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Unusual Behavior of Membrane Somatic Angiotensin-Converting Enzyme in a Reversed Micelle System

S. V. Grinshtein, A. V. Levashov, and O. A. Kost*

School of Chemistry, Lomonosov Moscow State University, Moscow, 119899 Russia; fax: (095) 939-5417; E-mail: kost@enzyme.chem.msu.ru

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Abstract—Properties of the membrane and soluble forms of somatic angiotensin-converting enzyme (ACE) were studied in the system of hydrated reversed micelles of aerosol OT (AOT) in octane. The membrane enzyme with a hydrophobic peptide anchor was more sensitive to anions and to changes in pH and composition of the medium than the soluble enzyme without anchor. The activity of both forms of the enzyme in the reversed micelles significantly depended on the molarity of the buffer added to the medium (Mes-Tris-buffer, 50 mM NaCl). The maximum activity of the soluble ACE was recorded at buffer concentration of 20-50 mM, whereas the membrane enzyme was most active at 2-10 mM buffer. At buffer concentrations above 20 mM, the rate of hydrolysis of the substrate furylacryloyl-L-phenylalanyl-glycylglycine by both ACE forms was maximal at pH 7.5 both in the reversed micelles and in aqueous solutions. However, at lower concentrations of the buffer (2-10 mM), the membrane enzyme had activity optimum at pH 5.5. Therefore, it is suggested that two conformers of the membrane ACE with differing pH optima for activity and limiting values of catalytic constants should exist in the reversed micelle system with various medium compositions. The data suggest that the activity of the membrane-bound somatic ACE can be regulated by changes in the microenvironment.

Key words: angiotensin-converting enzyme, membrane form, soluble form, reversed micelles, aerosol OT

Angiotensin-converting enzyme (ACE, peptidyl dipeptidase A, EC 3.4.15.1) is a physiologically important membrane glycoprotein of humans and animals. The somatic enzyme found in virtually all tissues of the body consists of two domains and contains two highly homologous active sites. This enzyme is mainly involved in the regulation of blood pressure and water-salt metabolism [1, 2]. In the body, ACE functions as a membrane-bound enzyme (m-ACE) that is bound to the plasma membrane of the cell [1, 3] through the C-terminal hydrophobic peptide anchor [4]. Under the influence of a specific secretase, the anchor (~8 kD) can be split off with the generation of a soluble form of the enzyme (s-ACE) that is released into biological fluids [4-6]. So far, the properties of the enzyme have been mainly studied in aqueous solutions, and no difference was found in the properties of the s-ACE and the m-ACE [7]. However, membrane enzymes are known to be very sensitive to their environment, and functions of the membrane-bound forms are suggested to have some specific features that fail to manifest themselves in homogenous aqueous solutions [8]. This suggestion is directly based on findings on the catalytic properties of the membrane-bound ACE in Chinese hamster ovary cell culture [9]. The catalytic constants of hydrolysis of the natural substrate (angiotensin I) on the N- and C-domains of the membrane-bound enzyme were found to differ from those in aqueous solutions [9].

We studied earlier properties of s- and m-ACE in a system of hydrated reversed micelles (AOT in octane) that simulates biological membranes, and the membrane enzyme, which actively interacted with the micellar matrix, was found to be more sensitive to the presence of chloride anions in the medium and to have a higher catalytic activity [10]. It was also shown that s-ACE could function as a monomer, a compact dimer, and a tetramer, whereas m-ACE functioned as three different dimers and a compact tetramer [10]. Moreover, ACE immobilized on carbohydrate carriers was more sensitive to changes in the environmental pH and to the presence of activating anions [11].

The purpose of the present work was to compare the properties of various oligomeric structures of the soluble and membrane somatic ACE in a system of reversed micelles (AOT in octane) at different concentrations of anions, pH values, and composition of the medium.

* To whom correspondence should be addressed.

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MATERIALS AND METHODS

Preparation of soluble and membrane forms of ACE. The enzymes were prepared from bovine lungs by an earlier developed method that included hydrophobic and affinity chromatography [12]. This method resulted in preparations of individual forms of the enzyme that were homogenous on SDS electrophoresis and that contained nearly 100% active molecules according to the data of titration with lisinopril [12]. The presence of a hydrophobic anchor in molecules of the membrane enzyme was confirmed by the somewhat decreased electrophoretic mobility of the m-ACE compared to the mobility of the s-ACE (185 and 180 kD, respectively) and also by a significantly higher hydrophobicity of the m-ACE on phase separation of the two forms of the enzyme in a solution of Triton X-114 [12].

Kinetics of ACE-catalyzed hydrolysis of furylacryloyl-L-phenylalanyl-glycylglycine (FA-Phe-Gly-Gly) in aqueous solution. The kinetic parameters of ACE-catalyzed hydrolysis of the substrate FA-Phe-Gly-Gly (Sigma, USA) were determined with varying substrate concentration in the range from 50 to 2000 μM as described in [10]. The activity was determined by the initial rate of hydrolysis of the substrate as described previously [13] using a Shimadzu UV-265FW spectrophotometer (Japan) equipped with a thermostatted cuvette section.

