The Stability of Deoxyribonucleic Acid Solutions under High Pressure *

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Exposure of DNA to a pressure of 10 000 atm does not cause denaturation. In the pressure range of 1—2700 atm, a stabilizing effect against heat denaturation was found, the denaturation temperature being ~ 6°C higher at 2700 atm than at 1 atm. In parallel with the denaturation temperature, the optimum temperature for renaturation of denatured DNA is raised.

The stability of several proteins and viruses under high pressure has been thoroughly investigated.[1-4] Most proteins seem to be irreversibly denatured in the region of 4000—9000 atm, while lower pressures, having no denaturing effect, cause stabilization against heat denaturation. With the exception of a brief review of some of the experiments reported here,[5] no similar studies have so far been reported on the pressure-sensitivity of nucleic acids. In view of recent studies on induction of lysogenic bacteria by means of high pressure treatment [6,7] it was of interest to study the stability under pressure of isolated DNA.

The data presented below show that exposure of DNA solutions to pressures up to 10 000 atm at room temperature does not cause irreversible changes in the native molecular structure. At a pressure of 2700 atm a significant stabilizing effect against heat denaturation was found.

MATERIAL AND METHODS

Apparatus. Pressure chambers without temperature control (for use up to 10 000 atm) and with temperature control (for use up to 2700 atm) were available.

The general design of the latter chamber is shown in Fig. 1. Accurate temperature measurement with a minimum time delay was achieved by using a small encapsulated thermistor (YSI series probe. Yellow Springs Instrument Co. Inc., Yellow Springs, Ohio) with the leads passing into the pressure chamber *via* a plug of nylon (*cf.* Fig. 1).
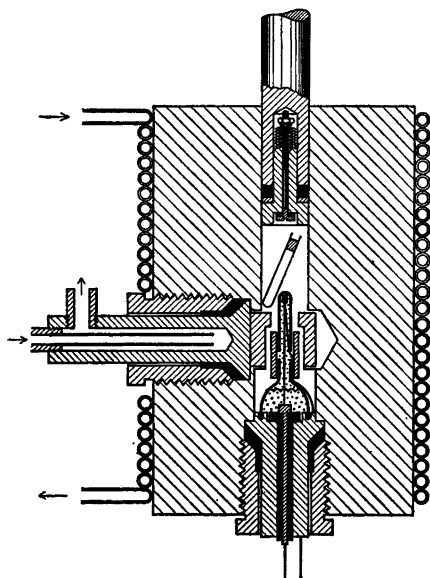

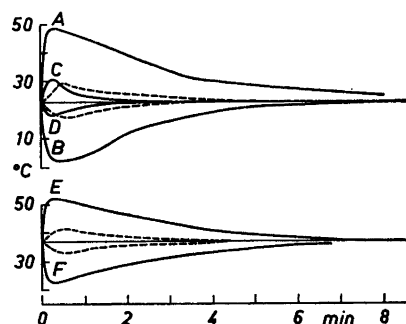Fig. 1. Cross-section of cylindrical pressure chamber with slanting specimen tube in axial waterfilled hole.

Fig. 2. Temperature pulses associated with compression/decompression of paraffin oil and water. A: Paraffin oil temperature under compression to 2700 kg/cm². B: Paraffin oil temperature under decompression from 2700 kg/cm². C: Water temperature under compression to 2700 kg/cm². D: Water temperature under decompression from 2700 kg/cm². E: Paraffin oil temperature under compression to 1350 kg/cm². F: Paraffin oil temperature under decompression from 1350 kg/cm². Broken curves show paraffin oil temperatures measured by thermistor in steel pocket.

