NATURE OF LYMPHOCYTE SUPERHELICAL DNA "WEIGHTING" DURING INCUBATION *IN VITRO*

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The writers demonstrated previously a substantial increase in the relative sedimentation mobility (Sr) of superhelical thymocyte DNA (sDNA) on incubation of the irradiated cells *in vitro* [3, 6]. "Weighting" of sDNA has also been observed by other workers on incubation of irradiated cells, including those of nonlymphoid origin [7, 8]. Inhibition of repair of sDNA by novobiocin in human intestinal carcinoma cells after UV-irradiation also was accompanied by an increase in its mobility in a sucrose gradient [10]. A similar effect was produced by incubation of intact HeLa cells under hyperthermic conditions (44-46°C) [11]. It can thus be postulated that the phenomenon of sDNA in the "weighting" is manifested during exposure of the cell to a variety of unphysiological conditions.

This paper describes an attempt to study the nature of "weighting" of sDNA and to assess its possible role in cell viability.

EXPERIMENTAL METHOD

Experiments were carried out on male (CBA × C57BL)F_1 mice aged 4-5 weeks. Thymocytes were isolated and fractionated in a stepwise human serum albumin density gradient as described previously [5]. The cells were irradiated with ^{60}Co γ-rays on the ÉGO-2 apparatus in a dose of 2.5 Gy, with a dose rate of 0.43 mA/kg. Nuclear sDNA was isolated from thymocytes and its sedimentation characteristics determined in a neutral sucrose density gradient as described previously [5]. The quantity of protein bound with sDNA of thymus lymphocytes was determined after preliminary labeling of the cells with [^{14}C]leucine (specific radioactivity 4.44 GBq/mmole) [4]. Electrophoresis of proteins bound with sDNA was carried out in polyacrylamide gel containing sodium dodecylsulfate by the method in [9]. Samples were prepared by the method described previously [4]. The molecular weights of the proteins were calculated by the method in [13]. Chromatin degradation was estimated from the yield of polydeoxyribonucleotides (PDN) after incubation of the thymocytes in Eagle's medium at 37°C [1]. The DNA concentration was determined spectrofluorometrically with 3,5-diaminobenzoic acid.

EXPERIMENTAL RESULTS

On incubation of both irradiated and unirradiated small thymocytes *in vitro* a substantial increase in sedimentation mobility of sDNA was observed relative to mobility of DNA of unirradiated and unincubated cells (Table 1). A similar, although less marked "weighting" of sDNA was observed when thymocytes were kept in Hanks' solution at 4°C for 4 h (Sr = 1.50 ± 0.05).

It can be tentatively suggested that the observed increase in sedimentation mobility of sDNA was due to a change in its conformation on account of either an increase in superhelicization density or a decrease in size of the superhelicization domains as a result of the formation of a larger number of points of attachment of DNA to the protein matrix of the nucleus. The formation of additional protein cross-linkage at sites of conformational constraints, in the formation of which nonhistone proteins with molecular weights of 50-60 and 75-87 kilodaltons (kD) take place, as the writers showed previously [4], is also possible.

The increase in sedimentation mobility of sDNA of HeLa cells after incubation under hyperthermic conditions was shown to correlate with an increase in the quantity of protein firmly bound with DNA [11]. However, as Table 1 shows, no increase was observed in the quantity

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TABLE 1. Changes in Sr of sDNA of Small Thymocytes and Content of Protein Bound with It during Incubation of Irradiated or Unirradiated Cells in Hanks' Solution at 37°C (M ± m)

Parameters studied	Duration of incubation of thymocytes, h			After incubation for 4 h preceded by irradiation in a dose of 2.5 Gy
	1	2	4	
Sr	1,00±0,05	1,01±0,06	1,73±0,09	1,97±0,05
Protein concentration, percent of total activity in gradient	8,0±3,4	4,7±1,8	7,5±1,8	7,2±3,5