The concentration of active sites of ACE was determined by a modified stoichiometric titration with a specific competitive inhibitor, N-(S-1-carboxy-3-phenylpropyl)-L-lysyl-L-proline (lisinopril, Sigma) as described earlier [14].

Activity of ACE in the reversed micelle system. The activity of ACE in reversed micelles of sodium di-(2-ethylhexyl)sulfosuccinate (aerosol OT, AOT) (Fluka, Austria) at various hydration degrees was determined by the initial rate of hydrolysis of FA-Phe-Gly-Gly using the Shimadzu UV-265FW spectrophotometer as described in [10]. The required hydration degree of AOT (w_0, $[H_2O]/[AOT]$) in the system was obtained by varying the amount of an aqueous buffer. As a rule, 1 ml of 0.3 M AOT solution in octane and the volume of buffer (105-270 μl) to provide the required value of w_0 (25-55) were placed into a spectrophotometric cuvette. The mixture was shaken vigorously until an optically transparent solution was produced, and the system was supplemented with 5-15 μl of acetonitrile. Then 20 μl of 0.5 μM solution of the enzyme was added. The reaction mixture was shaken repeatedly and incubated for 20 min at 25°C. Then 10 μl of 9-10 mM solution of the substrate was added to the final concentration of 80 μM, the mixture was shaken, and the rate of decrease in absorption at 328 nm was recorded.

Effect of anions on the activity of ACE in the reversed micelle system. Preparations of the enzyme were preliminarily desalted in an ultrafiltration cell, and buffers and substrate solutions were prepared in deionized water. Desalting was confirmed by the absence of enzymatic activity in chloride-free aqueous buffer. The activity of ACE in the presence of various concentrations of anions was determined in 0.3 M AOT micelles at various hydration degrees (w_0 from 27 to 50) as described earlier. In the enzymatic reaction, 20 mM Tris-CH_3COOH buffer (pH 7.5) containing 1 μM zinc acetate and the enzyme at 10 nM concentration were used. Contents of anions (Cl^- and NO_3^-) in the added buffer were varied from 0 to 150 mM.

The pH dependence of FA-Phe-Gly-Gly hydrolysis. The catalytic activities of s- and m-ACE in the reversed micelle system were determined for various pH values ranging from 5.5 to 8.5 and for various molarities (from 1 to 100 mM) of the universal Mes-Tris buffer containing 50 mM NaCl and 1 μM $ZnCl_2$. The activity was determined as described above. Moreover, the pH dependence of the activity of m-ACE over a wider range of pH (from 4 to 9) was studied using universal 5 mM Mes-Tris-acetate buffer containing 50 mM NaCl and 1 μM $ZnCl_2$.

Kinetic parameters of ACE-catalyzed hydrolysis of FA-Phe-Gly-Gly in the reversed micelle system. These parameters were determined at 0.05-0.3 M AOT in octane (w_0 from 27 to 50) as described earlier [10]. Mes-Tris buffer (5 or 20 mM, pH 5.5-7.5) containing 50 mM NaCl and 1 μM $ZnCl_2$ was used for the aqueous phase. The substrate concentration was varied from 50 to 500 μM, and the resulting dependences of the initial rates of substrate hydrolysis by both ACE forms on the initial concentration of the substrate followed Michaelis–Menten kinetics.

Catalytic constants were calculated using the concentration of the active enzyme in the micellar system determined by titration with lisinopril at different hydration degrees w_0: 27, 31, 47, and 50. About 60 nM ACE in the reversed micelles were incubated for 20 min, then lisinopril (0-100 nM in the reaction medium) was added, and the mixture was incubated for 30 min more. The residual enzymatic activity in this system was determined as described above.

Dependence of s- and m-ACE activity on the concentration of AOT in the reversed micelle system. These experiments were carried out at constant values of w_0 (27-50). The concentration of AOT in octane was varied from 0.05 to 0.3 M. The activity of ACE was characterized by the catalytic constants of FA-Phe-Gly-Gly hydrolysis.

Limiting value of the catalytic constant. The limiting value of the catalytic constant (k_{lim}) for m-ACE-catalyzed FA-Phe-Gly-Gly hydrolysis in the reversed micelle system was determined from dependences of k_{cat} values on the concentration of AOT. The k_{lim} value that characterizes the limiting catalytic activity of the enzyme in an isolated enzyme-containing micelle was calculated from the value of the ordinate-axis intercept on extrapolation to zero of the linear dependence $1/k_{cat}$ on [AOT] [15].

