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ON THE POSSIBLE MECHANISMS OF ACTIVATION CHANGES OF ENZYMES UNDER PULSED IRRADIATION

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Abstract

Classical radiation biology, radiation treatment of patients, and pulsed radiation chemistry usually deal with weak beams of radiation and operate in terms of dose (D). In this case, only the *absolute number of ionizing particles or photons* interacting with the object *independently* of one another is important. In this work, we resume our previous investigations of enzyme activation/inactivation produced by pulsed ionizing radiation from a dense plasma focus (DPF) device at a very short and intense irradiation process, when the concentrations of spurs and blobs are sufficiently dense to allow the micro-volumes to overlap each other. The time interval is small compared with the corresponding biochemical process. It is shown that the irradiation of biological objects for a few nanoseconds by beams of x-ray photons with a low dose ($D \sim 10^{-6} - 10^{-3}$ Gy) but at a high dose power ($D/\tau \sim 10^3 - 10^5$ Gy/s) may be of a collective nature and result in synergetic effects. In particular, it was found that a remarkable activation/inactivation of enzymes (horseradish peroxidase, angiotensin-converting-enzyme) takes place at low doses in these regimes. The results of irradiating the above-mentioned enzymes *in vitro* with x-rays of nanosecond pulse duration coming from a dense plasma focus are presented here. Neutrons (2.5 MeV, $10^3 - 10^5$ n/cm^2) and x-ray photons of different energy ranges (7–9 keV and 20–200 keV) together with dose power factors are analyzed as possible reasons for the activation/inactivation of enzymes in various dose ranges. Our results are compared to those of the same experiments conducted with continuous x-ray sources based on a classical x-ray tube and on a ^{137}Cs-isotope source ($D \sim 10^{-3} - 10^2$ Gy).

Keywords: enzyme activation/inactivation, x-ray irradiation, powerful x-ray source, dose, dose power, dense plasma focus.

1. Introduction

The illumination of biological objects by ionizing radiation is used in life sciences for diagnostics, e.g., microscopy and tomography, medical treatment, and for directed changes of structure, composition, and properties of the objects. Directed changes are aimed at investigating by brute force the role of different elements of a biological object and their complicated nonlinear interactions [1–3]. It also can help in modeling of various fundamental processes, e.g., the development of malignant tumors. "Ionizing radiation" can be fast electrons, x-rays, γ-radiation, neutron and ion beams, α-particles, nuclear fission

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fragments, and nuclear fusion products. In any of these cases, the minimal energy of an individual particle or photon must be at least equal to the ionization energy of an atom or the dissociation energy of a molecule (∼10 eV) of the biological object under irradiation. Very powerful beams of low-energy particles and photons such as solid-state lasers represent another form of ionizing radiation; however, the corresponding mechanisms and consequences are fundamentally different. We have concentrated our attention only on the first type of radiation.

Classical radiation biology, the radiation treatment of patients, and even "pulsed radiation chemistry" [4, 5] usually deal with "weak" beams of radiation (low flux density) and operate in terms of "dose" (D). In this case, only the *absolute number of ionizing particles or photons* interacting with the object is important. The primary assumption is that each fast particle or hard photon interacts with the object independently from the others.

Ideally, pulsed radiation biology should operate with "instantaneous" flashes of radiation. However, it is physically impossible to produce instantaneous pulses with $\tau = 0$, where τ is the pulse duration. As a result, we must take into account dose power or intensity averaged over the pulse duration ($P{=}D/\tau$) as well. The nomenclature term "dose rate" (*dD/dt),* which is used for quasicontinuous sources for characterization of their operation during time intervals, where this quantity is constant, is not convenient in the case of a bell-like structure of pulses. We shall compare quasicontinuous and pulsed sources by these two characteristics. And, generally speaking, the term "flash radiobiology" could be applicable to the interaction of ionizing radiation beams with the objects, provided that the interaction time (the beam pulse duration τ) is *short compared with the duration of a corresponding specific biological process.*

We shall operate also with beam energy (in [J]) and power flux density (or simply "flux" or "intensity") of the irradiating beam at the target (in [W/cm^2]) to describe physical characteristics of a source and irradiation conditions. These characteristics are commonly used in the field of physics of high-energy density. Taking into consideration the mean free path of fast particles of the beam within a biological tissue, we shall eventually discuss the problem in terms of a concentration of fast particles within the biological object during the irradiation process. In other words, we shall analyze the beam's influence in terms of absorbed dose and dose power simultaneously, i.e., the "chemical" characteristics of the source.

