Radiation-induced inactivation of angiotensin-converting enzyme in solutions
2.* The pH-dependence

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Radiation-induced inactivation of angiotensin-converting enzyme (ACE) in the low-dose range is characterized by the existence of a concentration- and pH-dependent induction period, activation, and inactivation. These effects are probably related to the change in the conformational flexibility of the active center in conjunction with the retention of the "autoprotective" multistep mechanism of the protection of the entire molecule. The schemes of radiation-induced reactions occurring in ACE are presented. The radiation-chemical yield of inactivation of ACE has a maximum at pH 7.5 independent of the initial concentration of the enzyme, which allows inference of a conformational transition in this pH range.

Key words: angiotensin-converting enzyme, radiation-induced inactivation; stability, pH-dependence.

Angiotensin-converting enzyme, ACE (EC 3.4.15.1), is a Zn-containing peptidase and a membrane-bound glycoprotein. It is known that this enzyme is involved in various regulatory physiological processes occurring in an organism (in particular, in the control of blood pressure), as hydrolyzing vasoactive peptides (angiotensin I and bradykinin).[2] Evidently, the processes of inactivation and activation of ACE can be of great importance for an organism, causing pathological changes. The radiation-induced inactivation is an *in vitro* model of changes of the enzyme *in vivo*.

In the previous paper,[1] possible reasons for an induction period on plots of dose dependences of radiation-induced inactivation of ACE obtained in >50 Gy dose ranges were discussed. Previously, we have not revealed any induction period in radiolysis of a series of proteinases,[3] such as chymotrypsin, trypsin, and subtilysine, but it occurred in radiolysis of horseradish peroxidase.[1,4]

It was suggested that the possible reasons for the observed induction period may be the large number of aromatic amino acid residues in the protein molecule including the surface residues that are not essential for execution of enzymatic functions; the presence of carbohydrate residues in ACE and horseradish peroxidase; and the probable formation of protein associates. It was suggested that the higher the degree of protection of a biomolecule (*i.e.*, the higher the "threshold" of the internal changes that are followed by destructive processes), the higher the observed rate of subsequent inactivation.[1] However, the character of the effect—dose dependence at low doses remained unclear, since the existence of the induction period in the dependences of the activity of the enzyme *vs.* dose did not signify the absence of changes in the protein molecule in this dose range. In fact, it was shown, for example, by the method of second derivatives of the UV spectra that at doses corresponding to the induction period, damage of aromatic amino acid residues occurred.[1]

In the present work, an attempt to study the dependence of radiation sensitivity of ACE on the pH of the medium and the concentration of the enzyme was undertaken; particular attention was focused on the effect of "low" doses that have not been considered previously. It was assumed that in the latter case, fine effects characterizing the responce of a complex biomolecule on the change in a ratio of reactive radicals produced in the radiolysis of water to the active enzyme molecules were possible. The most intersting is likely to study the change in dependence of the fine effects on the pH of the medium and their relations to the change in the active conformation of the enzyme.

Results and Discussion

The initial radiation doses usually employed in the method of radiation-induced inactivation are rather high (>50 Gy). However, at lower doses, one can expect to observe the fine effects that influence, to a great extent, conformational changes in the active center of the enzyme and in the whole molecule. In the present work, we used doses higher than 0.2 Gy.

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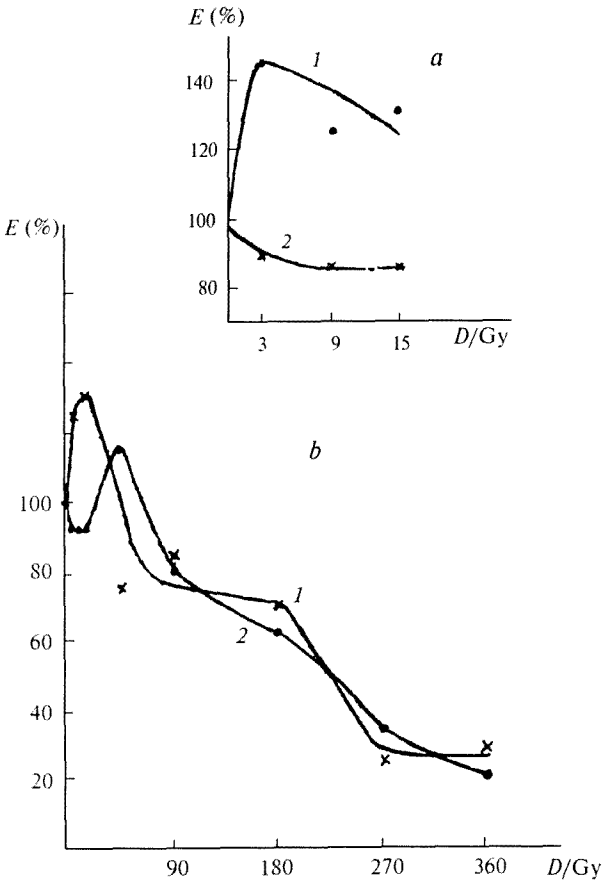


