R = $C_{12}H_{25}$) and **8** (n = 8, R = H) suppress the energy exchange in cells (Fig. 6, *b*). Since micelles of **5** possess a high binding ability toward phosphorus acid esters (see Table 2) compared to the enzymatic ability,[30] it can be assumed that SCA **5** form complexes with components of membranes or membrane proteins, inhibiting enzymes and violating metabolism of the cell. The fact that nonalkylated SCA **8** less efficiently affects plant cells than alkylated SCA **5** (see Fig. 6, *b*) is probably due to the lower ability of compound **8** to bind with plasmalemma because of the lower hydrophobicity.

Thus, the study of the modifying effect of a series of SCA on the membranes and energy exchange in wheat root cells showed that the biological activity of the SCA increases with an increase in the macrocycle size and hydrophobicity of the substituents. This results in substantial differences in the electrokinetic characteristics of nanoparticles of SCA of different structure and their binding ability and reactivity toward phosphorus acid esters, which provides fine control of bioeffects of the supra-

molecular systems based on these compounds. The results obtained create prerequisites for the further combined study of self-organization in mixed supramolecular systems "amphiphilic calixarene—biologically important compound" and bioeffects in these systems.

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