We shall show that, under certain conditions, the effect of short-pulsed radiation on an object will have essentially non-diffusive character and could be accompanied by synergetic (collective) effects. The final result will be formed by this "instant" volumetric creation of active products of water radiolysis and their subsequent transformation and diffusion.

The most important point is that, during irradiation, a concentration of "effective interaction volumes" (spurs and blobs) with a characteristic size of about one mean free path of active particles should be sufficiently dense to allow micro-volumes to overlap each other. And, as was mentioned above, this condition should be realized during a time interval (radiation pulse duration) that is less than the corresponding biochemical process.

The nonstationary development of biochemical processes has to be counted as an intrinsic feature of perfect flash radiation biology. For the above conditions, two issues become important. Do these photons or fast particles, when compressed in time and space to the proper degree, result in a mutual *collective* action? Will this result in catastrophic consequences for the object at an overall dose much lower than the upper classical allowable limit?

In this work, we resume our previous investigations of enzyme activation/inactivation by pulsed ionizing radiation [6] produced with a dense plasma focus (DPF) device. This device generates x-ray photons of wide energy range – from a hundred eV up to a few MeVs – and almost monoenergetic emission of 2.5-MeV

neutrons [7].

As an object under investigation, enzymes of different classes have been chosen. Namely, we worked with an angiotensin-converting enzyme (ACE) — dipeptidyl-dipeptidase — and horseradish peroxidase (HRP). These enzymes were chosen because of their biological importance and because they are metal-containing glycoproteins.

2. Apparatus

The ultimate goals in characterizing sources of ionizing radiation for the purpose of flash radiation biology and particularly radiation enzymology are:

(i) The availability of hard radiation flashes with different pulse duration over a wide range of time intervals both for the flashes and the pauses between them. They should range from atto-, femto-, and pico-, to nano-second time frames. These characteristic flash times and pauses refer to various processes taking place within biological objects — from *primary physical processes,* e.g., the production of photoelectrons, up to *simple chemical reactions*, including water radiolysis [8];

(ii) The ability to produce and combine different types of pulsed ionizing radiation (x-ray photons, neutrons, etc.) during one run of the apparatus;

(iii) The ability to tune parameters of the source such as pulse duration, coherence and spectrum, the source size or beam divergence, dose interval, and dose power.

At the present time, several new sources of hard radiation are available. They are capable of producing short pulses (less than 10^{-7} s) of ionizing radiation [9–12]. The two most prominent ones are dense plasma foci (DPF) [9] and high-voltage fast linear devices that utilize a system of exploding wire arrays [10]. All have advantages and disadvantages, but the DPF is the most economical, flexible, and ecologically clean source. In addition, the DPF produces very high-power ionizing radiation in nanosecond time ranges. These features, coupled with a wide spectrum of radiation, distinguish the DPF from the "Febetron"-type facilities [11], which are currently used in radiation chemistry and biology.

The DPF, which is related to the Z-pinch, is a powerful source of pulsed hard radiation of various types. During plasma compression by magnetic field pressure, it generates soft x-rays of varying spectra depending on the working gas, including quasimonochromatic line emissions. After this initial "pinching" process, magnetic energy is converted into beams of fast electrons and ions as a result of a number of turbulent phenomena. The interaction between the beams, anode, and plasma produces a hard x-ray flash, which includes a quasimonochromatic component owing to Auger processes, and neutron radiation.