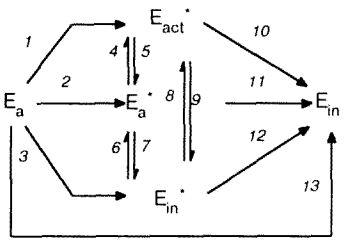
Fig. 1. Dependence of activity (E) of ACE at a 10^{-8} mol L^{-1} concentration (in 0.15 M solution of NaCl, phosphate-borate buffer) *vs.* dose of irradiation at pH 6.5 (*1*) and 8.5 (*2*): *a*, $D < 3$ Gy; *b*, whole range of doses.

It was found that in the region of the induction period observed previously[1] by us (at "low doses"), the dose dependences of the activity of ACE are in fact complex, while the shape of the curves depends on the pH of the medium and the $[R]/[E]_0$ ratio, where [R] is a concentration of reactive particles, the products of radiolysis of water, and $[E]_0$ is an initial concentration of the enzyme.

In Fig. 1, some effect *vs.* dose dependences obtained for irradiation of 10^{-8} M solutions of ACE are presented, where the fine effects characterized by the presence of regions of stability, activation, and inactivation are especially pronounced. It is the range of doses $D < D_{80}$ (*i.e.*, the dose, at which 80 % of the molecules remain active) where the so-called "defects" of the structure that predetermine the further stability or radiosensitivity of the enzyme molecule are formed.

It should be especially stressed that under definite conditions, low doses of ionizing radiation may increase the enzymatic activity of ACE. The process of activation, rather significant in some cases, is potentially physiologically important in relation to the functions of ACE in organism. It should be noted that the activation of ACE, depending on the conditions of irradiation is observed either prior ("primary" activation), or after the partial inactivation and/or the induction period ("secondary" activation) (see Fig. 1, *b*, curve *2*). Thus, the possible transformations of the ACE molecule in its interaction with the products of radiolysis of water are given in Scheme 1.

Scheme 1



Here E_a is an initial active enzyme molecule; E_a* is a molecule, which has reacted with a radical, but retained the initial enzymatic activity; E_{act}* is an activated molecule, which increased its activity due to the interaction with a radical; E_{in}* is a molecule reversibly deactivated due to the interaction with a radical; and E_{in} is an irreversibly inactivated molecule.

Changes in the enzyme concentration and/or pH of a solution result in differences in the observed fine effects at low doses of irradiation. The differences in behavior of the enzyme at doses lower than D_{80}, where the influence of conformational changes is the most significant, are given in Fig. 2.

At the initial concentration of the enzyme $[E]_0 = 10^{-8}$ mol L^{-1}, when the concentration of radicals is comparable with the concentration of the enzyme even at $D = 0.2$ Gy (see Experimental), in the range of relatively low pH (5.5—7.0), the primary activation is observed, the maximum value of which (up to 45 %) is observed at pH 6.5 (Fig. 3). Thus, the transformations of the enzyme are described by the formal scheme "activation → inactivation". Evidently, the processes *1*, (*2* + *4*), and (*3* + *8*) (see Scheme 1) most probably lead to the activation of the enzyme. At higher pH values, *i.e.*, with the shift to the alkaline area, the change in the type of fine effects (see Fig. 2, *a*) is observed: the "primary" activation disappears and replaces by the induction period, being the most prominent at pH 8.0—8.5 (Table 1). Possibly, this is primarily related to the increase in a contribution of process *2*, as well as (*3* + *6*) and (*1* + *5*). The same effect may be achieved through existence of an equilibrium between processes *1* and *3* (see Scheme 1) due to the change in the original conformation of the enzyme and accessibility of the active center for the radicals. It should be