The chamber was originally filled with paraffin, but, as shown in Fig. 2, this sample environment caused a highly undesirable heating pulse on compression and a cooling pulse on decompression. These pulses could be reduced to a very large extent by the use of water in the chamber. However, as the isolation of the thermistor was obviously damaged by the pressure cycles, it was provided with a thin paraffin cover and separated from the water by means of a glass-funnel. This consisted of two sections connected by a piece of rubber tubing which permitted the compression and expansion of the paraffin oil surrounding the probe. With this arrangement and the chamber set at the desired temperature by means of an outside coil and a temperature conduction "finger" made of copper, it was possible to raise the precooled sample as well as the chamber water to exactly the desired temperature when exposed to the pressure selected. However, the metal mass of the chamber made it impossible to carry out a rapid succession of tests with only one chamber. For this reason and in order to provide facilities for other lines of study in the department, a 6-fold hydraulic pressure bench was constructed. Fig. 3 shows this bench and Fig. 4 the circulation system provided for the temperature control of the chambers.
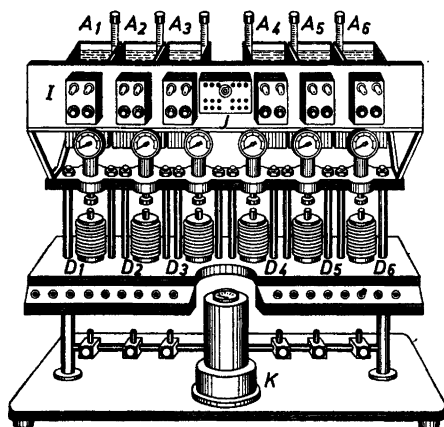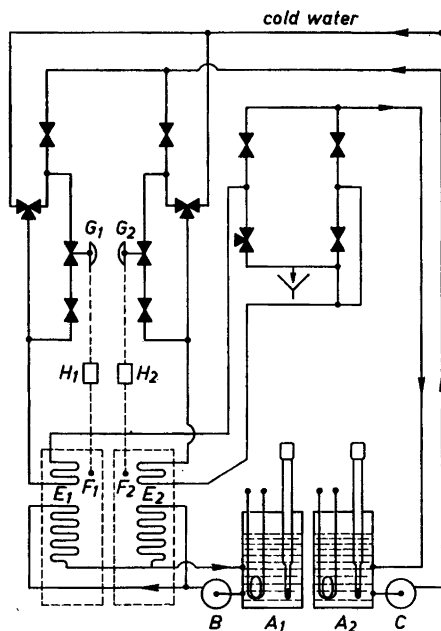
Fig. 3.   Fig. 4.

Figs. 3 and 4. Pressure unit with temperature control circuit.

Six thermostated water-baths (A_1, A_2, etc.) can be used. Three of these (A_1, A_3, and A_5) are connected *via* pumps (B) to coils of copper tubing serving as jackets (D_1, D_2, et .) for the pressure chambers. The water-baths A_2, A_4, and A_6 are connected *via* pumps (C) and a system of valves to the temperature control finger in the pressure chambers (E_1, E_2, etc.). Those can also be flushed with cold water when desired. Thermistors (F_1, F_2) in the chambers operate solenoid valves (G_1, G_2) *via* electronic switches (H_1, H_2) and thus control the finger temperatures. Since rubber hoses are used for the connection of the chambers to the piping, special types of arrangement can easily be achieved; the fingers and jackets were run in series in the highest temperature experiments.

I is one of the electronic switches, J, the selector for connecting any desired thermistor to an indicator instrument and K the hydraulic pump.

DNA preparations. DNA, isolated from a prototrophic strain of *Bacillus subtilis* by means of a conventional detergent-phenol procedure,[8] was used in most experiments. This DNA showed high transforming activity, and a limiting viscosity number of 5000.

In experiments on the hyperchromic effect of DNA under pressure, a commercial thymus DNA (Worthington) was used.

Methods. Transformation tests on *B. subtilis* DNA were performed according to Anagnostopoulos and Spizizen [9] with the strains * 168 (*ind⁻*) and SB 1 (*ind⁻*, *hist⁻*), the latter strain permitting the simultaneous transformation of two unlinked markers, independence of indole and histidine.