We shall now list the most important characteristics of the two devices used in our experiments [7]):

(a) The generation of many types of radiation coupled with the possibility of tuning the output within a wide spectral range, e.g., fast electrons, x-rays (100 eV–100 keV), neutrons (monochromatic, 2.45 or 14.0 MeV). It can also produce fast ion and plasma jets, which are not used in the current experiments;

(b) A high efficiency (10 % for soft x-rays and fast particles, up to 1 % for K_α-lines and 0.1 % for hard x-ray components), high brightness, and high repetition rate;

(c) A wide range of feeding energy and relatively compact size of the device (in the present experiments, 200 J and 2 kJ, the first one being portable and the second one transportable);

(d) A small size of radiating zones of the source (1 cm–1 μm);

(e) The generation of nanosecond pulses (with picosecond substructure);

(f) A relatively low charging voltage of the capacitor bank (∼10–20 kV);

(g) A relatively ecologically clean, safe, and inexpensive device.

The overall x-ray doses for these two devices measured by thermo-luminescent detectors and gas-ionization dosimeters in the range 8 keV–3 MeV at a distance of 20 cm from the DPF chamber were $10^{-5} - 10^{-3}$ Gy and $10^{-3} - 10^{-1}$ Gy, respectively. The neutron yields were 10^6 and 10^8 neutrons per pulse, respectively (2.5 MeV neutrons with deuterium as the working gas). For each device, the pulse duration for the two types of radiation was several nanoseconds.

Previous experiments of such a type show that crystals give the appearance of irreversible structural damage and formation of defects within the sample [13–15] — contrary to the case of the prolonged irradiation with the same dose. As for organic materials, the results show that certain reconstruction mechanisms take place side-by-side with the defect formation. For living tissues, we may expect not only reconstruction but also certain reparation mechanisms. Unfortunately, there is not enough experimental evidence at this point to draw hard conclusions within the framework of flash radiobiology. Therefore, the problem can be formulated as follows: which ratio of dose power P or dose rate dD/dt and absorbed dose D results in obvious effects (defects) at low doses received in one very short pulse? Alternatively, what is the critical dose power (P) for living tissues if the radiation is delivered by extremely short and powerful flashes of ionizing radiation at a low absolute dose (D)?

At the present time, the scientific community admits that in development of radiation disease the main role is played by biochemical reactions with the assistance of free radicals. In discussing the difference between ultrashort pulses and continuous radiation, we must compare the radiation pulse duration with time intervals characteristic for various physical-chemical processes. Matter responds to irradiation via several stages [8].

During the first stage (physical), the primary processes of water radiolysis take place:

– production of photoelectrons;

– their free traveling and diffusion, inelastic collisions and energy transfer ("electronic activation");

– creation of δ-electrons and their solvation;

– production of recoil atoms and radicals and their migration, scattering, and relaxation, very complicated dynamics, and eventually its recombination.

No less important are the time intervals between these processes, i.e., their repetition rate.

The physical-chemical stage consists of the transformation (spontaneous or due to collisions) of initial radiolysis products into intermediate ones, such as ions and free radicals. The chemical reactions of the products with each other and their environment occupy a more prolonged period of time and form a third stage — the chemical one. The fourth or biological stage is occupied by reactions of the whole

organism. The time duration of the most important processes relevant to radiation damage of living objects is presented in [14].

A typical DPF radiation source has pulses comparable with the duration of all the above physical and physical-chemical processes. A DPF can produce pulses of 10^{-9} s probably with picosecond substructure and very high brightness. Free radicals are produced during this time interval; however, reactions involving these products take much longer ($\sim$1 μs).

We examine two possibilities: a certain dose at low dose power (long pulse) versus the same dose at high power (short pulse). The last case may result in latent (taking into consideration the non-threshold action of radiation [2]) or visible damages.

3. Experiment

The search for an optimal ratio between dose and dose power in the field of radiation biology is important because of possible applications of the DPF in medicine. Indeed, for safety reasons, a high dose power device at low absolute dose such as a DPF could be used for x-ray diagnostics. In the opposite case, such a device can be exploited for low-dose radiotherapy.

Now we describe our experiments on irradiation of several types of enzymes *in vitro* using several sources of hard radiation with differing dose, dose power, and spectrum. These experiments are a continuation of our previous work [6].

