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Inactivation of simian immunodeficiency virus by hydrostatic pressure

(retrovirus inactivation/pressure inactivation)

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ABSTRACT **The inactivation of the simian immunodeficiency viruses SIVmac251 and SIVagm by pressures of 150 and 250 MPa was determined. The extent of inactivation depended on the time that the virus was subjected to compression as well as the level of the pressure and at 150 Mpa reached 5 $\log_{10}$ dilution units after ≈10 hr. The inactivations, which were uniformly carried out at room temperature, were independent of the concentration of the virus. Possible applications of pressure inactivation for molecular biological and clinical use are discussed.**

The development of effective methods for eliminating unwanted or harmful components, such as viruses, present in biological samples and preparations is an important scientific endeavor with many possible practical applications. Sterilizing biological preparations and introducing new vaccines are obvious motives; moreover, all procedures that modify the biological activity of viruses can lead to a better understanding of their requirements for successful infectivity. Key considerations in practical, large-scale applications are the simplicity and reproducibility of the procedures. Physical methods are not always highly selective but they are simple, easy to reproduce, universally applicable, and relatively easy to apply on a large scale. High pressure can be applied to almost all biological preparations, is readily implemented routinely and safely in a laboratory environment, and is often selective in its action on macromolecular structures. In this report we present results showing that subjecting virus samples to pressures under 250 MPa (1 MPa = 10 atmospheres) can inactivate the simian immunodeficiency viruses SIVmac251 and SIVagm. Pressure often perturbs selectively the properties of biological molecules and complex biological systems by disrupting noncovalent associations, while leaving unaffected the covalent architecture of the separate components (1–3). This is of particular interest in prospective viral vaccines because the precise relations of the capsid proteins with each other, with the lipid membrane when there is one, and with the nucleic acids must be responsible for the specific infectivity, whereas an intact covalent framework of the proteins is necessary for the proper immune response. Hydrostatic pressures under 300 MPa can disrupt icosahedral viruses (4) and a membrane-enveloped animal virus, causing loss of infectivity with retention of the immunogenic capacity (5, 6). Therefore hydrostatic pressure procedures may by themselves, or in conjunction with other physical, chemical, or biochemical techniques, offer a suitable procedure for the preparation of vaccines.

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MATERIALS AND METHODS

SIVmac251 was isolated from a rhesus monkey (*Macaca mulatta*) (7) and SIVagm Tyo 7 was isolated from an African green monkey (*Cercopithecus aethiops*) (8). The simian immunodeficiency viruses were grown in the CEM human T-cell line. The medium for cell growth and for virus inactivation was RPMI 1640 supplemented with 20% fetal bovine serum. The high-pressure inactivation of the virus was performed in cell-free supernatant.

Incubation at high pressure and room temperature (21.5°C) was performed in a Nova Swiss high-pressure apparatus (Nova Swiss, Effretikon, Switzerland) using absolute ethanol as the pressure-transmitting solution. Samples (≈300 μl) of cell supernatant containing SIVmac251 or SIVagm were placed in plastic tubes; the tubes were sealed with tight-fitting movable plunger seals, and each tube was hermetically sealed in a plastic sack filled with medium to avoid the escape of virus and to completely isolate the viral solution from the ethanol.

Serial dilutions of cell-free SIVmac251 or SIVagm virus solutions that had been pressure treated were examined by infection of 3×10^4 MT-4 cells per well in a microtiter plate (9). The RPMI 1640 medium used for the dilutions contained 25 mM Hepes and 20% fetal bovine serum. Four days after infection 0.1 μCi (3.7 kBq) of [*methyl*-^{3}H]thymidine was added. Twenty hours later the cells were harvested on glass fiber filters and [^{3}H]thymidine incorporation into the cellular DNA was measured. The activity of the virus was always compared with that of a control sample not subjected to pressure.

RESULTS AND DISCUSSION

The extent of inactivation of SIVmac251 is dependent on the level of the hydrostatic pressure and the time that the virus is subjected to it. Fig. 1 shows the effect of 1-hr incubations of SIVmac251 at various pressures. A pressure of 250 MPa is sufficient to decrease the infectivity of the virus by at least 5 $\log_{10}$ dilution units, whereas lower pressures are progressively less effective. Below 100 MPa no inactivation occurs within 1 hr. The concentration of the virus in solution during the application of high pressure did not affect the extent or rate of inactivation. Virus samples within a 100-fold range of dilutions incubated for 1 hr at 200 MPa followed the same inactivation profile (data not shown). This result indicates that with respect to pressure the viral population behaves as individual units that undergo independent inactivation (18). The inactivation is unlikely to be directly related to the dissociation into subunits, as observed in icosahedral viruses (brome mosaic, cowpea) but rather to effects produced in the nucleoproteins under the compressible lipid membrane, as observed in vesicular stomatitis virus (5). The level of inactivation depends on the time that the virus solution is maintained at high pressure; longer times elicit greater extents of inactivation and the rate of inactivation

