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INHIBITORY EFFECT OF STRONGLY ACID POLYPEPTIDES ON REPAIR OF DOUBLE-STRANDED DNA BREAKS INDUCED BY γ-IRRADIATION IN CHINESE HAMSTER FIBROBLAST'S

A. I. Medvedev, G. I. Revina, and A. M. Kuzin

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During the search for natural metabolites involved in radiobiological effects, biologically active glutamyl-peptides with closely similar physicochemical properties, possessing cytogenetic activity and capable of potentiating the mutagenic action of ionizing radiation, were found in rabbit bone marrow tissue [1, 2]. The ability of natural and synthetic strongly acid polypeptides to increase the reproductive death rate of cells during irradiation and to interact with DNA of irradiated cells, was demonstrated subsequently. The binding capacity of the polypeptides was significantly increased by simultaneous administration of modifiers and by γ-irradiation of the cells, indicating that modifiers may probably interact with rapidly repaired DNA defects. Testing the ability of strongly acid polypeptides to inhibit repair of γ-induced single-stranded DNA breaks confirmed this hypothesis [3, 4].

The aim of the present investigation was to study the effect of synthetic analogs of a natural modifier, namely the polypeptides aspartyl-glutamate (AG) and polyglutamate (PG), on repair of another rapidly repaired chromatin defect, namely double-stranded DNA breaks induced by irradiation of a culture of Chinese hamster fibroblasts.

EXPERIMENTAL METHOD

AG was obtained by the method in [2] and the mean molecular weight of the AG preparation with molecular weight range of 1500-3500 daltons was 2365 daltons. A commercial preparation ("Ferak") of PG also was used; its mean numerical molecular weight for a preparation with molecular weight range of 2000-15,000 daltons was 3952 daltons. A 2-day monolayer culture of Chinese hamster cells of clones 237 and 431, grown on Eagle's medium with the addition of 10% bovine serum, was used in the experiments. Immediately after seeding of the cells ^{3}H-thymidine (0.5 μCi/ml) was added to the medium for 24 h. The medium with labeled thymidine was poured off 16 h before the beginning of the experiment and the cells were washed 3 times with Earle's medium and incubated in medium with serum. The cells were treated by the method in [3]. They were washed with physiological saline and incubated in solutions of polypeptides in the concentrations given below, and irradiated at a dose rate of 3.6 Gy/min on the GUBÉ apparatus (^{60}Co) at 37 or 4°C. After the end of irradiation and treatment with polypeptide or simultaneous treatment with polypeptides and irradiation, the cells were fixed in phosphate buffer at 4°C for 30 min or washed with physiological saline and transferred to medium with serum and incubated for 1-4 h at 37°C, and then fixed in phosphate buffer at 4°C. The cells were then removed mechanically. Double-stranded breaks in DNA were determined by the method in [7]. For this purpose a cell suspension (50,000-100,000) was washed on a membrane filter, and lyzed; the degraded DNA was eluted. DNA remaining on the filter was removed by treatment with 0.4 ml of 0.4 M NaOH for 12 h at 37°C, and then neutralized with 1.5 M hydrochloric acid. Radioactivity was counted in toluene–Triton scintillation fluid. Polynuclear Lavsan filters with

Laboratory of Molecular Radiobiology, Institute of Biological Physics, Academy of Sciences of the USSR, Pushchino.
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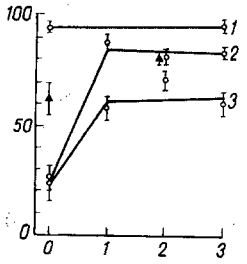

Fig. 1

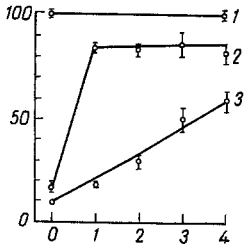

Fig. 2

Fig. 1. Repair of double-stranded DNA breaks in Chinese hamster cells of clone 431 under the influence of irradiation (100 Gy) and PG. 1) Incubation with PG, repair after rinsing out PG; 2) irradiation at 4°C in the absence of PG, repair at 37°C; 3) irradiation in the presence of PG at 37°C, repair at 37°C after rinsing out PG; 4) △, irradiation at 37°C in presence of PG, repair at 37°C. Abscissa: ○) fixation immediately after irradiation or irradiation in presence of PG; numbers indicate time of repair at 37°C (in h); ordinate quantity of DNA remaining on filter (in %).