3.1. ACE

We irradiated an isolated and purified electrophoretically homogeneous ACE (MW 180 kDa) from bovine lungs. The enzyme contained circa 98 % of active molecules as determined by stoichiometric titration with a specific competitive inhibitor. ACE enzymatic activity was determined with 10^{-5} M carbobenzoxy-L-phenylalanyl-L-histidyl-L-leucine (Cbz-Phe-His-Leu) (Serva, Germany) as a substrate in 0.05 M phosphate buffer, pH 7.5, containing 0.15 M NaCl, 25°C, using o-phthalaldehyde modification of His-Leu as a reaction product.

3.2. Peroxidases

We used native horseradish peroxidase C (HRP, MW 44 kDa) purchased from Biozyme and recombinant wild-type HRP (MW 34 kDa) produced from *Escherichia coli* inclusion bodies by the method developed by us earlier (see references in [4]). The measurements of peroxidase activity were performed with $2,2'$-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) as substrate using a Shimadzu UV 120-02 spectrophotometer (Japan) at 25°C by the following method:

To 2 ml of 0.1 M Na-acetate buffer (pH 5.0) we added 0.05 ml of ABTS solution (8 mg/ml) and an aliquot of the enzyme, and the reaction was initiated by the addition of 0.1 ml of hydrogen peroxide (100 mM). The molar absorptivity of the ABTS oxidation product was taken equal to 36,800 liters/(mol·cm) at 405 nm.

3.3. Irradiation

We have used several sources of ionizing radiation:

– standard ^{137}Cs γ-source: $E_{h\nu} = 662$ keV, $D = 0.1$–200 Gy, and a dose rate of $5{\cdot}10^{-2}$ Gy/s;

– standard x-ray tube used in clinics for patient's body examination working in three regimes: $U_{\text{tube}} =$ 50, 60, and 90 kV, $E_{h\nu,\max} \approx$ 35, 40, and 60 keV, respectively, a pulse duration of ∼0.1–1.0 s, a number of pulses during a séance of 1–10, and $D = 10^{-4} - 10{-}1$ Gy;

– isotope calibration β-source of DRG3-01 (Sr-Y), which produces x-ray photons due to conversion of electron energy to x-ray photons at an Al foil with x-ray energy spectrum in the range of 8–2000 keV; its dose rate is much less than 10^{-3} Gy/s;

– miniature DPF device "PF-0.2": energy storage equal to 100 J, $0.1 < E_{h\nu} < 100$ keV, x-ray pulse duration equal to 4 ns, x-ray yield ∼0.1–1.0 J/shot, $D = 10^{-6} - 10^{-3}$ Gy/shot, and neutron yield $\sim 10^6$ n/pulse ($E_n = 2.45$ MeV);

– medium-size DPF device "PF-2.0": energy storage equal to 2.0 kJ, $0.1 < E_{h\nu} < 200$ keV, x-ray pulse duration ∼10 ns, x-ray yield ∼1.0–10.0 J/shot, $D = 10^{-5} - 10^{-2}$ Gy/shot, and neutron yield $\sim 10^8$ n/pulse ($E_n = 2.45$ MeV).

The irradiation was performed at 18–20°C. The absorbed dose was determined by thermo-luminescent detectors based on LiF activated with Mg, Cu, and P with the help of a Harshaw TLD system-4000 analyzer. The x-ray spectrum of sources and its pulse shape in the case of DPF were monitored by the filter method [16, 17]. Detectors in these measurements included a Roentgen-γ dosimeter 27040 (Germany), calibrated x-ray films, SNFT photomultipliers (Russia) with plastic scintillators (of 2-ns time resolution), and individual dosimeters (gas-ionizing chambers).

