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Effects of Ionizing Radiation on Arylesterase and Cholinesterase

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The effects of ^{60}Co γ-irradiation on arylesterase and cholinesterase of human blood plasma were compared using solid preparations of purified enzymes containing various amounts of water. In the case of cholinesterase a water content of 12 % exerted maximum protection against irradiation. Such a protection by water was not observed with arylesterase. In aqueous solutions cholinesterase was more resistant to irradiation by γ-rays than was arylesterase when irradiation was performed in an atmosphere of nitrogen.

It is generally believed that ionizing radiation inactivates enzymes by direct action in dried preparations, and by means of free radicals formed from the ionization of water in dilute aqueous solutions [1]. In the present study the effects of ^{60}Co γ-irradiation on two esterases, acetylarylesterase and butyrylcholinesterase, were compared using solid preparations of partly purified esterases equilibrated to different water contents. Arylesterase was recently shown to be a sulphydryl enzyme in contrast to cholinesterase, the activity of which is not dependent on the presence of free —SH groups in the same high degree as arylesterase [2].

MATERIALS AND METHODS

Materials. Arylesterase was prepared from human serum according to a partly modified method No. 6 of Cohn *et al.*[3]; a homogeneous, finely powdered sample of this preparation was used throughout the experiments. The cholinesterase preparation was obtained from the same source by the same fractionation procedure and was similarly homogeneous. Both preparations contained one single esterase, and neither preparation showed any activity of the other. These preparations were dried over silica-gel at room-temperature and equilibrated to different water contents (see below). After irradiation they were dissolved in a bicarbonate-CO_2 buffer, the composition of which was dependent on the esterase studied, to give a concentration of 0.15 mg arylesterase and 0.50 mg cholinesterase per ml, respectively (unless otherwise stated), corrections being made for the water content. In order to get clear solutions of the arylesterase preparation these were gently shaken under running water (40 – 41°C) for one min. The cholinesterase prepara-

tion was readily soluble in water, except for preparations subjected to irradiation in the presence of NO. For esterase determinations, 0.4 ml portions of these enzyme solutions were used.

In the experiments with irradiation of esterase in aqueous solutions, these were prepared similarly before irradiation.

Phenyl acetate was used as substrate for arylesterase, solutions being prepared with a bicarbonate-CO_2 buffer containing 0.0336 M $NaHCO_3$ and 0.001 M $CaCl_2$. In the experiments with cholinesterase, acetylcholine chloride was used as substrate, solutions being prepared in a bicarbonate-CO_2 buffer containing 0.115 M NaCl, 0.0336 M $NaHCO_3$ and 0.0012 M $MgCl_2$. The final concentration of each substrate (1.6 ml used for esterase determination) in the reaction mixture during activity measurement was 0.01 M (total reaction mixture, 2.00 ml).

Equilibration of esterase preparations to different water contents. Before irradiation the esterase samples, in small polyethylene tubes, were kept for 48 h in desiccators containing solutions giving different humidities. To each desiccator two wash-bottles, containing the same solution, were connected and a slow stream of air was pumped through the system. The driest samples were equilibrated by means of concentrated sulphuric acid, and the other water contents were obtained by means of solutions of potassium hydroxide in distilled water.

Radiation source. Irradiation of the esterase samples was performed in a ^{60}Co γ-source ("Hot Pot" from Picker, Cleveland, Ohio, USA) containing 2 750 Curies of ^{60}Co and with a maximum dose rate of 670 000 r/h. The dose rate in the experiments described was 570 000 r/h. The temperature measured with a N. T. C. resistor did not exceed 37°C during the irradiation, and the controls were also kept under similar temperature conditions.

Irradiation performance. The polyethylene tubes, containing 12—15 mg of the esterase preparation to be irradiated, were transformed to vacuum glass tubes (Thunberg tubes) allowing the samples to be kept in any desired gas atmosphere during irradiation. The standard procedure involved three washings with nitrogen and 10 min evacuation with an oil pump between the nitrogen fillings after which the desired gas could be filled in. After irradiation for various lengths of time, depending on the required dose, the gas atmosphere was not altered for 6—8 h before enzyme activity measurements, thus allowing the after-effects to occur in the irradiation atmosphere.

In the experiments with aqueous solutions, 2—4 ml was irradiated in glass tubes. When irradiation was performed in N_2-atmosphere, the gas was bubbled through the solutions in small wash-bottles for 5 min before the bottles were carefully sealed and placed in the irradiation chamber.

Control experiments have shown that the composition of the tubes (glass, polyethylene, *etc.*) has no measurable influence, *i.e.*, electronequilibrium is present.

Enzyme activity measurements. Manometric determinations were made at 25°C by the Warburg technique [2]. Esterase activity was expressed in μl CO_2 evolved in 30 min, corrections being made for spontaneous hydrolysis of the substrates. The percentage of remaining esterase activity after irradiation was plotted as a function of irradiation dose (in r) on semilogarithmic paper.

RESULTS

Activity-dose relationship for solid preparations and aqueous solutions. Solid preparations with known water content (4—12 %) of partly purified esterases were irradiated with doses varying from 1.0 to 11.0×10^6 r. In all experiments performed the activities decreased exponentially in relation to the dose. A similar relationship was found with aqueous solutions of the same preparation (*cf. Fig. 2*). This indicated that both esterase preparations used were homogeneous as far as the active component was concerned. The dose corresponding to 37 % remaining activity was extrapolated from these dose curves and used when the irradiation effects on preparations with various water contents were compared.