Fig. 2. Repair of double-stranded DNA breaks in Chinese hamster cells of clone 237 under the influence of irradiation (100 Gy) and AG. Legend as to Fig. 1.

pore diameter 0.95-1.0 μ were used for elution Preliminary tests showed that polycarbonate membrane filters [7] with pore diameter 1.0 μ (BioRad Laboratories, USA) and Soviet polynuclear [6] filters are equally suitable for the analysis of double-stranded DNA breaks by the neutral elution method. The elution time in all experiments was 4 h and each experiment was repeated 4-5 times.

EXPERIMENTAL RESULTS

Figure 1 gives data on repair of double-stranded DNA breaks induced by irradiation of Chinese hamster cells, clone 431, in a dose of 100 Gy at 4 and 37°C, and in the presence of PG in a concentration of 20 μM. As Fig. 1 shows, when the cells were irradiated at 37°C most of the breaks were repaired actually in the course of irradiation (which continued about 30 min), in agreement with previous data [7]. The greatest degree of DNA degradation was observed during irradiation at 4°C, which completely suppresses repair of double-stranded DNA breaks [9]. DNA degradation of equal magnitude was observed in the case of irradiation of the cells at 37°C in the presence of PG, indicating complete inhibition of repair of double-stranded breaks by the modifier under the experimental conditions mentioned. If incubation of the irradiated cells was continued at 4°C or in the presence of PG at 37°C, the degree of DNA degradation remained at the same level.

The effects of inhibition of repair of DNA breaks at a temperature of 4°C and with a chemical modifier were reversible. During transfer of the irradiated cells into incubation medium at 37°C or rinsing cells incubated with PG to remove the free form of the modifier, not bound with them, repair of the breaks began. After only 1 h of incubation, the degree of degradation of DNA in cells irradiated at 4 and 37°C (Fig, 1, curve 2) was equalized and remained at about the same level during the next 2 h of incubation, A similar repair kinetics was observed also with cells irradiated in the presence of PG (Fig. 1, curve 3), the only difference being that the degree of degradation of DNA in these cells was significantly greater.

The results show (Fig. 1, curves 2 and 3, repair time 2-3 h) that irradiation causes the formation of unrepaired lesions, whereas the modifier significantly enhances this effect. It will be clear from Fig. 1, curve 1, that the modifier itself does not induce marked degradation of DNA if determined either immediately after the end of incubation of the cells with PG or after 3 h of incubation in the absence of the polypeptide, at 37°C.

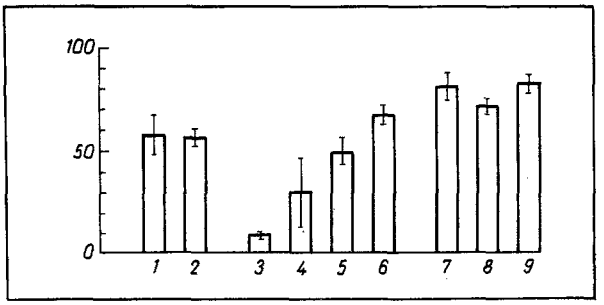

Fig. 3. Repair of double-stranded DNA breaks in Chinese hamster cells of clone 237 under the influence of irradiation (100 Gy) and AG in various concentrations. 1, 2) Irradiation in presence of AG in concentrations of 300 and 100 μM, fixation after 4 h of repair; 3, 4, 5) irradiation in presence of AG in concentrations of 100, 11, and 3.7 μM respectively, and fixation immediately after irradiation; 6) fixation immediately after irradiation at 37°C in absence of AG; 7, 8, 9) fixation after 2 h of repair after rinsing out AG (7 — irradiation at 37°C in absence of AG, 8, 9 — irradiation in presence of AG in concentrations of 11 and 3.7 μM respectively). Ordinate: quantity of DNA remaining on filter (in %).

Similar results also were observed when another modifier was used, namely AG in a concentration of 100 μM, and irradiation of Chinese hamster cells of clone 237 in a dose of 100 Gy. It follows from Fig. 2 that AG, in this concentration, like PG, did not induce any appreciable formation of double-stranded DNA breaks, Irradiation of the cells in the presence of AG similarly induced complete inhibition of repair of double-stranded DNA breaks. Rinsing the cells to remove free AG led to restoration of repair, but its rate, by contrast with the rate of repair of cells irradiated and treated with PG, was slowed. However, the final degree of DNA degradation recorded after 4 h of repair coincided with that measured when PG was used (compare Figs. 1 and 2, curves 3), Comparison of curves 2 in Figs. 1 and 2 shows agreement between the levels of unrepaired breaks for cells of both clones, when irradiated at 4°C.