All enzymes were irradiated in the dose range from 10^{-6} up to 10^2 Gy and dose power (dose rate for quasi-continuous sources) from 10^{-2} up to 10^6 Gy/s using the following methods:

– by the ^{137}Cs isotope quasi-continuous source at various time periods lasting from seconds till hours;

– by the isotope calibration β-source of DRG3-01 (Sr-Y) at various time periods lasting from minutes till hours;

– by classical x-ray tubes in the above-mentioned regimes with one to ten times irradiation per séance for a specimen;

– by both DPF sources at various distances and with 1 to 100 times irradiation ("shots") per séance for a specimen;

– by both DPF sources with two types of x-rays filters – Al and Cu foils;

– by both DPF sources with two types of n/γ filters transparent for x-rays with energy above 10 keV and opaque for neutrons (boron polyethylene slab "Neutron-stop") or, on the contrary, transparent for neutrons and opaque for x-rays (1-cm lead plate).

4. Results

The typical results of radiation-induced changes in enzyme activity found in our experiments are illustrated in Fig. 1. Because of the broad spectrum covered in the experiments, we present only the most representative points taken from the original curves [6] and subsequent experiments. In two cases (irradiation by ^{137}Cs and DPF sources), we have registered activation and inactivation of enzymes as well.

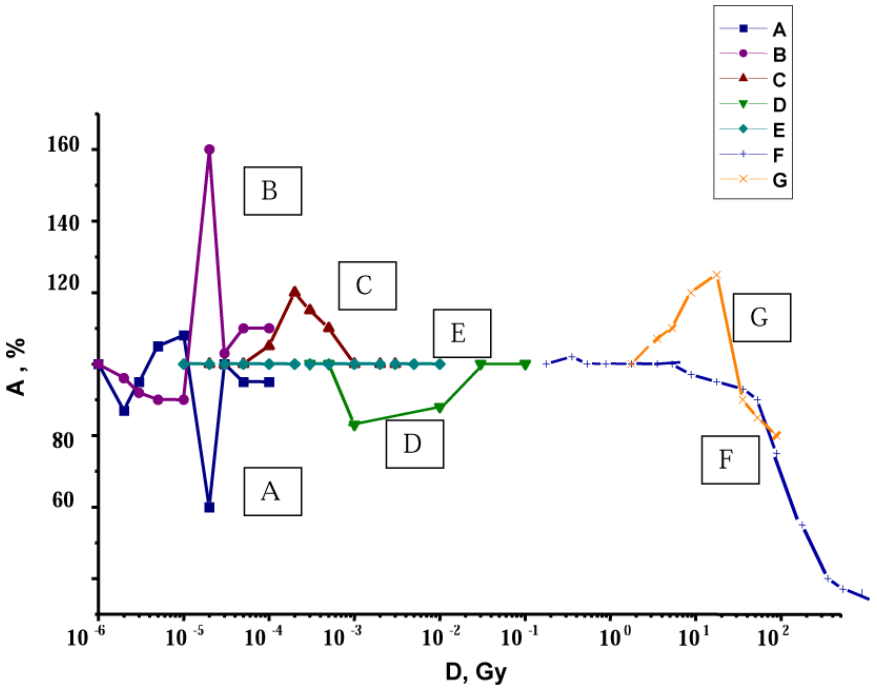

Fig. 1. Dependence of enzyme activation of HRP C (curves A, D, and F) and ACE (curves B, C, E, and G) on x-ray dose with different sources: miniature "PF-0.2" with energy storage equal to 200 J, Cu filter, 7 keV $< E_{h\nu} <$ 100 keV (curves A and B); medium-sized "PF-2.0" with energy storage equal to 2.0 kJ, Cu filter, 7 keV $< E_{h\nu} <$ 200 keV (curve C); standard x-ray tube with U= 50 kV, $E_{h\nu,\max} \approx$ 35 keV (curve D); isotope calibration β-source of DRG 3-01 (Sr-Y) at 8 keV $< E_{h\nu} <$ 2000 KeV and miniature "PF-0.2" with Al foil at 18 keV $< E_{h\nu} <$ 100 keV (curve E); and standard γ-source ^{137}Cs with $E_{h\nu}$ = 662 keV (curves F and G.)

When irradiated by x-ray tubes small inactivation of enzyme has been found in two séances only in one regime, namely, when we used the lowest voltage on the tube (50 kV). Our experiments with a β-active isotope source gave negative results (no change in enzyme activity) despite the fact that we overlapped the range of doses $10^{-5} - 10^{-2}$ Gy. In all regimes, the activity changes appeared to be irreversible, at least for several days.