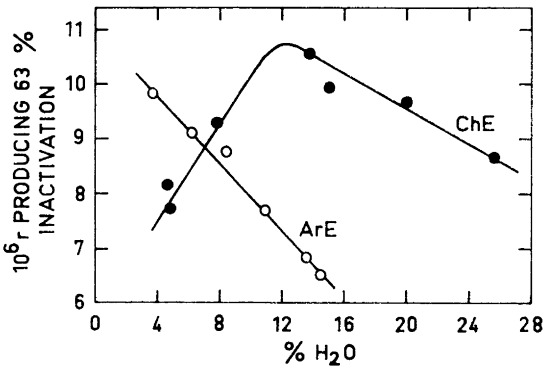

Fig. 1. Influence of the water content on inactivation of the esterases by ^{60}Co γ-irradiation. The 63 % inactivation values were extrapolated from dose-activity curves obtained in irradiation experiments with a series of solid preparations (ArE, arylesterase; ChE, cholinesterase) containing the same amount of water.

Influence of the water content on the irradiation effect. With increasing water content the sensitivity to γ-irradiation of the arylesterase preparations was increased. Fig. 1 illustrates the linear relationship between the water content and the irradiation dose which produces 63 % esterase inactivation. The influence of the water content in the case of cholinesterase was strikingly different. With increasing water content, up to about 12 %, the esterase was increasingly protected from inactivation. In the presence of higher (> 12 %) water contents this enzyme behaved similarly to arylesterase, *i.e.*, the inactivation by γ-irradiation increased proportionally to the amount of water present. These were the experimental results when irradiation was performed in an atmosphere of air.

Due to practical difficulties in obtaining reproducible water contents of the preparations when in equilibrium with other atmcspheres than air, similar irradiation experiments performed in atmospheres of N_2 or NO have not yet given conclusive results. However, enzyme inactivation by γ-irradiation of arylesterase did not seem to differ greatly when irradiation was performed in air, N_2 and NO with water contents varying between 4 and 8 %. In the case of cholinesterase, the irradiation had less effect when carried out in the absence of air (preparations in equilibrium with N_2) with water contents varying between 5 and 12 %. The solid cholinesterase preparation used seemed to be partially denaturated when irradiated in an atmosphere of NO.

Loss of esterase activity on irradiation of aqueous solutions. Fig. 2 illustrates the loss of esterase activity in aqueous solutions of arylesterase and cholinesterase subjected to ^{60}Co γ-irradiation, as a function of dose. With increasing dose the activity decreased more rapidly the lower the initail enzyme concentration. When the solutions were irradiated in the absence of air (solutions equilibrated with N_2; curve C) cholinesterase was relatively more resistant to the inactivating effect of γ-rays than was arylesterase.

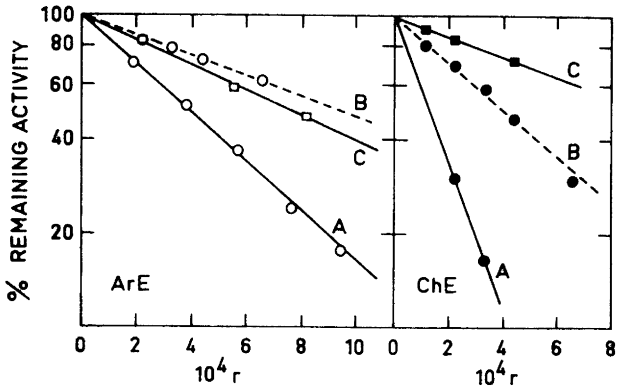

Fig. 2. Decrease of esterase activity of aqueous solutions of purified preparations of arylesterase (ArE) and cholinesterase (ChE) after irradiation with ^{60}Co γ-rays. A and B refer to irradiation performed in an atmosphere of air, C in that of N_2. Protein concentration in A and C was 0.15 mg/ml, in B 0.50 mg/ml.

DISCUSSION

The results reported above with irradiation of solid enzymes may be of some importance in view of the fact that a maximum protection by water against radiation has been found also in seeds [4] and starch [5] at about the same water content as in the case of cholinesterase. This seems to indicate that the same or similar effects are involved. With arylesterase, such a protection by water was not observed, which may be due to the dependence on —SH groups for the activity of this enzyme. It is well-known that these groups are sensitive to irradiation. An increasing degree of hydration, therefore, may render the energy transfer from the "hit points" in the molecule to the —SH groups easier, thus increasing the inactivation of the enzyme.

Cholinesterase activity is not as dependent on —SH groups as arylesterase activity, but the same theory may also be used to explain the increasing protective effect of water up to 12 % H_2O for cholinesterase. The increasing water content makes the energy transfer to the —SH groups easier, thus protecting the more essential part(s) of the active enzyme surface. This difference between the two enzymes supports the hypothetical explanations of the water effect given by Ehrenberg [4,5]. The increase in sensitivity with increasing water content higher than 12 % is not yet possible to explain clearly. Other types of reactions are possibly becoming predominant in this case, *i.e.*, the energy transfer takes other pathways which leads to a destruction of the sensitive sites in the active surface before energy can be trapped by —SH groups.

Arylesterase and cholinesterase behaved differently also when irradiated in aqueous solutions. The difference in resistance to irradiation, performed in an atmosphere of nitrogen, needs further studies in order to be explained. It indicates, however, that there is some fundamental difference in the mechanism of action of ionizing radiation for the two esterases studied. The difference in behaviour may also be of some value in understanding the role of

—SH groups in enzyme action, which has been found to differ for the two types of esterase by other experiments.

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