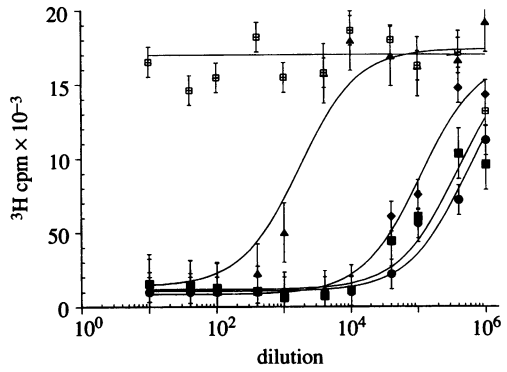

FIG. 1. Plot of [^{3}H]thymidine incorporation vs. $\log_{10}$ of the sample dilution (i.e., the serial dilution used for the cellular assay) following a 1-hr incubation of the virus at a pressure of 0.1 MPa (□), 100 MPa (▲), 150 MPa (◆), 200 MPa (■), or 250 MPa (●). Abscissa shows dilution of the virus sample subsequent to the pressure application, prior to the addition to the cellular assay mixture. Ordinate shows radioactive thymidine cpm incorporated after 20 hr (see text). The lines are fits of the data at each pressure to the following function: background cpm + 17,000 $[x/(K_{1/2} + x)]$, where x is the dilution of the virus solution and $K_{1/2}$ is the dilution that produces thymidine incorporation equal to half of the control value.

depends on the level of the pressure. Suppression of the infectivity to $1/10^5$ is achieved at 200 MPa within 3 hr, whereas >8 hr of incubation at 150 MPa is necessary to attain a similar decrease in infectivity (Fig. 2). Provided that the incubation time is sufficient, pressures of 50 MPa or higher can inactivate the retrovirus to the same extent as higher pressures do in shorter times. Only the total time that the sample is subjected to the pressure affects the extent of inactivation significantly. Control experiments involving the repeated application and removal of pressure reveal that raising and lowering the pressure, which would be expected to bring about pressure gradients within the sample, do not play an important role in the inactivation.

From the data shown in Fig. 2 it can be deduced that 100 hr at 150 Mpa would reduce the infectivity to 10^{-8} to 10^{-9} of the original sample and provide therefore a safe vaccine preparation. A similar result would be obtained with compression at 200 Mpa for 30 hr.

To examine the generality of the pressure inactivation with immunodeficiency retroviruses, we applied high-pressure incubation cycles to the related virus SIVagm Tyo7. The biological behavior of the viral peptide diverges considerably from SIVmac251 (10, 11) and the pathogenicity *in vivo* is very different for the two viruses (12–14). The inactivation of SIVagm achieved after 3 hr of incubation at 200 MPa is the same as that with SIVmac251 under identical incubation conditions. The mode of pressure inactivation of these two viruses seems to be associated with a common property of the viruses that is not directly related to their considerably divergent sequences or different pathogenicities. The molecular and structural perturbations underlying the viral inactivation reported above are not clear, but the usefulness of this simple physical method for inactivation of simian immunodeficiency virus is evident, especially in light of the lack of adequate treatment or vaccines against human immunodeficiency virus, the causative agent of AIDS. It is important to emphasize that the assay (after the application of pressure) takes about 1 week, and the inactivated virus does not regain its activity within this time. On application of pressure the effects on complex molecules are far easier to control and to interpret than those of temperature (1–3). The effective inactivation of simian immunodeficiency virus by pressure is probably similar to the inactivation of vesicular stomatitis virus (5), in which case electron microscopy has shown displacement of the capsid subunits retained under the very compressible viral membrane. Disruption of the relations between the capsid proteins may make it impossible for the virus to interact with the cell receptors in the manner necessary for the penetration of the viral RNA into the cell. Pressures of the magnitude employed do not affect the chemical structure of nucleic acid components or their degree of polymerization (15), so that it is most unlikely that the pressure inactivation directly involves the destruction of the genetic information.

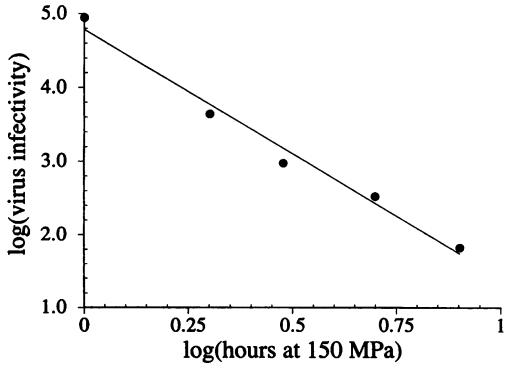

FIG. 2. Plot of the dilution of a series of virus samples required to reduce the viability of cells by 50% vs. the time (0, 1, 2, 3, 5, and 8 hr) that 150-MPa pressure was applied. The scales of both axes are decimal logarithmic. See Fig. 1 and text for the cellular assay procedures.

This report concerns the inactivation of a retrovirus by high pressure. Several previous investigations have dealt with the effect of high pressure on the infectivity and structure of other viruses (5), and the survival of a great variety of biological cells and organisms at pressures between 0.1 and 300 MPa has been determined in numerous studies (16, 17). Every biological system reacts to the application of high pressure in an individualistic way; however, we have shown that two related retrovirus isolates are inactivated to the same extent by pressure despite their divergent biological properties.

The employment of high pressure is a relatively safe and simple procedure for technical personnel, and pressures less than 300 MPa do not significantly irreversibly affect the tertiary structure of many macromolecules. The results reported here highlight another application of high-pressure techniques in addition to aiding in the development of improved preventive, diagnostic, and perhaps even clinical tools. Depending on the generality of successful inactivation of viruses with high pressures without destruction of the structural details of other critical molecular agents, the method could prove effective in sterilization of biological preparations such as vaccines. This would provide an additional measure of safety that could be carried out easily on almost any preparation.

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