The main differences between changes in the activity of the enzymes irradiated by isotope ^{137}Cs γ-sources (curves F and G) and ones illuminated by PF-0.2 (A and B) are as follows.

Although the amplitudes of the activity changes are about the same in all cases, the changes are measurable in the Cs-source case only at doses above 1.0 Gy. In the DPF case, however, irradiation is registered at a dose less than five or six orders of magnitude. The dose power in the DPF case was 4–5 orders higher compared with the Cs-source.

When the dose was decreased below the above critical levels (for the isotope, lower than 10^{-1} Gy and for the DPF, lower than 10^{-5} Gy) no changes in enzyme activity were detected.

As expected, an increase in dose in the case of isotope irradiation resulted in higher enzyme inactivation. Contrary to this, a dose increase under the DPF irradiation during one shot (by 1–2 orders of magnitude) results in a situation where the activity of both enzymes returned to the initial one.

To clarify the situation, we undertook several additional experiments. At first, we checked the influence of neutrons on the results. We positioned filters between the source (PF-0.2 and PF-2.0) and the specimens,

effectively cutting off the x-rays while allowing the transmission of the neutrons. This did not show any appreciable effect of neutrons on the activity of the enzymes in this case. Analysis of the shots with high neutron/low x-rays and low neutron/high x-rays resulted in the same conclusion. At this stage, we suspect that the neutrons played little or no role in the phenomenon (unless we have compensation effects).

Second, as mentioned above, the use of classical x-ray tubes for irradiation in the dose range of 10^{-5} through 10^{-1} Gy also did not show any effect. The only exception was found when we performed irradiation at the lowest voltage at the x-ray tube (50 kV). In this case, in two experiments we found a weak peak of the same character, i.e., activity decrease (1 and 3 shots) and then its restoration with the dose increase (10 shots). In comparison with the DPF experiments, the effect was found at $\sim 10^{-2}$ Gy and it was ~12–15 % by amplitude (so very close to the accuracy of our measurements of 10 %).

Third, we changed the x-ray dose from the DPF source by a factor of ten by increasing the distance from the source (PF-2.0 in this case) to the specimen (likewise for the dose power as the pulse duration was about the same). At the same time, we increased the number of shots. We expected to see the same narrow peak in the dose scale as seen in Fig. 1 (curve C) with the séance consisting of 10 shots. However, it was displayed at a dose of 100 times greater (100 shots), not 10 times (at $\sim 2\times 10^{-4}$ Gy) than in the case of higher dose power (curves A and B).

Finally, we changed the filter in our DPF window from a Cu foil to an Al one. All attempts to repeat the above results failed in this case.

5. Discussion

To analyze the situation and deduce any conclusions, we must take into consideration a number of important conditions of our experiments. The overall spectrum of x-rays generated by DPF consists of two parts. The first is x-ray photons of plasma thermal radiation nature, which peak around 1–2 keV and exponentially decrease to higher photon energy. The production efficiency in these experiments was ~1 %. The second is x-ray photons radiated by accelerated electrons at their dissipation on the anode. Their spectrum has a power-like law decreasing in the high-energy wing and possesses much lower efficiency. Our DPF has an anode (where a relativistic electron beam generates x-ray photons) made of copper. Near the photon energy of 9 keV, the fast electrons produce several bright lines. The highest brilliance is represented by the Cu K_α line [16]. After filtration by Al or Cu foils positioned at the outlet window of the DPF, the resulting spectra exhibit different properties.

The difference in the radiation spectra in the two cases of Al-foil filter and Cu-foil filter (Fig. 2) is due to the Cu foil having a "window of transparency" (K-edge) at around 9 keV (for the Cu K_α line) in a scale of photon energies. The Al filter contributes nothing in the low-energy wing and about the same total energy in the range above 18 keV.