A solution of 1 µg/ml *B. subtilis* DNA in 0.15 M NaCl and 0.015 M trisodium citrate was used for high pressure treatment. This low DNA-concentration was chosen as a precaution against possible concentration dependence effects. In some experiments 2 M

* Kindly provided by Dr. J. Spizizen.

NaCl and 0.001 M trisodium citrate has also been used as solvent to check the influence of ionic strength on the heat denaturation curves. The salt solutions were heat-sterilized and de-aerated before use.

Experiments on the hyperchromic effect accompanying heat denaturation were performed with thymus DNA in 0.15 M NaCl and 0.015 M trisodium citrate. 0.1 volume of 10 % formaldehyde in 0.1 M 'tris'-HCl-buffer, pH 7.6, was added to these samples before treatment to prevent re-formation of hydrogen bonds after cooling.[10] 'Tris'-buffer was preferred to phosphate buffer, as the changes in the degree of ionization in the organic amine type of buffers are accompanied by very small changes in molecular volume, while the dissociation of the $H_2PO_4^-$ ion is accompanied by an exceptionally large volume decrease, leading to an acid shift at high pressures.[3] The optical density at 260 mμ after pressure-temperature treatment was read with microcuvettes in a Zeiss PMQ II spectrophotometer.

Renaturation experiments were performed on *B. subtilis* DNA at a concentration of 20 μg/ml in 0.3 M NaCl and 0.03 M trisodium citrate.[11] The DNA solution was denatured by heating to 100°C for 15 min. followed by rapid cooling. The denatured DNA solution was more or less renatured by heating for 60 min at different temperatures under 1 atm and 2700 atm, respectively. The residual transforming activity was measured.

When exposed to pressure, the DNA solution was kept in a capillary Pyrex-glass tube, containing 0.25 ml. The tube was closed in one of three ways:

a) a rubber tube closed at its upper end by a glass bead, the space above the DNA solution being filled with argon,

b) the same as a), but with a thin layer of paraffin oil floating on the DNA solution, preventing contact with the gas,

c) the same as b), but with silicone oil instead of paraffin oil.

It was found upon comparison that heat-denaturation curves, which were identical in other respects, were not affected in any way by these procedures.

Samples for pressure treatment were precooled together with the chamber water to an empirically found temperature level in order to compensate for the heating pulse during compression. The temperature in the pressure chamber was followed continuously during and immediately after compression. The compression was rapid in all experiments (less than 1 min). Advantage was taken of the cooling pulse during decompression to rapidly remove the samples while still $\sim$ 10°C cooler than the previously used temperature without using external cooling of the pressure chamber. In some control experiments, though, the whole stainless-steel cylinder was cooled with circulating tap water before the pressure was released.

No correction for increase in the ionic strength of the samples under pressure has been made. For ionic systems, corrections for the pressure-dependence of the activity coefficients in water solutions are actually negligible. In systems of lower dielectric constant they are, however, important.[12] On the other hand, a small increase in the salt concentration of the sample will occur when the water is compressed to a smaller volume. The overestimation of the stabilizing pressure effects for this reason is probably less than 1°C.

Table 1. Transforming activity of *B. subtilis* DNA after exposure to high pressure in 0.15 M NaCl and 0.015 M trisodium citrate for 30 min.
DNA concentration in the transformation experiment 0.01 μg/m'. Samples from the bacterial cultures were diluted 1:10. 0.1 ml was then plated out.

Atm	Number of colonies	
	ind⁻-marker	*hist⁻*-marker
1	170 152	269 291
10 000	166 156	275 297

RESULTS

As shown in Table 1, no decrease in transforming activity occurs when *B. subtilis* DNA is exposed to 10 000 atm for 30 min at room temperature. It should be mentioned that the absence of a specific temperature control in this particular experiment means that the sample has been exposed to a short heating pulse of 20°C—30°C above room temperature due to adiabatic compression.