RESULTS AND DISCUSSION

The sensitivity of the membrane form of ACE in the reversed micelle system of AOT in octane to the presence of anions and to changes in pH and composition of the medium was higher than that of the soluble enzyme. These results were obtained for various oligomeric structures of ACE generated in this system [10]. The dependence of the catalytic activity of both forms of the enzyme on the hydration degree (w_0, the micelle size) under standard conditions (pH 7.5, 50 mM NaCl, 1 μM $ZnCl_2$) and sedimentation analysis of the protein-containing reversed micelles showed that the s-ACE could function as a monomer (w_0 27), a compact dimer (w_0 31), and a tetramer, whereas the m-ACE could function as three dimers (w_0 31, 35, and 40) and as a rather compact tetramer (w_0 47) [10].

Effect of anions on activity of s- and m-ACE in the reversed micelle system. A characteristic feature of ACE is the dependence of its activity on the presence of activating anions, in particular Cl^- [1]. The specific activation of somatic ACE by chloride was significantly different in the micellar medium compared to that in aqueous solutions (Figs. 1 and 2, the curves *1*).

Addition of NaCl into the buffer had no effect on the activities of the monomer, dimer, and tetramer of s-ACE in the reversed micelle system (a typical dependence is presented in Fig. 2), i.e., any oligomeric structure of the enzyme manifested the maximum activity even in the absence of a classical activating anion. Various explanations are suggested for this phenomenon. First, the maximum activity of the enzyme might be provided by a high local concentration of charged sulfate groups of AOT in the micelle inner cavity because high concentrations of sulfate anions markedly activate the enzyme in aqueous solutions [10]. Second, this phenomenon might be caused by the rigid structure of the micelle itself maintaining the active conformation of the enzyme [16], and in this case the enzymatic activity could be independent of the activator presence in the system.

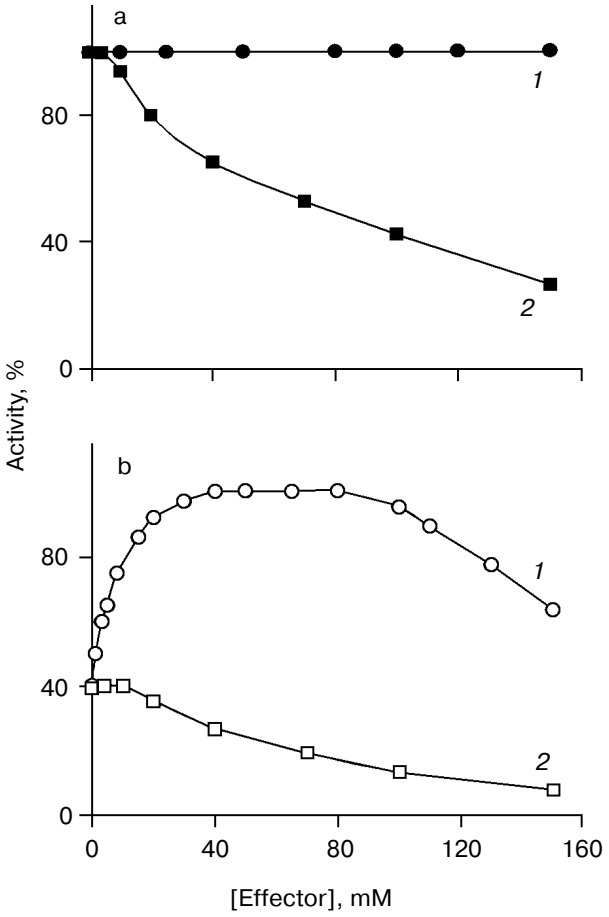

Fig. 2. Dependence of activity of dimers of s- (a) and m- (b) somatic ACE enclosed in the reversed micelle system on the concentration of chloride (*1*) and nitrate (*2*) calculated per aqueous phase of the system. The maximum enzymatic activity was taken as 100%. Conditions: 0.3 M AOT in octane; w_0 31; aqueous phase, 20 mM Tris-CH_3COOH buffer (pH 7.5), 1 μM $Zn(CH_3COO)_2$.

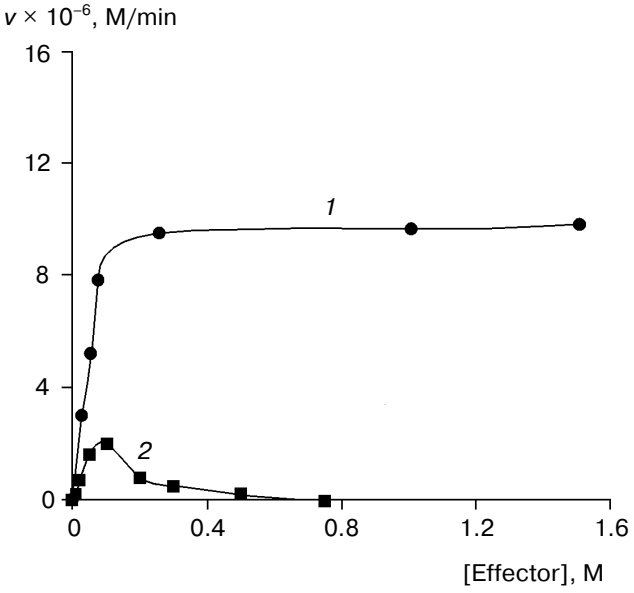

Fig. 1. Dependence of activity of ACE on the concentration of chloride (*1*) and nitrate (*2*) in aqueous medium. Conditions: 50 mM Tris-CH_3COOH buffer (pH 7.5), 1 μM $Zn(CH_3COO)_2$, enzyme concentration 3 nM, substrate (FA-Phe-Gly-Gly) concentration 60 μM.