The enzymes with the strongest activation/inactivation effects had atoms of Zn or Fe in their structure. The spectral absorption curves of Zn and Fe atoms overlap with the Cu K-edge curve. Therefore, it is reasonable to suppose that the presence of an atom of Zn in ACE and of Fe in HRP might favor the above activity oscillation.

The thickness of the enzyme solution layer under irradiation (3 mm) was about the mean free path of the 9-keV photons (~1 mm). It is, however, considerably lower than the mean free path of the 30-keV photons (peak of the high-energy component of the DPF spectrum) and much lower than the 662-keV photons irradiated by the ^{137}Cs source.

As is well known [4, 8], photons below 1 MeV spend almost all their energy on the creation of spurs

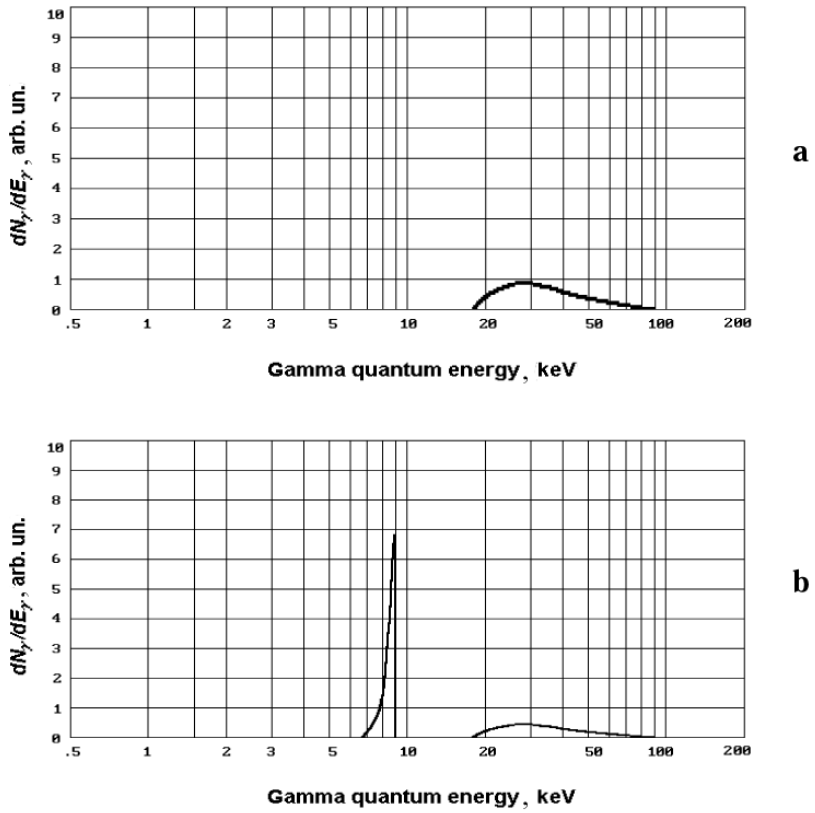

Fig. 2. X-ray photon spectrum of DPF through Cu (a) and Al (b) filters.

(size ∼10 nm) and blobs. For x-ray photons below about the 5-keV limit, mainly blobs are produced. Their characteristic size is ∼100 nm.

The fast electrons in the case of the isotope source (662 keV) and the DPF's hard component (30–100 keV) are of Compton nature. The 9-keV photons produce primarily photoelectrons. When these electrons penetrate aqueous solutions they never produce δ-electrons with an energy of more than 1 keV (the main part of them has an energy of the order of the ionization potential of the water molecules) [8]. It is easy to estimate the mean distance between blobs (in the case of 9-keV photons) and spurs (in the cases of the hard component of the DPF and isotope γ-quanta) produced in the aqueous solutions of enzymes. It appears that in the case where the DPF-based source is positioned 10 cm apart from the specimen and equipped with a copper foil (i.e., predominantly with x-ray photons of $E_{h\nu} \sim 9$ keV) this distance is equal to or less than the blob size. This implies that our ionization zones are overlapped, i.e., the density of radiolysis water products is highly increased during the period of x-ray pulse.