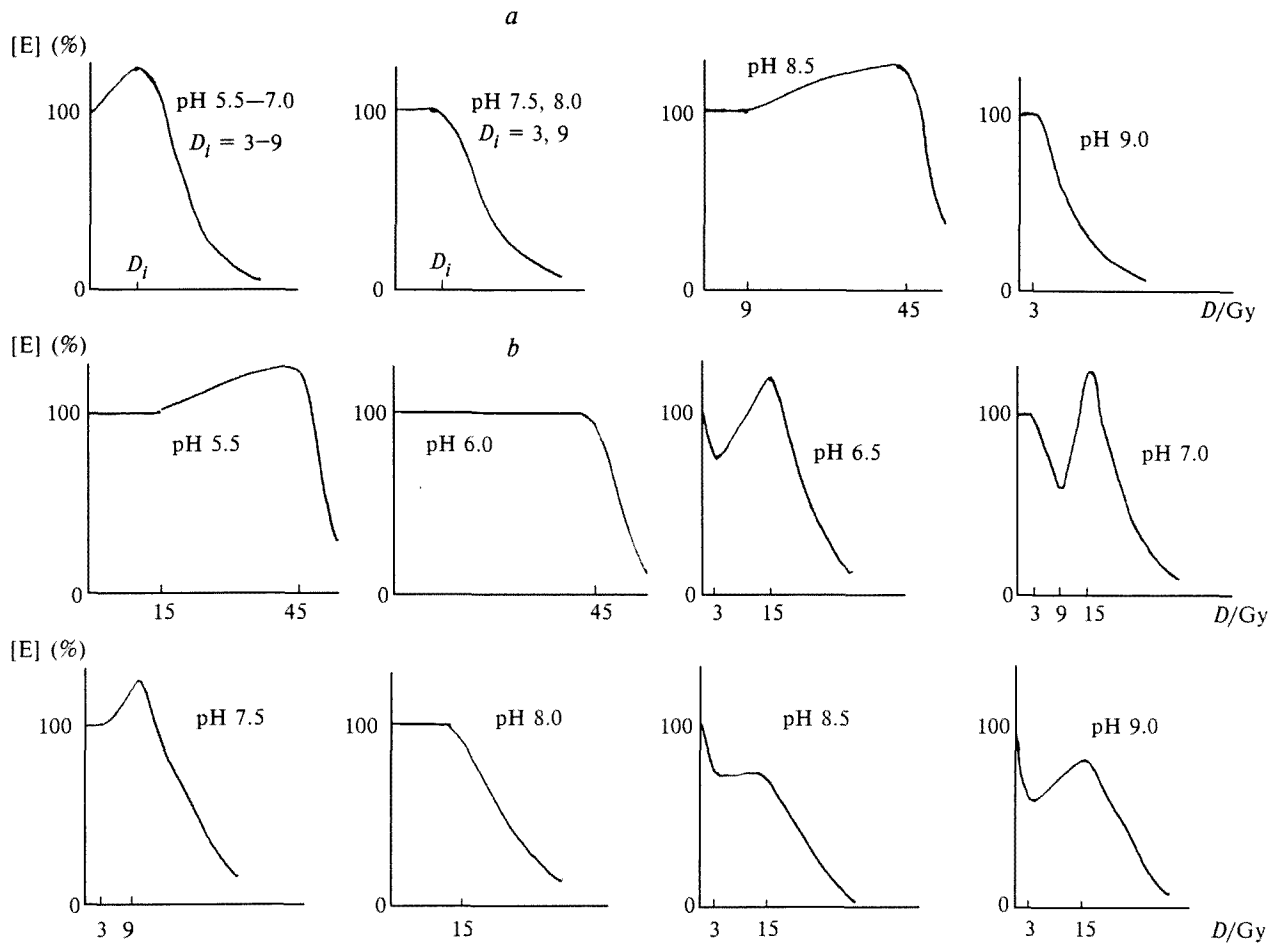


Fig. 2. Formal schemes of processes occurring under γ-irradiation of ACE at various pH and concentrations of the enzyme of 10^{-8} mol L^{-1} (*a*) and 10^{-7} mol L^{-1} (*b*) (in 0.15 *M* solution of NaCl, phosphate-borate buffer).

