Description of the activation—inactivation processes in enzymes

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The process of activation of an angiotensin-converting enzyme by ionizing radiation was studied in terms of a kinetic model suggesting the existence of at least one activated form of the enzyme. The kinetic decaying oscillations induced by an exciting force were obtained and analyzed. It was shown that the probability of a periodic process is lower than that of the appearance and reactions of one activated form of the enzyme.

Key words: radiation inactivation, angiotensin-converting enzyme, kinetic equation, equations of decaying oscillations induced by an exciting force.

It is known that irradiation of a living tissue at a strictly defined wavelength can cause resonance phenomena in an organism, leading to lethal outcomes for cells. This principle provides the basis for the operation of medical lasers. During radiotherapy, this selectivity of treatment is not attained and the resonance has not been observed yet.

However, during irradiation of an angiotensin-converting enzyme (ACE)[1] with a γ-radiation source at the dose rate $P_\gamma = 0.05$ Gy s^{-1} under conditions when the dose was accumulated for a certain period of time, an activation effect was observed (*i.e.*, the efficiency of catalysis with respect to some of substrates increased), whose appearance cannot be explained by simple reasons. Previously,[1,2] we have analyzed the factors that could account for this effect. Apparently, a special role in this phenomenon is played by tryptophan residues and carbohydrate sites, whose excitation and modification can result in an increase in the conformational flexibility of the active site of the enzyme; this enables an increase in the enzymic activity at low radiation doses.

It seems fairly probable that the interaction of the aromatic ring of the tryptophan residue (in some cases, of a combination of tryptophan (Trp) and tyrosine (Tyr) residues) with active products of water radiolysis, especially with $H^\cdot$ and $OH^\cdot$ radicals, can afford aromatic radicals; this might change the spatial arrangement of the ring plane and, as a consequence, change the conformation of the active site of the enzyme molecule. This applies to those Trp (Tyr) residues that are either located in the area significant for the functioning of the active site or can get into this area upon radiation-induced conformation changes. Previously,[2] we came out with the suggestion, which has later been partially confirmed experimentally, that when the enzyme molecule contains only one tryptophan residue, this residue should necessarily play a crucial role in the conformational changes of the molecule.

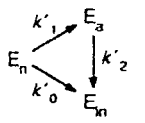
In this work, we attempt to perform mathematical analysis of the dose curve of the variation of the catalytic activity and to elucidate the possibility of appearance of peaks corresponding to enzyme activation on this curve. The analysis is based on the transformation of conventional kinetic equations into an equation of decaying oscillations induced by an exciting force, which implies the occurrence of oscillations on the dose curve under certain conditions.

Results and Discussion

Figure 1 shows the previously reported[1] variation of the catalytic activity of the ACE under conditions when "primary" activation takes place. This curve will serve as the basis for our description of the activation process.

Let us assume that there exists only one activated form of the enzyme E_a and that the apparent specific activities (V) of the native and the activated enzymes are equal to 1 and greater than 1, respectively. The processes taken into account in the radiolysis of the enzyme and the enzyme forms thus arising are shown in Scheme 1, where E_n is the native, E_a is the activated, and E_{in} is the inactivated form of the enzyme; and k'_0, k'_1, and k'_2 are the rate constants for the formation of the respective enzyme forms from the native form under irradiation. The apparent specific activities of the corresponding forms of the enzyme have the following values

Scheme 1



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$V_a > 1$, $V_n = 1$, $V_{in} = 0$. This characterizes the fundamental difference between their functioning.

The set of differential equations corresponding to the given scheme of the enzyme radiolysis in a simplified form can be written as follows:

$$\left.\begin{aligned} d[E_n]/dt &= -(k'_1 + k'_0)[E_n][R], \quad (1a)\\ d[E_a]/dt &= (-k'_2[E_a] + k'_1[E_n])[R], \quad (1b)\end{aligned}\right\} (1)$$

where [R] is the concentration of radicals (it is assumed that the concentrations of other components responsible for the change in the enzyme activity remain constant). At the initial instant, $[E_n] = 1$; $[E_a] = 0$.