If a solution of *B. subtilis* DNA in 0.15 M NaCl and 0.015 M trisodium citrate is heated at 1 atm, the biological activity of the DNA is rapidly lost above 90°—91°C, the *hist⁻*-marker being somewhat more temperature-resistant than the *ind⁻*-marker. The decrease in activity probably reflects a single-stranding of the DNA double helix. When the same experiment is performed under a pressure of 2700 atm, no heat denaturation takes place below 96°C (Fig. 5).
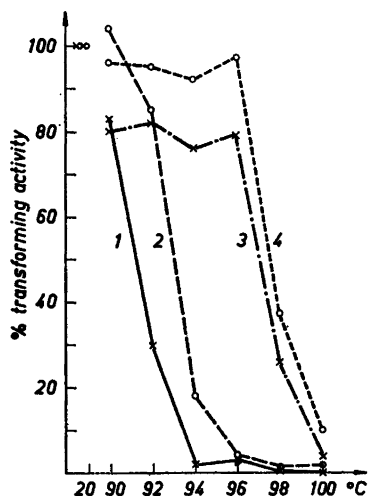

Fig. 5. Heat inactivation of transforming DNA from *B. subtilis* at two different pressures. The solvent was 0.15 M NaCl and 0.015 M trisodium citrate and the exposure time 30 min for each sample. 1 = *ind⁻*, 1 atm.; 2 = *hist⁻*, 1 atm.; 3 = *ind⁻*, 2700 atm.; 4 = *hist⁻*, 2700 atm.

In 2 M NaCl plus 0.001 M trisodium citrate, a stabilizing effect of the same magnitude is obtained, the critical temperatures being 94°C at 1 atm and 102° at 2700 atm.

The increased stability against heat denaturation at high pressures is further shown in Figs. 6 and 7. A minimum pressure of 2000 atm is necessary for complete stabilization of the native secondary structure of DNA in 2 M NaCl plus 0.001 M trisodium citrate at 100°C. Exposure to 100°C under high pressure for even longer times does not cause denaturation as does the same temperature exposure at atmospheric pressure.

A slow, first-order rate decrease in biological activity, more or less independent of the secondary configuration of the DNA, always takes place at these high temperatures. It mainly depends on the hydrolytic splitting-off of purine bases from the DNA molecule.[13] This slow decrease in activity provides a background when the rapid break-down of the helix structure is studied. The primary structure of DNA in the denatured state is degraded somewhat
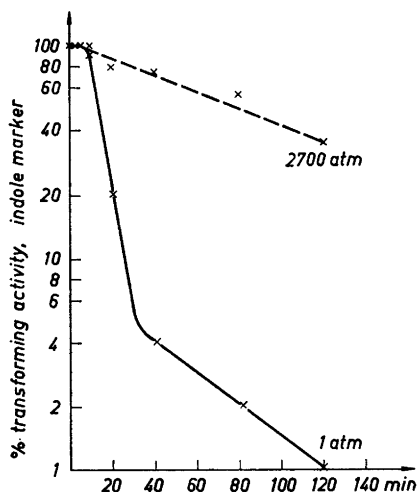
Fig. 6. Heat inactivation of transforming DNA from *B. subtilis*. The temperature was 100°C and the solvent 2 M NaCl and 0.001 M trisodium citrate.
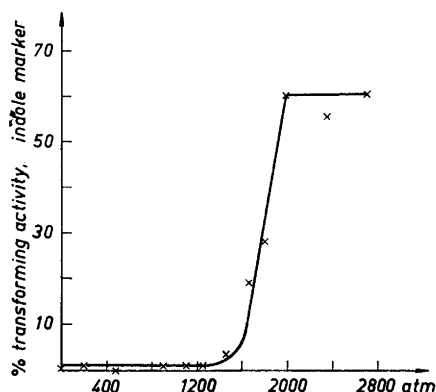

Fig. 7. Residual transforming activity of DNA from *B. subtilis* after heating to 100°C at different pressures. The solvent was 2 M NaCl and 0.001 M trisodium citrate and the exposure time 30 min.

faster than in helical DNA (Fig. 6). It seems reasonable to assume that the double-helix structure may to some extent prevent solvent-base interactions.