noted that under these conditions, the accessibility of the active center for the substrate remains virtually the same, since in a 0.15 *M* solution of NaCl, the value of Michaelis constant is almost unchanged with increase in pH. At pH 8.5, the "secondary" activation (in accordance with the scheme: induction period → activation → inactivation) is observed, it apparently illustrates the competition of several processes that activate and inactivae the enzyme (see Fig. 3 and also Fig. 2). At this pH of the medium, the highest "threshold" for irreversible changes is revealed; it can be characterized by the D_{dl} value (above this dose the alternation of fine effects is terminated and irreversible inactivation begins) equal to 45 Gy (see Table 1). Thus, with a small concentration of ACE and an excess of active radicals, it is at pH 6.5 and 8.5 that irradiation-induced processes of activation of ACE are most prominent.

An increase in the initial concentration of the enzyme by an order of magnitude (to 10^{-7} mol L^{-1}) and, hence, a decrease in the $[R]/[E]_0$ ratio makes the effect *vs.* dose dependences more complex (see Fig. 2, *b*). Over the whole pH range studied, at a concentration of

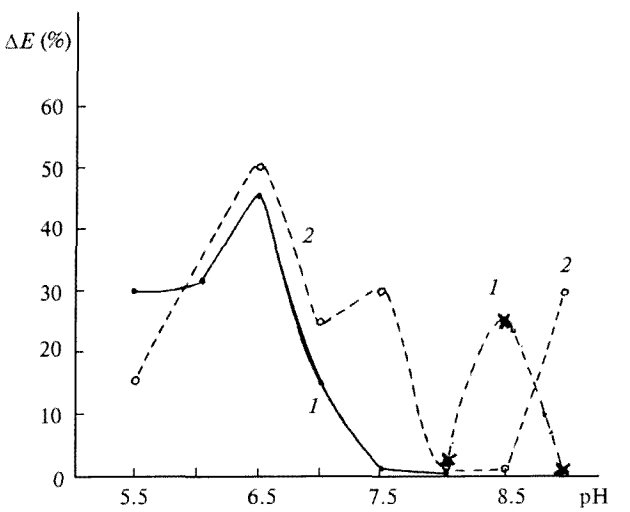


Fig. 3. pH-Dependence of activation (Δ*E*) of ACE at concentrations of 10^{-8} mol L^{-1} (*1*) and 10^{-7} mol L^{-1} (*2*) (in 0.15 *M* solution of NaCl, phosphate-borate buffer). Primary activation is given by the solid line; secondary activation is depicted by the dashed line.

Table 1. Dose values that characterize radiolysis of the ACE (10^{-7} *M* and 10^{-8} *M* solutions) at various pH (0.15 *M* solution of NaCl, 0.025 *M* phosphate-borate buffer) and at various concentrations of enzyme

pH	D_{act} of peak		D_{ind}		$D_{ind.tot}$		D_{dl}	
	10^{-7} *M*	10^{-8} *M*	10^{-7} *M*	10^{-8} *M*	10^{-7} *M*	10^{-8} *M*	10^{-7} *M*	10^{-8} *M*
5.5	45*	9	15	—	60	135	45	9
6.0	—	9	45	—	60	45	45	9
6.5	15*	3	—	—	180	45	15	3
7.0	15*	3	3	—	150	90	15	3
7.5	9*	—	3	3	180	6	9	3
8.0	—	—	15	9	20	30	15	9
8.5	—	45*	—	9	15	45	15	45
9.0	15*	—	—	3	30	10	15	3

* "Secondary" activation.

the enzyme equal to 10^{-7} mol L^{-1}, "primary" activation was not observed in the "effect—dose" plots, but rather "secondary" activation followed the induction period or initial inactivation (see Fig. 2, *b*). It is difficult to reveal the regularity in changes in the type of observed effects with changes in the pH of the irradiation medium; this is definitely related to the variety of elementary processes occurring under these conditions and their competition due to the closeness of the [R] and $[E]_0$ values (the $[R]/[E_0]$ ratio is close to 1 at $D \approx 1$ Gy). Note that under different conditions "secondary" activation may result in the enzymatic activity of ACE either exceeding the initial value or only the preceding level (see Fig. 2). The value of the observed "secondary" activation depends on the pH of the medium (see Fig. 3), and, as in the cases with 10^{-8} *M* solutions of ACE, its maximum value falls at pH 6.5, while at pH 8.5 in 10^{-7} *M* solutions of ACE no activation is observed. One can conclude that to make the activation significant with an increased initial concentration of the enzyme, an increase in dose or dose power (*i.e.*, the number of radicals per protein molecule) is required.