The simplification lies in the fact that we do not consider variation of the radical concentration during the reactions thus assuming it to be constant. Then we can introduce the designations: $k'_1[R] = K_1$; $k'_2[R] = K_2$; $k'_0[R] = K_0$. This is valid in the case of large dose rates and very short irradiation times. In our experiments, these conditions are fulfilled almost completely when irradiation is carried out using a setup of the plasma-focus type, in which the enzyme is exposed to radiation with a dose of $\sim 10^{-5}$ Gy over a period of $\sim 10^{-9}$ s at a very high dose rate of the source, or when the irradiation is performed with a γ-source, the irradiation time is short, and no radical-trapping admixtures are present.

The exact solution of set (1) for $V_E = [E_n] + [E_a]V_a$, where E is the catalytic activity of the activated enzyme, has the form

$$V_E = B\exp(-K_2 t) + (1 - B)\exp[-(K_0 + K_1)t], \quad (2)$$

where $B = V_a K_1/(K_0 + K_1 - K_2)$.

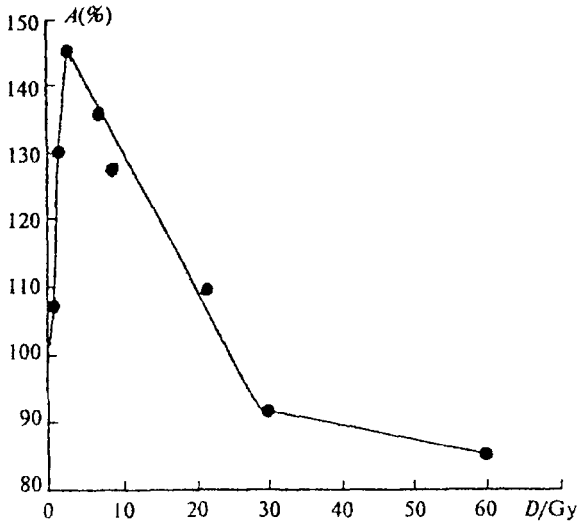


Fig. 1. Catalytic activity (*A*) of a γ-irradiated 10^{-8} *M* solution of an angiotensin-converting enzyme (phosphate—borate buffer, 0.15 *M* NaCl, pH 6.0, carbobenzoxy-L-Phe-His-Leu as the substrate) as a function of irradiation dose (*D*).

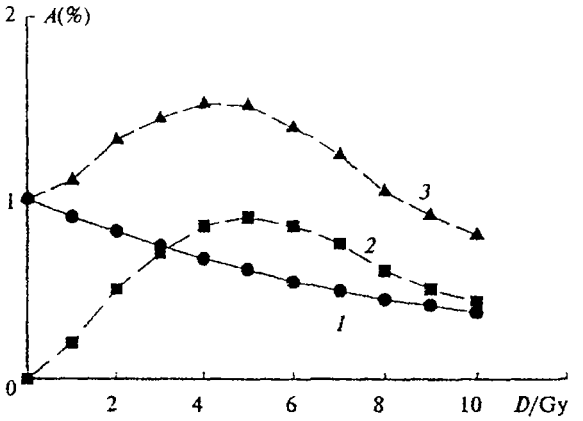


Fig. 2. Catalytic activities (*A*) due to the contribution of the native (*1*) and activated (*2*) forms of the enzyme in accordance with Scheme 1 as functions of irradiation dose (*D*); *3* is the total contribution of both enzyme forms.

It is reasonable to assume that the rate constants for the inactivation of the native and activated enzymes are close, *i.e.*, $K_0 \approx K_2$ and $K_0 - K_2 << K_1$.