Furthermore, this overlapping takes place during a time interval (a few nanoseconds) on the order of the time intervals (10^{-12}–10^{-8} s [8]) of the creation of water radiolysis products. However, it is much less than the end of reaction with radicals (10^{-6} s [8]). Therefore *all the products* instantly start to diffuse and interact chiefly with enzyme molecules, not with each other, as the enzyme molecule is very large.

We note that certain synergetic effects might accompany this short-lasting process. A quite possibly more important consequence of this "instantaneous" event is the creation of the high concentration of

intermediate products (radicals) and the excitation of Zn or Fe atoms of enzyme *simultaneously*. This ensures the efficient interaction of the radiolysis products with such an enzyme molecule, which has excited Zn/Fe atoms, and during each short pulse. Indeed, the interaction of the above products with this enzyme molecule likely must result in conformational changes of the molecule.

Contrary to this situation, the distance between spurs created by photons of the hard component of the DPF spectrum as well as by γ-quanta from the isotope sources are much larger than their own size [8]. In these cases, there is also no known spectrally selective influence on Zn/Fe atoms of the enzyme. Thus, it is extremely unlikely that a very long pulse of low intensity could produce an excitation of these atoms when an individual track of a fast electron crosses the site of an enzyme molecule in the presence of a radical in its vicinity. That is why we need a much higher dose (much larger statistics) in this case to reach the necessary coincidence.

The most difficult phenomenon to understand is the disappearance of the effect with an increase in dose, which takes place at high-power irradiation. This means that the effects of low dose [3] were also displayed in the experiments.

In view of the above discussion, it seems reasonable to take into account not just the dose D or dose power P separately, but their product. It is particularly interesting to plot the data of Fig. 1 versus this parameter. It appears that in such a graph all our peaks for pulsed sources having a low-energy x-ray component will be collected around $PD \approx 10^{-2}$ Gy^2/s. Contrary to this, ^{137}Cs isotope-based source will produce the effect only at this parameter of two orders of magnitude larger. Unfortunately, we cannot present any physical conception for the PD value at this time.

Given that a well-documented explanation of the low-dose effect is currently absent, the authors wish to propose the following hypothesis.

It seems clear that the effects presented in Fig. 1 by curves A, B, C (and probably D) on the one hand and F and G on the other hand depend on the complexity of the enzyme molecule, the characteristics of the radiation spectrum, and the dose value. The main difference lies in the dose power — we receive the same effect as reached at high dose for the same enzyme and radiation spectrum only at high concentration of radiolysis products produced by high dose power at very low doses.

It is quite possible that at high dose power any dose increase above a certain critical level will result in an effect known in laser physics as the saturation effect of a two-level system under high photon pumping [18].

We suppose that a large number of possible conformational states of the initial enzyme molecule exist in chemical equilibrium that have numerous experimental confirmations. Under irradiation, a shift of equilibrium might take place, particularly in the direction of a superactive state. At the high-power source (DPF), with a power increase, a balance between activation and inactivation processes might be reached. This will result in the saturation of conformational changes and eventually in the insensitivity to a low-dose radiation. In the case of a low power source such as ^{137}Cs, this effect is impossible. But prolonged irradiation increases the dose and finally leads to a further modification and destruction of the molecule. Only in the case of an "intermediate" dose and dose power – within a certain dose and dose power range – shall we have such an effect. It is clear that, to verify or refute this hypothesis, many experiments to investigate conformational changes in enzyme molecules should be conducted. This is so, in particular, because at the moment no data on micro-monitoring parameters at pulsed irradiation of enzymes have been obtained.

6. Conclusions

In experiments on irradiation of enzymes by x-ray photons from sources with continuous (isotopes) and short-pulsed (DPF) action, it was found that activation–inactivation strongly depends on the dose power and the spectrum of x-ray radiation. In particular, the above effect is seen at doses five to six orders of magnitude lower for a powerful radiation source having a low energy (resonance) component than in the case of a prolonged action of hard radiation having low intensity. It seems that the product of dose and dose power (PD) is the most adequate characteristic of the action. A hypothesis based on a saturation effect in a conformational change of enzyme molecule is proposed for the explanation of the above oscillations.

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