It should be noted, that the increase in the concentration of enzyme in some cases causes primary inactivation at low doses of irradiation in contrast to the activation occurring (see Fig. 2) in more dilute solutions (10^{-8} mol L^{-1}). This indicates the predominance of additional processes at the increased concentration that lead to inactivation. The appearance of these processes in 10^{-7} *M* solutions of ACE is noticeable at pH 6.5—7.0 and 8.5—9.0. Apparently, the contribution of reactions *3* and *13*, as well as (*1* + *10*), (*2* + *11*), and (*3* + *12*) in comparison with reaction *1* can increase (see Scheme 1). The considered change in the fine radiation-induced effects in pH-dependences makes it possible to assume that the predominance of the processes causing the increase in efficiency of catalysis or in reversible and/or irreversible inactivation is related to conformational flexibility of the active center. Apparently, it should be maximum at pH 6.5, while in alkaline medium, the conformation of the active center of ACE becomes more rigid. This assumption is in a good agreement with the literature data[5]: at pH < 7.0, dissociation of the Zn ion from the active center of the enzyme occurs. This dissociation must certainly result in loosening the structure of the latter.

In the case of complex dependence of dose curve at low doses of irradiation, the "threshold" value D_{dl} may serve as a convenient radiation-chemical parameter to characterize the stability of the molecule. Over the whole pH range studied, the D_{dl} values for radiolysis of 10^{-7} *M* solutions of ACE exceeds significantly the corresponding values obtained for radiolysis of 10^{-8} *M* solutions of the enzyme (except for D_{dl} at pH 8.5, which is anomalously high for 10^{-8} *M* solutions of ACE). In both cases, the lowest value of D_{dl} is observed at pH 7.5, and the highest value is observed at lower pH (5.5 and 6.0).

The comparison of changes in radiation-chemical yields of inactivation (G_{in}) calculated for $D > D_{dl}$, indicates the significance of initial damages for the subsequent destruction of the enzyme molecule. In 10^{-8} *M* solutions of ACE (Fig. 4), the maximum surface of the molecule accessible to radicals is achieved at pH 6.0, 7.5, and 8.5 as a result of "conformational disbalance" under the action of "low" doses of irradiation, which is manifested in the highest G_{in}. As the concentration of the enzyme increases to 10^{-7} mol L^{-1}, only one peak of the increase in G_{in} is revealed at pH 7.5. Apparently, at this pH, a significant rearrangement of the enzyme molecule, *i.e.*, conformational transition, occurs. This is also confirmed by the existence of minimum of D_{dl} exactly in this pH range.

The further increase in the concentration of ACE to 10^{-6} mol L^{-1} results only in the increase in total radiation stability of the enzyme. The dose dependences are characterized by a distinct induction period, but fine effects and, in particular, activation of the enzyme are not observed. One cannot exclude a possibility of cross-linking of the enzyme molecules in the range of "low" doses of irradiation in more concentrated solutions. It is

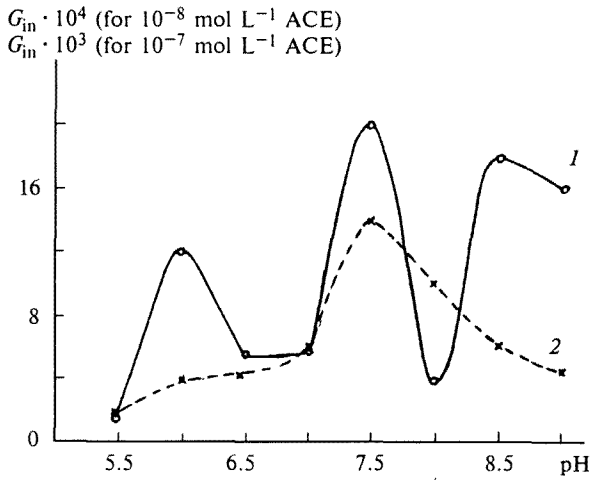


Fig. 4. Dependence of radiation-chemical yield (G_{in}) of inactivation of ACE on pH (in 0.15 *M* solution of NaCl, phosphate-borate buffer) at concentrations of the enzyme of 10^{-8} mol L^{-1} (*1*) and 10^{-7} mol L^{-1} (*2*).

known[6,7] that these reactions are essential for radiolysis of polymers and proteins (for example, gelatin). In the case of ACE, these reactions may decrease the flexibility of the active center and, hence, increase the contribution of processes (*2* + *11*) (see Scheme 1).