However, the activity of m-ACE in the micellar system depended on the presence of chloride (Fig. 2). In the absence of Cl^-, the membrane ACE, like the soluble ACE form, demonstrated catalytic activity (about 40% of the maximal activity). Addition of anions increased the activity of the enzyme up to the maximum value in the presence of 50 mM activator, whereas in aqueous

solutions the maximum activity of the enzyme was recorded in the presence of no less than 150 mM NaCl (Fig. 1). Further increase in the concentration of NaCl in the micellar system was accompanied by inhibition of the enzymatic activity. Note, that various dimers and the tetramer of m-ACE exhibited similar dependence on chloride. This change in the characteristic activation of the enzyme by Cl^- was earlier recorded for ACE immobilized on solid carbohydrate carriers: the enzyme manifested a certain catalytic activity in the absence of activating anions, and the optima of enzymatic activity were shifted to lower Cl^- concentrations [11].

Is this activation-inactivation of the membrane enzyme by Cl^- in the reversed micelle system a specific effect or is it caused by the ionic strength of the buffer solution used? To answer this question, we studied the effect of sodium nitrate concentration on the activity of ACE in this system. Nitrate had virtually no activating effect of ACE in aqueous buffer solution (Fig. 1, the curve *2*). But in the micellar system, increased concentration of sodium nitrate in the buffer solution decreased the activity of both s- and m-ACE (Fig. 2, curves *2*). The inactivation of both enzyme forms was probably due to an inhibiting effect of ionic strength. The increase in ionic strength was probably associated with changes in the properties of the system itself, in particular, destabilization of micelles could occur, as described earlier [17].

Thus, it was concluded that the activation of the membrane ACE in the reversed micelle system in the presence of chloride anions was specific, whereas the inactivation at the increased concentration of salt to more than 100 mM seemed to be associated with the inhibiting effect of ionic strength. However, on increasing the concentration of chloride to 150 mM, no decrease in the activity of s-ACE was recorded in the micelle system (Fig. 2). This seems to indicate that a cancellation occurred between the chloride-induced activation and the inactivation caused by ionic strength.

Note that all subsequent determinations of ACE activity in the system of reversed micelles of AOT in octane, except the determinations described in this section, were carried out in the presence of 50 mM NaCl in the added buffer, i.e., at the optimum chloride content providing the functions of both forms of the enzyme.

Effects of environmental features (pH and molarity of buffer used for the aqueous phase) on activity of s- and m-ACE in the reversed micelle system. The hydrolysis of FA-Phe-Gly-Gly in the system of micelles under standard conditions (20 mM Mes-Tris buffer (pH 7.5), 50 mM NaCl, 1 μM $ZnCl_2$) by s- and m-ACE was characterized by the same pH optimum (pH 7.5) in the aqueous solution (Fig. 3, the curves *2*). However, varying the molarity of the buffer (Mes-Tris buffer containing 50 mM NaCl) had a significant effect on the activity of both forms of the enzyme (Fig. 4) and on the pH dependence of m-ACE (but not s-ACE!) (Fig. 3).

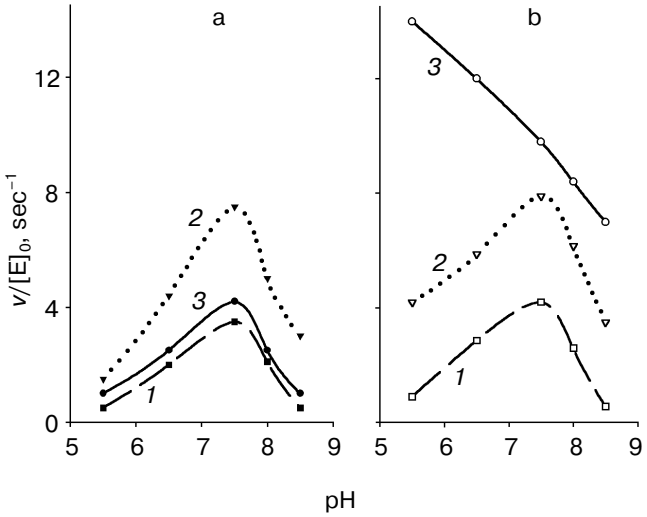

Fig. 3. Dependence of the catalytic activity of the soluble (a) and membrane (b) somatic ACE on pH of the buffer in the reversed micelle system at 100 (*1*), 20 (*2*), and 5 mM (*3*) buffer concentrations. Conditions: 0.3 M AOT in octane; w_0 31; for the aqueous phase universal Mes-Tris buffer, 50 mM NaCl, 1 μM $ZnCl_2$ was used; enzyme concentration, 10 nM; substrate, 80 μM FA-Phe-Gly-Gly.