The hyperchromic effect occurring when a DNA solution is heated also takes place at a higher temperature under high pressure, the mean denaturation temperature, T_m, being 5°—6°C higher at 2700 atm than at 1 atm (Fig. 8).

The optimum temperature for renaturation of denatured DNA is found at about 25°C below the denaturation temperature, T_m. It might be expected
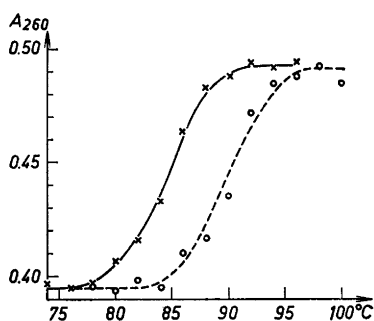

Fig. 8. Optical density of a thymus DNA solution after heating at two different pressures. Each sample was heated for 15 min. ——— = 1 atm, ----- = 2700 atm.
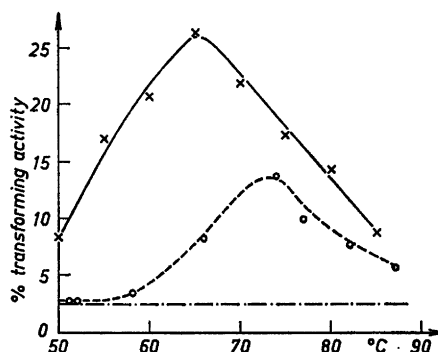

Fig. 9. Renaturation of denatured transforming DNA from *B. subtilis* at two different pressures. —·—·—· indicates the transforming activity before renaturation. ——— = 1 atm, ----- = 2700 atm.

that the optimum temperature for renaturation, like the T_m, will be shifted upwards under pressure. This is indeed the case, as seen from Fig. 9. The optimum renaturation temperature for *B. subtilis* DNA at 1 atm is $65° - 66°C$, a value in close agreement with that found by Marmur and Doty,[11] while at 2700 atm the optimum temperature is $73°C$. For reasons unknown, the renaturation is markedly inhibited by the pressure treatment.

DISCUSSION

The stabilizing effect of high pressure against heat denaturation of DNA is perhaps most easily explained as a counter-action of an increase in molecular volume associated with the change to a disordered state. This would seem to be contradicted by the fact that single-stranded DNA has a higher apparent density than native DNA when ultracentrifuged in a CsCl density gradient.[14] It is, however, possible that the heat-denaturation of DNA proceeds *via* a partly unfolded state of relative large molecular volume, while the final product occupies a smaller molecular volume. It is also possible, as pointed out by Kauzmann,[15] that denatured, flexible macromolecules might have a much larger coefficient of thermal expansion than native ones, leading to a volume increase on denaturation at high temperatures (retarded by high pressure) but to a volume decrease at low temperatures (accelerated by high pressure).

The T_m-curve for thymus DNA, while being shifted to a $6°C$ higher temperature level does not change its shape detectably under high pressure. A preferential pressure stabilization of any one of the two base-pairs in DNA (adenine-thymine or guanine-cytosine) is therefore unlikely. While the T_m-curve probably reflects the disruption of base-pairs in the DNA helix, the decrease in transforming activity, which takes place at a higher temperature, corresponds to the final melting-out of the most thermo-stable (guanine-cytosine-rich) regions of the molecule. The magnitude of the pressure-stabilization in both cases is the same, again contradicting a preferential stabilization of one of the base-pairs.

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