Radiolysis of 10^{-6} *M* solutions of ACE at pH 6.5 and 8.5 was studied additionally by the method of the second derivatives of UV spectra. This method allows to determine the decrease in the content of aromatic amino acids in an enzyme upon radiolysis from the change in the intensity of the corresponding absorption peaks.[1] In addition, the position of the absorption maximum characterizes the accessibility of aromatic amino acid residues in the protein molecule for a solvent and its change upon radiolysis.[8]

Analysis of the results obtained (Fig. 5) shows that the destruction of the most radiation-sensitive aromatic amino acid residues, tyrosine and tryptophan, occurs in the ACE molecule at practically the same rate.

The activity of the enzyme at pH 6.5 decreases after 7 h of radiolysis (D = 1.26 kGy) only to 65 % of the initial level against 20 % for pH 8.5, *i.e.*, at pH 6.5 the active center of ACE is more resistant to radiolysis. The change in the accessibility of the tryptophan residues for the solvent is also different: at pH 6.5, it smoothly falls from 40 % in the starting sample to 20 % at 1.26 kGy irradiation dose, but at pH 8.5, a decrease in the relative amount of the exposed residues from 40 to 25 % is observed even at D = 0.54 kGy; this state remains at further irradiation, *i.e.*, the loss of surface residues during radiolysis is compensated by the exposure of previously hidden residues due to unwrapping of the protein globule.

It is known that the molecule of ACE from bovine lungs contains 32 tryptophan residues.[9] In the native enzyme, at pH 6.5 and 8.5, 13 residues are accessible

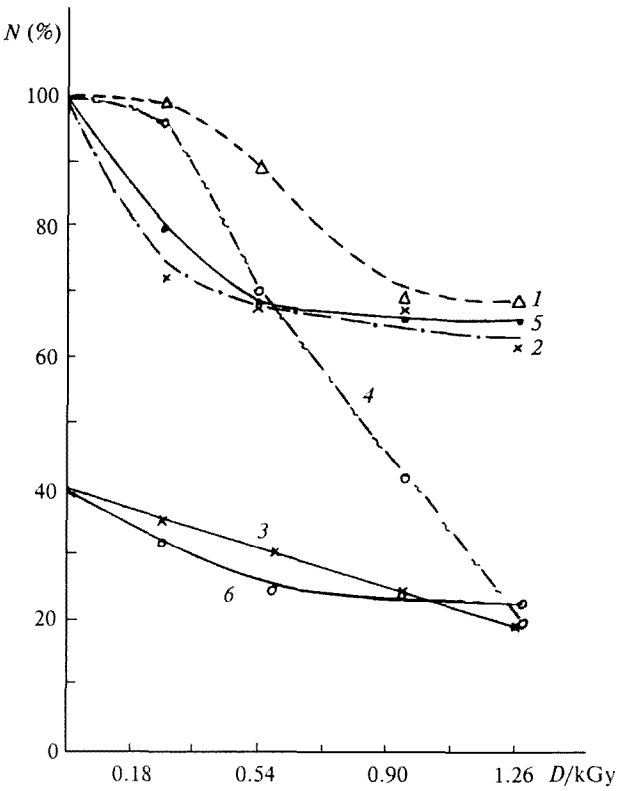


Fig. 5. Radiolysis of 10^{-6} *M* solution of ACE at pH 6.5 (*1—3*) and pH 8.5 (*4—6*); *1* and *4*, change in enzymatic activity; *2* and *5*, destruction of Tyr and Trp residues; *3* and *6*, accessibility of Trp residues in the ACE molecule to a solvent.

for a solvent, and 19 residues are inaccessible. Under radiolysis of the ACE solution at pH 8.5 for 3 h (D = 0.54 kGy), *ca.* 30 % of Tyr and Trp residues are destroyed (see Fig. 5), *i.e.*, *ca.* 22 tryptophan residues remain intact. The decrease in accessibility of the residues for a solvent from 40 to 25 % after 3 h of radiolysis corresponds to five to six accessible tryptophan residues *vs.* 17 inaccessible residues. It should be noted that the activity of ACE drops by *ca.* 30 % thereupon.