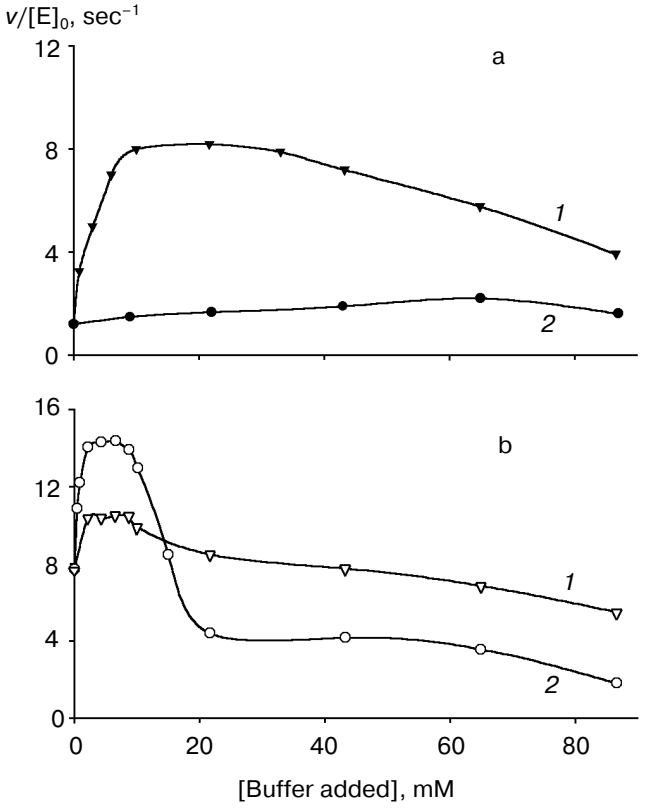

Fig. 4. The dependence of the activity of soluble (a) and membrane (b) somatic ACE in the reversed micelle system on concentration of the buffer at pH 7.5 (*1*) and 5.5 (*2*) (for conditions, see legend to Fig. 3).

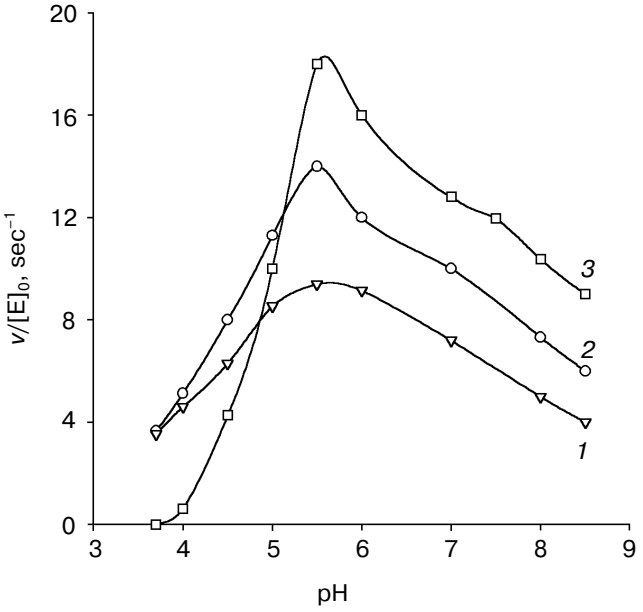

Fig. 5. Dependence of the catalytic activity of the monomer (w_0 27) (*1*), dimer (w_0 31) (*2*), and tetramer (w_0 47) (*3*) of m-ACE in the reversed micelle system on pH value of the buffer solution added to the system. Conditions: 0.3 M AOT in octane; aqueous phase, 5 mM universal Mes-Tris-acetate buffer, 50 mM NaCl, 1 μM $ZnCl_2$.

The activity of the enzyme with and without the anchor depended differently on the buffer molarity. The activity of s-ACE was maximal in the presence of 20-50 mM buffer, whereas the maximal activity of m-ASE was found over a narrower range and at lower concentrations of the buffer: from 2 to 10 mM (Fig. 4). Increasing the buffer concentration to 100 mM was accompanied by a monotonous decrease in the activity of each enzyme (Fig. 4); this was suggested to be caused by a destabilization of the system [17].

The buffer molarity dependence profile of ACE activity also depended on the pH of the medium. Thus, at pH 5.5 the activity of s-ACE only slightly depended on the buffer concentration, whereas the activity of m-ACE sharply increased with decrease in buffer concentration to <20 mM (Fig. 4, the curves *2*). The activity maximum of the membrane enzyme was also recorded at buffer concentration of 2-10 mM, as it was at pH 7.5, but the enzymatic activity was even higher than at pH 7.5.

