BIOCHEMICAL LETTERS

DNA fragmentation in permeabilized cells

Miller *et al.* (1978, 1979) developed a method of permeabilization of mammalian cells that has been useful in studying the synthesis of macromolecules. They showed that in permeabilized cells from exponentially growing cultures DNA is synthesized in a normal replicative fashion for at least 2 h. Recently, Pawlak *et al.* (1986) reported that incorporation of labelled nucleotides into nucleic acids in permeabilized cells was not consistent with semiconservative DNA replication or RNA transcription. Authentic DNA synthesis required the addition of exogenous DNA. The authors suggested that DNA in permeabilized cells is impaired in its function as a template for replication and transcription. Recently we found that DNA undergoes progressive fragmentation after cell permeabilization.

Experiments were performed with two cell lines, cultured Ehrlich ascites tumour cells and human lymphoblastoid cells (RB 6176, obtained from Dr J. H. Robbins). Cells were cultured as previously described (Li & Kaminskas, 1984; Otsuka *et al.*, 1985) and were in exponential growth phase. Cells were labelled with [2-¹⁴C]thymidine (0.01 μ Ci/ml) for 24 h and then chased in fresh medium for 16 h. Permeabilization of cells was carried out essentially by the method of Miller *et al.* (1978, 1979). Briefly, duplicate or triplicate samples (3×10^6 cells) were collected by centrifugation in 1.5 ml tubes in an Eppendorf microcentrifuge. The residual medium along the wall of the tube was removed with lintless tissue. After 25 min in an ice/water bath, 0.1 ml of ice-cold L- α -lysophosphatidylcholine (Sigma type I) (150 μ g/ml) in solution A (150 mM-sucrose, 80 mM-KCl, 35 mM-Hepes, 7.5 mM-K₂HPO₄, 4 mM-MgCl₂, 1 mM-CaCl₂, pH 7.4) was added to the cell pellet and the cells were suspended by mixing briefly three times with a vortex-mixer. After a 2 min incubation in an ice/water bath, 1.4 ml of the DNA synthesis medium (1.5 mM-ATP, 0.15 mM each of CTP, GTP, UTP, 0.25 mM each of dTTP, dATP, dGTP, dCTP and 20 mM-phosphoenolpyruvate in solution A) was added to the cell suspension. The extent of permeabilization (generally 94–99%) was determined by the uptake of Trypan Blue. Cells were then incubated at 37 °C for 0–2 h before DNA was analysed by alkaline elution. Control cells were treated in the same manner, except that L- α -lysophosphatidylcholine was not added. Alkaline elution was carried out by the procedure described previously (Kohn *et al.*, 1981). This assay consists of lysing cells on inert filters under non-denaturing conditions (usually pH 9–10) and then slowly eluting DNA as single strands at denaturing conditions (usually pH 12.1). The rate of elution of DNA from the filter is dependent on the strand length: the shorter the strand, the faster its elution. Thus, the rate of elution is related to the frequency of single-strand breaks in DNA molecules. The range of DNA single-strand lengths that can be discriminated by this

Fig. 1. Alkaline elution patterns of parallel cultures of cells that were not permeabilized (○), 99% permeabilized (△) or 99% permeabilized and incubated for 15 min at 37 °C (▲)

method is 2.7×10^8 – 10^{10} Da (Kohn *et al.*, 1976). Briefly, $(0.5$ – $1.0) \times 10^6$ cells were deposited on a polycarbonate membrane (25 mm diameter, 2 μ m pore size; Nuclepore) and then lysed with 5 ml of lysis solution (50 mM-Tris, 50 mM-glycine, 25 mM-disodium EDTA and 2% SDS, pH 9.6). DNA was eluted at 0.04 ml/min with a solution containing 20 mM-EDTA (acid form), 0.1% SDS and sufficient tetrapropylammonium hydroxide to raise the pH to 12.1. Six 5 ml fractions were collected. The radioactivity in each fraction of the eluate was used for the calculation of the percentage of DNA retained on the filter at that fraction.

As shown in Fig. 1, there was an extensive accumulation of DNA strand breaks in permeabilized cells after 15 min of incubation at 37 °C. This extent of strand breakage is approximately equivalent to that resulting from X-irradiation with 400 rad at 0 °C (Kohn *et al.*, 1981). After a 2 h incubation of the permeabilized cells at 37 °C, DNA was largely composed of small fragments, since 70–80% of DNA was eluted in the first fraction. The results with lymphoblastoid cells and with Ehrlich ascites tumour cells were essentially the same.

Why DNA fragmentation occurs after permeabilization is not clear. Permeabilization may disrupt structures that are necessary to maintain the integrity of DNA. Alternatively, permeabilization may cause a release of degradative enzymes. In either case, the present findings should be taken into consideration when interpreting data on DNA synthesis in permeabilized cells.

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