In the radiolysis of the ACE solution at pH 6.5, the decrease in enzymatic activity also by 30 % takes place at higher doses, D = 1.26 kGy (7 h), while *ca.* 35 % of aromatic amino acid residues are destroyed, *i.e.*, *ca.* 21 tryptophan residues in the protein molecule remain intact. The accessibility of 20 % of tryptophan residues for a solvent corresponds to 4 accessible and 17 inaccessible residues, *i.e.*, like in the former case, the decrease in the enzymatic activity by 30 % is observed when the number of inaccessible residues decreases from 19 to 17.

Thus, the simple calculation given above shows that in the radiolysis of the ACE solutions (pH 6.5 and 8.5), the significant destruction of surface (accessible) tryptophan residues occurs first of all; apparently, they are

not essential for the enzymatic activity. However, the loss of the activity is evidently related to destruction of inaccessible tryptophan residues, *i.e.*, the central nucleus of the molecule.

It is known[3,7] that the radiation-induced inactivation of the enzyme may result from two processes. The first involves the damage of the general structure of the molecule, which is of a stochastic character and increases with dose. This process affects significantly the activity (especially under conditions of the autoprotecting mechanism of protection for ACE) at $D > D_{80}$. The second process involves conformational changes in the region of the active center, which prevail at "low" doses ($D < D_{80}$). Evidently, it is these changes that can manifest themselves in fine effects and, in particular, in the activation of the enzyme found by us. However, the overall result will depend on the ratio of these processes. The measure of the total protecting effect may be conveniently estimated by the change in the parameter, which can be arbitrarily named as a total induction period ($t_{ind.tot}$). This is the time (or dose) of irradiation, during which fine effects occur; after the effects are completed, the activity drops irreversibly to that lower than 95 % of initial (see Table 1). It should be noted that the minimum $t_{ind.tot}$ value is at pH 7.5 for a 10^{-8} *M* solution of ACE and at pH 8.5 for a 10^{-7} *M* solution stresses additionally the specific conformational states of the molecule of ACE at these pH.

An increase in irradiation dose ($D > D_{80}$) promotes irreversible inactivation. As has been shown previously,[1] this occurs after a definite percentage of aromatic amino acid residues has been decomposed. However, it appears that even at these higher doses and with the corresponding degree of damage to the molecule, even a slow decrease in enzymatic activity was not observed, thus indicating the multistep character of the process of destruction of ACE under irradiation, evidently, due to the existence of several mechanisms of protection.

Thus, at "low" doses of irradiation ($D < D_{80}$) of ACE solutions under conditions of the physiological concentration of NaCl, the conformational disbalance is observed, which causes inactivation and activation processes related to the increase or decrease in conformational flexibility of the active center against a background of the "autoprotective" mechanism of protection of the whole molecule. The area of maximum flexibility of the active center for ACE is apparently located at pH 6.5, and at pH 7.5 the conformational transition occurs, the latter makes the protein globule more rigid.

Experimental

Electrophoretically homogeneous ACE from bovine lungs was isolated and purified using an original procedure.[10] The homogeneity of the enzyme preparations was tested by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE); it contained practically 100 % of active molecules (determined by the method of stoichiometric titration[11,12] with lysinopril, a specific competitive inhibitor (Sigma, USA)). The ACE content was determined spectrophotometrically.[13] Determination of the enzymatic activity of ACE was performed spectrofluorometrically using carbobenzoxy-L-phenylalanyl-L-histidyl-L-leucine (10^{-5} mol L^{-1}) (Serva, FRG) as a substrate in 0.05 *M* phosphate buffer (pH 7.5) containing 0.15 mol L^{-1} NaCl (see Ref. 14).

Irradiations were performed with a γ-source with P_γ = 0.05 Gy s^{-1} at 18—20 °C. Solutions of proteins (10^{-8}—10^{-6} mol L^{-1}) for irradiation were prepared in a 0.025 *M* phosphate-borate buffer having the required pH in 0.15 *M* solutions of NaCl. After irradiation, the enzymatic activities of the samples were measured *vs.* the initial ones. No post-radiation effects were observed for ACE.