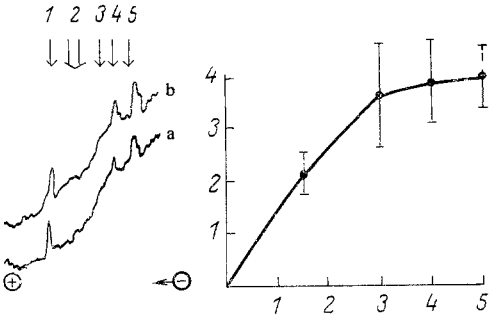

Fig. 1

Fig. 2

Fig. 1. Densitograms of proteins bound with sDNA of small thymocytes before (a) and after (b) irradiation of cells in a dose of 2.5 Gy. Irradiated cells were incubated in Hanks' solution at 37°C for 4 h. Cytochrome C (1) was added to each tube of gel. Arrows indicate position of marker proteins in neighboring tube of gel: total preparation of calf thymus histones (2), creatine phosphokinase (3), glutamate dehydrogenase (4), and bovine serum albumin (5). Direction of movement of proteins from cathode to anode.

Fig. 2. Degradation of chromatin of small thymocytes during incubation of cells at 37°C. Abscissa, incubation time (in h); ordinate, yield of PDN. Level of PDN yield in cells incubated for 15 min was taken as 1.

of protein bound with thymocyte sDNA during at least 4 h of incubation at 37°C of both irradiated and unirradiated cells. No qualitative change likewise was found in the composition of proteins bound with sDNA after incubation of small thymocytes, irradiated in a dose of 2.5 Gy, after 4 h of incubation (Fig. 1).

The hypothesis concerning "weighting" of sDNA due to a decrease in size of the superhelicization domains is unlikely to be true. For instance, the writers showed previously that sDNA of small thymocytes sediments faster in a sucrose density gradient than sDNA of large thymocytes [5], although the size of the superhelical domains of DNA of small thymus lymphocytes is about an order of magnitude greater than that of the domains of DNA from large cells [6].

It will be clear from Table 2 that values of the maxima and minima of relaxation of sDNA from both large and small thymocytes, repaired after γ-irradiation, are indistinguishable from the controls, evidence of maintenance of the original superhelicization density of the DNA. Other workers likewise found no change in the superhelicization density of DNA of HeLa cells after incubation under hyperthermic conditions, although the sedimentation mobility of the nucleotides was increased in this case [11].

TABLE 2. Effect of Ethidium Bromide on Sr of sDNA of Large and Small Thymocytes before and after Irradiation (M ± m)

Thymocytes	Concentration of ethidium bromide in gradient, μg/ml	
	4,9	31,0
Unirradiated:		
Large	0,67±0,04	0,88±0,05
Small	0,63±0,03	0,87±0,05
Irradiated:		
Large	0,64±0,02	0,82±0,05
Small	0,59±0,05	0,80±0,06

Legend. Cells irradiated at 0°C in a dose of 2.5 Gy were incubated at 37°C in Hanks' solution for 3 h (large thymocytes) and 4 h (small thymocytes).

The possibility thus cannot be ruled out that the "weighting" of sDNA of the lymphocytes observed in the present experiments was due to the denser grouping of superhelicization domains, possibly through the formation of extra protein–protein interactions of cross-linkage type at sites of conformational constraints in the domains.

It can be tentatively suggested that the "weighting" of sDNA observed after exposure of the cells to such unphysiological factors as radiation, hyperthermia, or prolonged incubation at 37°C or at 4°C in minimal medium reflects a phenomenon of condensation of lymphocytes that is characteristic of dying cells. It has been shown, for instance, that postradiation death of lymphocytes is accompanied by a decrease in their size and by condensation of the cells, but with an increase in the dose of irradiation the number of dense, dying cells increases [15]. The increase in weight of thymocytes of the lower fraction relative to the total of the upper fractions in a Ficoli–Hypaque gradient also was observed after γ-irradiation of the cells in a dose of 5 Gy followed by their incubation at 37°C for 3 h [2]. "Weighting" of the cells in that case also was observed after incubation of unirradiated thymocytes for 3 h at 37°C. According to our own data, incubation of unirradiated thymocytes at 37°c is accompanied by progressive destruction of chromatin and migration of PDN from the nucleus (Fig. 2). Migration of PDN from the nuclei of lymphoid cells is known to be a characteristic biochemical indicator of their death [12].

"Weighting" of sDNA observed during incubation of cells exposed to certain unphysiological factors (irradiation, hyperthermia, incubation in minimal medium) thus probably reflects processes of chromatin condensation, preceding what is called the apoptic form of death, characteristic primarily of lymphoid cells [14].

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