It was found that the usual pH optimum of ACE at 7.5 was maintained for both forms of the enzyme at buffer concentration >20 mM (Fig. 3, the curves *1* and *2*). But the pH profile of the membrane enzyme, unlike that of the soluble enzyme, was significantly changed at low concentration of the buffer (5 mM) (Fig. 3, the curves *3*). The findings indicate that the enzyme with the anchor is more sensitive to changes in the microenvironment than the enzyme without anchor.

The pH optimum of activity of all ACE oligomeric structures in 5 mM buffer in the reversed micelle system was shifted to more acidic values, to pH 5.5. Figure 5 shows findings for the monomer, dimer, and tetramer of the enzyme. Note, that the changes recorded in the pH profile were not associated with changes in stability of the membrane enzyme or in its characteristic activation by chloride anions under these conditions. Thus, the activation profile of m-ACE by chloride in 5 mM buffer (pH 5.5) virtually coincided with the profile recorded in 20 mM buffer (pH 7.5).

Moreover, under these conditions (5 mM buffer, pH 5.5), the dependence of activity of the membrane enzyme on hydration degree was changed (Fig. 6). In this case, a peak of activity of the monomer of somatic ACE (w_0 27) was found (Fig. 6b) that was not specific for this enzyme under standard conditions, i.e., in 20 mM buffer [10] (Fig. 6a). Besides, the dependence curve had only two peaks of ACE dimer activity, at w_0 values of 31 and 35, and a maximum at w_0 47 that was specific for the tetramer of the enzyme. These findings suggest that varying the buffer molarity is accompanied by changes in the structure of

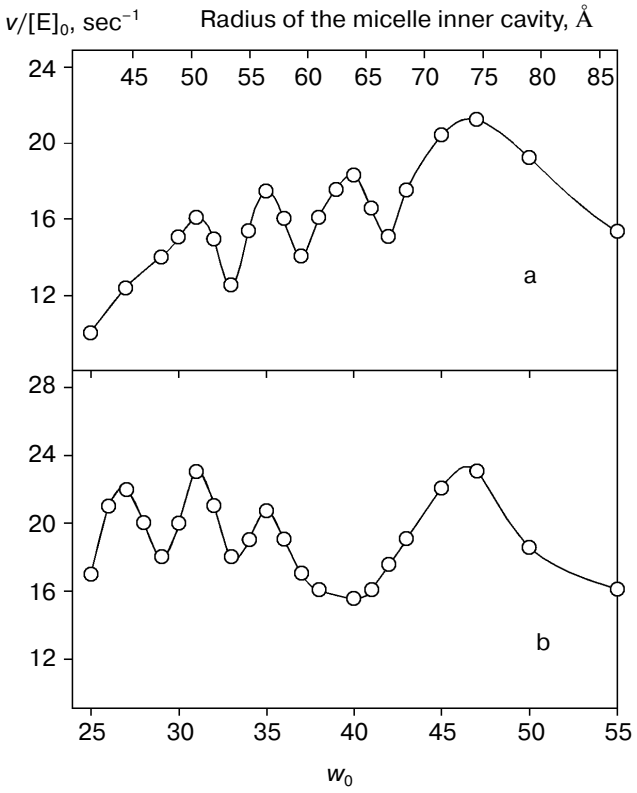

Fig. 6. Dependence of the catalytic activity of the membrane somatic ACE on the hydration degree of AOT in the reversed micelle system. Conditions: 0.3 M AOT in octane; 20 mM Mes-Tris buffer (pH 7.5), 50 mM NaCl, 1 μM $ZnCl_2$ (a); 5 mM Mes-Tris buffer (pH 5.5), 50 mM NaCl, 1 μM $ZnCl_2$ (b); the enzyme concentration in the system 10 nM; the substrate FA-Phe-Gly-Gly concentration 80 μM.