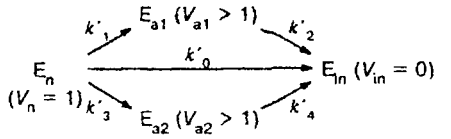
Then the expression for the variation of the activity as a function of dose at an initial period of time has the following form:

$$dV_E/dt_0 = -V_a K_2 + (V_a - 1)(K_0 + K_1) = K_1 V_a - K_0 - K_1. \quad (3)$$

From this it follows that activation at an initial period of time is determined by the following criterion: $K_1/K_0 > 1/V_a$. Most likely, when only one form of activated enzyme exists, activation can be observed only once during the kinetic dose accumulation, when the process involving the activated enzyme predominates among the competing activation and inactivation reactions. The plots representing the contributions of the activated and inactivated enzyme forms to the enzyme activity at various irradiation doses have the shapes shown in Fig. 2.

To ensure the possibility that two activation peaks would appear on the dose curves, we should consider a scheme for the kinetic processes responsible for the variation of the enzyme activity involving two activated forms of the enzyme (Scheme 2).

Scheme 2



Correspondingly, this scheme is matched by the following set of equations:

$$\left.\begin{aligned} d[E_n]/dt &= -(k'_1 + k'_3 + k'_0)[E_n][R], \\ d[E_{a1}]/dt &= (-k'_2 \cdot E_1 + k'_1[E_n])[R], \\ d[E_{a2}]/dt &= (-k'_4 E_2 + k'_3[E_n])[R] \end{aligned}\right\} \quad (4)$$

with the initial conditions $[E_n] = 1$, $[E_1] = 0$, $[E_2] = 0$. Provided that $[R] >> [E_0]$ (the dose rate is very high and the irradiation time is very short), the following designations can be introduced $K_1 = k'_1[R]$; $K_2 = k'_2[R]$; $K_0 = k'_0[R]$; $K_4 = k'_4[R]$.

The exact solution of this set for $V_E = [E_n] + [E_{a1}]V_{a1} + [E_{a2}]V_{a2}$ has the form

$$V_E = (1 - C - D)\exp[-(K_1 + K_3 + K_0)t] + C\exp(-K_2 t) + D\exp(-K_4 t), \quad (5)$$

where $C = V_{1a}K_1/[(K_1 + K_3 + K_0) - K_2]$, $D = V_{2a}K_3/[(K_1 + K_3 + K_0) - K_4]$.

In this case, the pattern of the variation of enzyme activity as a function of irradiation dose is more complicated, which is due to superposition of the activation peaks of two activated forms of the enzyme (Fig. 3). This variant of the solution demonstrates that for a relatively physiologically complex enzyme, especially for one having two active sites (like ACE) and thus providing the possibility of formation of two activated forms, the above-mentioned curve can exhibit several activation peaks. Perhaps, it is this fact that accounts for the appearance of the "secondary" activation reported in the literature.[1] The presence of two activation peaks can also be explained by a certain periodicity of the process, which apparently characterizes a system with one activated form of the enzyme. Now we consider set (1) in terms of this assumption. Let us differentiate both equations and combine them, after multiplying Eq. (1b) by V_a. Then we have

$$d^2V_E/dt^2 + K_2V_a \cdot d[E_a]/dt + [K_0 - K_1(V_a - 1)]d[E_n]/dt = 0. \quad (6)$$

By adding Eq. (1b) multiplied by $[V_a(K_0 + K_1 - K_1V_a)]$ to the resulting equation (6) we obtain Eq. (7)

$$d^2V_E/dt^2 + (K_0 + K_1 - K_1V_a)dV_E/dt + [K_1V_a^2 + K_2V_a - V_a(K_0 + K_1)]\{K_1[E_n] - K_2[E_a]\} = 0, \quad (7)$$

whose transformation gives the desired expression (8) for $[E(0)] = 1$:

$$d^2V_E/dt^2 + [K_0 + K_1(1 - V_a)]dV_E/dt + K_2[K_0 + K_1(1 - V_a) - K_2]V_E = (V_aK_1 + K_2)[K_0 + K_1(1 - V_a) - K_2]\exp[-(K_0 + K_1)t]. \quad (8)$$

This expression is a linear inhomogeneous equation with constant coefficients, which can be solved by the known method.[3] Time conversion of the $t \rightarrow \rho t$ type, where ρ corresponds to the change in the dose rate, can be done; after that, the plot for time variation of the enzyme activity does not change qualitatively but is either compressed or expanded, depending on the ρ value (all the coefficients introduced previously are assumed to be independent of the radiation power). This shows the possibility of displacement and change of the activation peak on the dose curve.