The results of investigations into the effect of different concentrations of AG on repair of double-stranded DNA breaks induced in Chinese hamster cells of clone 237 by irradiation in a dose of 100 Gy are given in Fig. 3. They show that an increase in AG concentration to 300 μM did not increase the degree of accumulation of breaks unrepaired after incubation for 4 h. With a decrease in the AG concentration to 11 and 3.7 μM, inhibition of repair of the breaks became less effective (Fig. 3), if the cells were fixed immediately after the end of irradiation. A tenfold decrease in the AG concentration (Fig. 3) still led to an appreciable increase in accumulation of breaks unrepaired after incubation for 2 h. with a decrease in the AG concentration to 3.7 μM, the inhibitory action of the polypeptide was no longer observed according to this criterion.

A similar result to those shown in Figs. 1 and 2 also were observed in HeLa cells irradiated in a dose of 100 Gy and treated with AG in the same concentration. In these experiments also, the level of accumulation of unrepaired breaks during irradiation or irradiation and treatment with the modifier coincided with the level of breaks determined in Chinese hamster cells. Comparison of the degree of DNA degradation observed after 4 h of repair and irradiation of HeLa cells in doses of up to 200 Gy showed that treatment with the modifier approximately doubled the efficacy of irradiation, according to the number of unrepaired breaks which accumulated.

A similar degree of potentiation of the action of radiation also was observed in Chinese hamster cells irradiated in a small dose (1 Gy) and treated with AG in the same concentration, according to the criterion of formation of structural chromosomal aberrations. Under these circumstances, the cells were fixed in the course of the first and two subsequent mitoses.

The results thus demonstrate the ability of both modifiers used to inhibit repair of γ-induced double-stranded DNA breaks. These results also explain the effect of increased reproductive mortality of cells, demonstrated previously, in the course of combined exposure to irradiation and strongly acid polypeptides [2].

In conclusion, the comparatively high biological activity of strongly acid polypeptides, suggested for use as inhibitors of repair of double-stranded DNA breaks [5], must be noted. For instance, complete inhibition of repair of double stranded DNA breaks in irradiated Chinese hamster cells was achieved by the use of EDTA in a concentration of 5000-10,000 μM [9]. With

these concentrations of EDTA, the integrity of the cells was already disturbed. Complete suppression of repair of double-stranded DNA breaks also was observed when arabinofuranosyl adenine, a specific inhibitor of DNA-polymerases, was used in a suspension of irradiated ascite cells; the concentration in this case was 400 μM and the duration of irradiation reached 1 h. Unlike the polypeptides used, arabinofuranosyl adenine has a much stronger toxic action on cells [8]. The nontoxicity of strongly acid polypeptides, and the total inhibition of repair of double-stranded DNA breaks which they produce, as demonstrated on eukaryotic cells, make the search for ways of using them in tumor radiotherapy promising.

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EFFECT OF DISORGANIZATION OF CARBOHYDRATE–PROTEIN COMPLEXES OF THE GROUND SUBSTANCE ON STRUCTURE AND PHYSICOCHEMICAL PROPERTIES OF HUMAN HYALINE CARTILAGE

V. A. Dubinskaya, S. S. Nikolaeva, Yu. A. Khoroshkov, and O. A. Koroleva

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Diseases accompanied by changes in cartilage tissue occupy an important place in human pathology [4, 14]. Cartilage tissue, being a complex composite system, contains cartilage cells, a fibrous carcass, and ground substance, represented mainly by proteoglycans consisting of several different glycosaminoglycans (GAG) [7, 9]. In this system the fibrous carcass performs a reinforcing function, whereas the ground substance is a unique filling agent and performs integrative and buffering functions. The ground substance is also responsible for binding and transport of water into the tissues, and it is also involved in the formation of its elastic properties [3-5]. Pathological changes in cartilage tissue are often based on autoimmune processes, accompanied by enzymic disorganization of the carbohydrate protein complexes of the ground substance [4, 12].

The aim of this investigation was an experimental study of the character of the possible effect of disorganization of the carbohydrate–protein complexes of the ground substance on the structure, water-capacity, and biomechanical properties of cartilage.

Research Laboratory of Biological Structures, Ministry of Health of the USSR, Moscow (Presented by Academician of the Academy of Medical Sciences of the USSR S. S. Debov.)

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