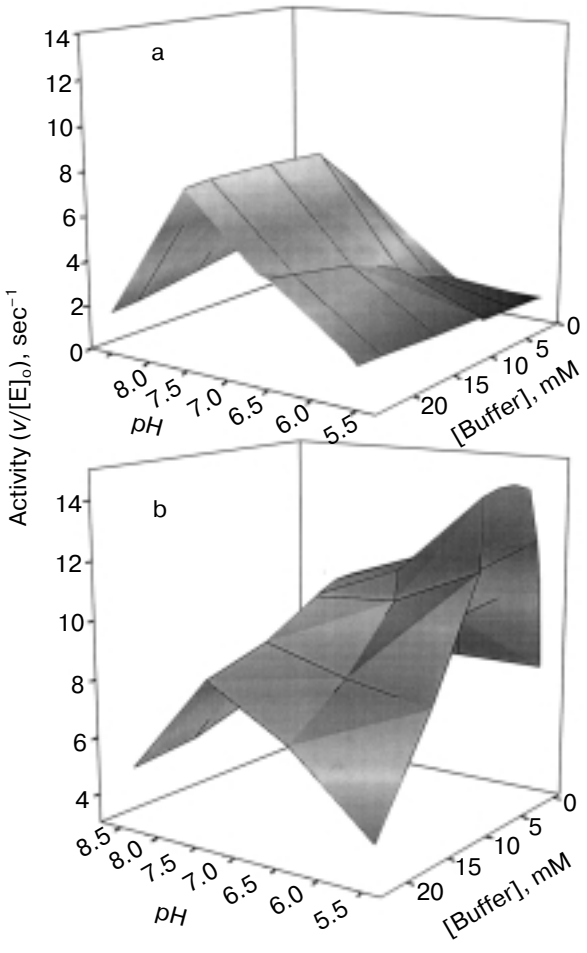

Fig. 7. Activity dependence of the soluble (a) and membrane (b) somatic ACE on molarity and pH of the buffer solution added into the system of reversed micelles AOT in octane. Conditions: 0.3 M AOT in octane; w_0 31; aqueous phase, universal Mes-Tris buffer, 50 mM NaCl, 1 μM $ZnCl_2$; enzyme concentration, 10 nM; substrate, 80 μM FA-Phe-Gly-Gly.

the somatic m-ACE resulting in changes in its oligomerization and in activity.

The shift of the pH profile of the membrane enzyme activity is probably caused by low capacity of the low-molarity buffer and thus, by its inability to maintain constant pH in the inner cavity of the micelles [17]. However, the pH dependence of s-ACE was not changed when 5 mM buffer was used (Fig. 3, the curve *3*); thus, the buffer capacity was sufficient. Consequently, it is unlikely that the surface membrane potential induced the shift of the pH profile of the membrane enzyme.

To explain these findings, it was suggested that in the reversed micelle system there are at least two different conformations of the membrane ACE that are different in catalytic activity and in pH optimum. A possible conversion of one conformation of ACE into another can be easily observed upon analysis of the surface demonstrating the effects of the pH and molarity of the buffer on the enzyme activity in the reversed micelle system (Fig. 7). For the sake of convenience, let us denote the m-ACE conformation specified by the optimum pH 7.5 as conformer I, and the conformation specified by the optimum enzymatic activity at pH 5.5 as conformer II.

Catalytic parameters of FA-Phe-Gly-Gly hydrolysis by soluble and membrane ACE in the reversed micelle system. To compare catalytic properties of all oligomeric structures of the s- and m-ACE in the reversed micelle system, kinetic parameters of the enzymatic hydrolysis of FA-Phe-Gly-Gly were determined at various concentrations of AOT. In the case of m-ACE, the study was carried out not only under standard conditions (20 mM Mes-Tris buffer, pH 7.5) but also under the optimal conditions to

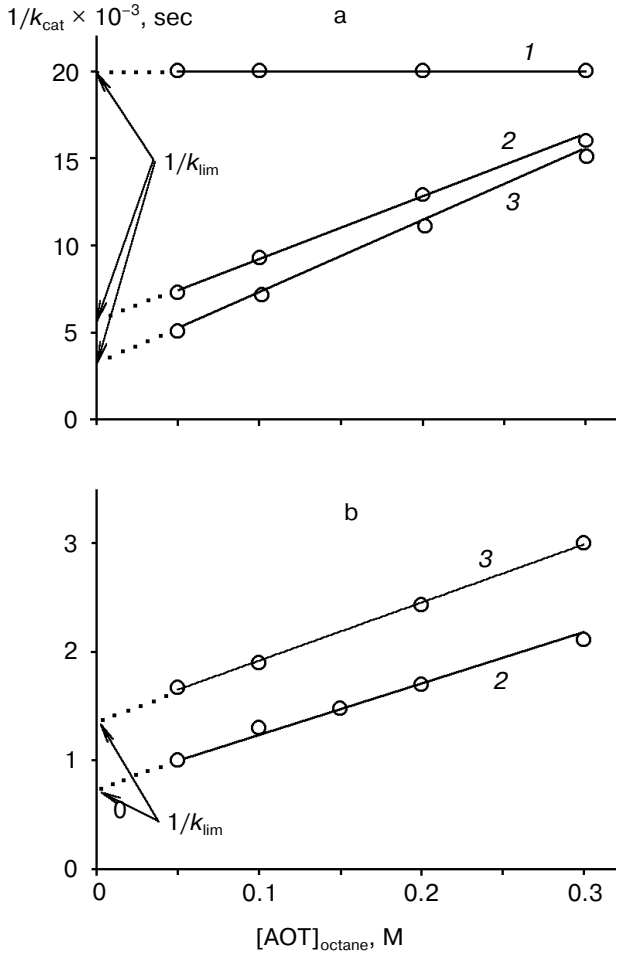

Fig. 8. Dependence of the catalytic constant (k_{cat}) of FA-Phe-Gly-Gly hydrolysis by s-ACE (*1*) and m-ACE (*2* and *3*, dimer and tetramer, respectively) on AOT concentration in the reversed micelle system with 20 mM buffer, pH 7.5 (a), and 5 mM buffer, pH 5.5 (b), as the aqueous phase. Conditions: aqueous phase, universal Mes-Tris buffer containing 50 mM NaCl and 1 μM $ZnCl_2$; enzyme concentration, 10 nM.