Damage to aromatic amino acid residues was determined quantitatively by following the changes in the peak intensities in the second derivative of the spectra of the proteins at λ = 290.5 nm for tryptophan and λ = 284.2 nm for tyrosine.[17] The spectrum of ACE (0.15 mg mL^{-1}) and its second derivative were obtained with a Shimadzu 265FW spectrophotometer. The measurements were carried out at 20 °C in the 300 to 275 nm wavelength range using a 1 nm slit width and the "Slow" scanning rate.

The ratio of active particles formed in radiolysis of water was: $OH : e^-_{aq} : H : H_2O_2 = 2.7 : 2.7 : 2.75 : 0.8$ (see Refs. 7 and 15). The number of active particles increases from $15 \cdot 10^{12}$ to $9 \cdot 10^{16}$ with the increase in the irradiation dose from 0.2 to 15 Gy (without regard to their expense in the reaction). The numbers of enzyme molecules are $6 \cdot 10^{12}$, $6 \cdot 10^{13}$, and $6 \cdot 10^{14}$, for concentrations 10^{-8}, 10^{-7}, and 10^{-6} mol L^{-1}, respectively.

The radiation-chemical yield of inactivation (G_{in}) was calculated as the tangent to the dose curve (after D_{dl}):

$$G_{in} = 0.96 \cdot 10^6 \, \Delta E/\Delta D,$$

where G_{in} is the number of inactivated molecules per 100 eV of absorbed energy, E is the molar concentration of enzyme, and D is a dose of irradiation (kRad).

References

1. M. A. Orlova, O. A. Kost, I. I. Nikol'skaya, N. N. Troshina, and A. A. Shevchenko, *Izv. Akad. Nauk, Ser. Khim.*, 1994, 1322 [*Russ. Chem. Bull.*, 1994, **43**, 1260 (Engl. Transl.)].
2. M. R. Ehlers and J. F. Riordan, *Biochemistry*, 1989, **28**, 5311.
3. M. A. Orlova, *Usp. Khim.*, 1993, **62**, 529 [*Russ. Chem. Rev.*, 1993, **62** (Engl. Transl.)].
4. M. A. Orlova, E. A. Mareeva, V. V. Doseeva, and I. G. Gasaryan, *Izv. Akad. Nauk, Ser. Khim.*, 1994, 2230 [*Russ. Chem. Bull.*, 1994, **43**, 2110 (Engl. Transl.)].
5. P. Bunning, B. Holenquist, and J. F. Riordan, *Biochemistry*, 1983, **22**, 103.
6. M. A. Orlova, I. I. Nikolskaya, I. V. Golubtsov, and N. F. Kazanskaya, *Radiat. Eff. Lett.*, 1984, **85**, 143.
7. I. V. Timofeev-Ressovsky, A. V. Savich, and M. I. Shal'nov, *Vvedenie v molekulyarnuyu radiobiologiyu* [*Introduction to Molecular Radiobiology*], Meditsina, Moscow, 1981 (in Russian).
8. A. A. Shevchenko, O. A. Kost, and N. F. Kazanskaya, *Biokhimiya*, 1994, **59**, 1269 [*Biochemistry (Moscow)*, 1994, **59** (Engl. Transl.)].

9. R. B. Harris and I. B. Wilson, *Int. J. Pept. Protein Res.*, 1982, **20**, 167.
10. O. A. Kost, T. Z. Sharafutdinov, N. A. Lamzina, and N. F. Kazanskaya, *Vestn. Mosk. Univ., Ser. Khim.* [*Bull. Moscow State Univ., Div. Chem. Sci.*], 1990, **31**, 82 (in Russian).
11. M. R. W. Ehlers, J.-N. P. Chen, and J. F. Riordan, *Biochemistry*, 1991, **30**, 1009.1
12. N. I. Larionova, E. V. Maslov, and Yu. E. Eliseeva, *Biokhimiya*, 1982, **47**, 1332 [*Biochemistry*, 1982, **47** (Engl. Transl.)].
13. R. M. C. Dawson, D. C. Elliot, W. H. Elliot, and K. H. Jones, *Data for Biochemical Research*, Oxford, 3rd Ed., 1986.
14. Yu. E. Eliseeva, V. N. Orekhovich, and L. V. Pavlikhina, *Biokhimiya*, 1976, **41**, 506 [*Biochemistry*, 1976, **41** (Engl. Transl.)].
15. A. J. Swallow, *Radiation Chemistry of Organic Compounds*, Pergamon Press, Oxford—London—New York—Paris, 1960.

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