Thus, equation set (1) was transformed to obtain Eq. (8) for decaying oscillations induced by an exciting force.

Let us introduce the following designations

$\alpha = [K_0 + K_1(1 - V_a)]/2$ and $w_0^2 = [K_0 + K_1(1 - V_a) - K_2]K_2$.

Then to find the general solution of a homogeneous equation corresponding to Eq. (8), the homogeneous equation can be written as follows:

$$E'' + 2\alpha E' + w_0^2 E = 0. \quad (9)$$

If we try the solution in the form $E = \exp(-wt)$, then to find ω, we have

$w^2 + 2\alpha w + w_0^2 = 0$,

whence $w_{1,2} = -\alpha \pm (\alpha^2 - w_0^2)^{1/2}$.

Let us analyze the possible solutions.

a. if $\alpha > 0$, $w_0^2 > 0$, and $\alpha^2 > w_0^2$, the solution is represented by two decaying exponents $E = a\exp(-w_1 t) + b\exp(-w_2 t)$. This equation describes inactivation as an exponential drop of the enzyme activity upon an increase in the irradiation dose.

b. If $\alpha > 0$, $w_0^2 > 0$, and $w_0^2 > \alpha^2$, the solution is represented by an equation for decaying oscillations. This equation describes an uneven dependence of the enzyme inactivation on the dose.

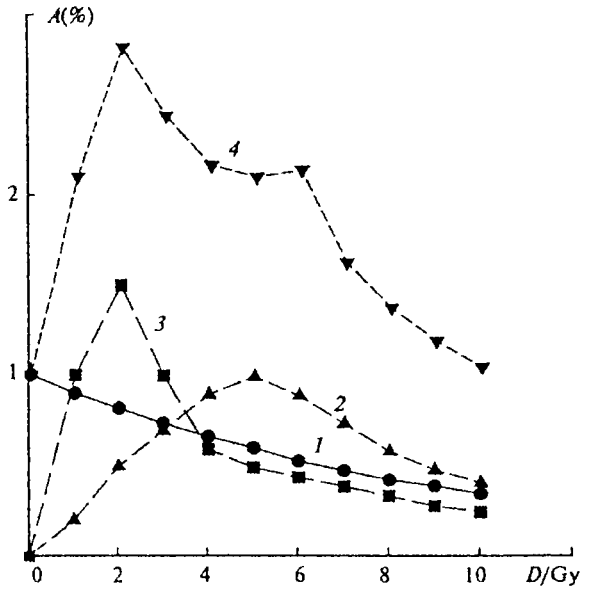


Fig. 3. Catalytic activities (*A*) due to the contribution of the native (*1*), activated-1 (*2*), and activated-2 (*3*) forms of the enzyme in accordance with Scheme 2 as functions of irradiation dose (*D*); (*4*) is the total contribution of both enzyme forms.

c. If $\alpha < 0$, $w_0^2 > 0$, and $w_0^2 < \alpha^2$, the solution is represented by an equation containing two increasing exponents.

d. If $\alpha < 0$, $w_0^2 > 0$, and $w_0^2 > \alpha^2$, the solution is an equation for increasing oscillations.

e. If $w_0^2 < 0$, the equation has one decaying and one increasing exponent, whose combination describes an increase in the activity.

Thus, the three last cases (c—e) can describe the appearance of activation; in the case (d), the process is periodical. Evidently, the appearance and disappearance of activation and the dose at which the activation occurs change as functions of the irradiation conditions (in particular, when the rate constants for the enzyme reactions vary) and the enzyme purity. By varying the exciting force (dose rate and, hence, the density of radicals), one can influence the character of variation of the activity of an enzyme exposed to radiation with doses considered to be low *in vitro* or with real low doses.

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