Limiting values of catalytic constants (k_{lim}) of FA-Phe-Gly-Gly hydrolysis by various structural forms of bovine somatic ACE in the system of reversed micelles AOT in octane

Oligomeric form of enzyme	Buffer concentration and pH optimum	k_{lim}, sec^{-1}
	s-ACE	(280 ± 20)
Monomer	20 mM, pH 7.5	50 ± 4
Compact dimer	20 mM, pH 7.5	55 ± 6
Tetramer	20 mM, pH 7.5	62 ± 8
	m-ACE	(300 ± 20)
Conformer I		
Compact dimer	20 mM, pH 7.5	190 ± 20
Tetramer	20 mM, pH 7.5	370 ± 50
Conformer II		
Monomer	5 mM, pH 5.5	570 ± 60
Compact dimer	5 mM, pH 5.5	1230 ± 130
Tetramer	5 mM, pH 5.5	715 ± 75

Note: The aqueous phase of the reversed micelles was provided by Mes-Tris buffer containing 50 mM NaCl and 1 μM $ZnCl_2$ (25°C). In parentheses: values of the catalytic constants obtained under the optimum conditions for the enzyme functioning in aqueous solutions (50 mM Tris buffer, pH 7.5, 150 mM NaCl, 1 μM $ZnCl_2$; 25°C).

provide functions of the hypothetical conformer II of the enzyme (5 mM Mes-Tris buffer, pH 5.5).

Catalytic constants of hydrolysis of the substrate by various oligomers of the soluble ACE (monomer, a compact dimer, and tetramer) did not depend on the concentration of AOT in the system (a typical curve is presented in Fig. 8a). However, the enzymatic activity of the membrane ACE significantly depended on the concentration of AOT (typical curves are presented in Fig. 8, a and b). Such behavior is specific for enzymes that have "anchor" groups and are able to interact with the micellar matrix [18]. A decrease in the content of the surface active substance AOT in the system at constant value of w_0, i.e., dilution of the micellar system with octane, was accompanied by an increase in the activities of the compact dimer and tetramer of conformer I and also by an increase in the activities of the dimer and tetramer of conformer II (Fig. 8, a and b). Consequently, independently of the properties of the medium, various structural forms of the membrane enzyme could interact with the micellar matrix.

The dependence of catalytic activity of the membrane ACE on concentration of AOT was linear in coordinates $1/k_{cat}$ against [AOT] (Fig. 8, a and b), and this allowed us to calculate the limiting values of catalytic constants (k_{lim}) with the AOT concentration tending to zero. The k_{lim} value characterizes the true activity of the membrane enzyme in an isolated micelle. The data are summarized in the table.

The data presented show that the catalytic properties of s- and m-ACE were significantly different in the reversed micelle system. On the whole, the catalytic activity of the membrane ACE was significantly higher than the activity of the soluble enzyme. While values of the catalytic constants of the monomer, compact dimer, and tetramer of s-ACE were similar, i.e., the activity of the soluble enzyme did not change during the generation of the homo-oligomers, the activity of m-ACE significantly depended on the oligomeric state of the enzyme. Thus, the limiting catalytic constants of FA-Phe-Gly-Gly hydrolysis by the compact dimers of conformers I and II of the membrane ACE were 3.5 and 22 times higher, respectively, than the corresponding value describing the activity of the dimer of the soluble enzyme. In the case of the tetramer, a similar comparison results in 6- and 12-fold difference. The significant difference in the activities of the s- and m-ACE found in the system modeling the membrane microenvironment suggests that a similar phenomenon could exist *in vivo* upon the interaction of this enzyme with biological membranes.

The table shows that in the reversed micelle system the values of k_{lim} for all structural forms (monomer, dimer, tetramer) of conformer II of the membrane ACE were higher than the k_{cat} value characteristic for the enzyme in aqueous solutions. The activity of the monomer of m-ACE was also lower than the activity of the oligomeric structures of the enzyme, i.e., the membrane ACE was more efficient in aggregates.

Overall, all oligomeric structures of the suggested conformer II of ACE were more active in the system of reversed micelles than the corresponding oligomeric forms of conformer I (table). In the case of conformer II, the compact dimer was the most active, and in the case of conformer I, the tetramer was most active. Consequently, various supramolecular structures of the membrane ACE manifested the highest activity depending on environmental conditions (pH of the medium and composition of the aqueous phase).

Thus, the findings suggest that the activity of m-ACE is regulated by varying both the composition of the medium and the oligomeric state of the enzyme. Obviously, changes in the environment (pH and composition) should be a powerful regulator of ACE activity *in vivo*.

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